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Enterobacteriaceae in ventilated PICU-patients: impact of drug resistances and influence on VAP-acquisition

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Abbreviations

BAL – bronchoalveolar lavage

BMI – body mass index

CDC – centre for disease control

CRT – classification and regression tree

CVL – central venous line

ECDC – European centre for disease control

ECMO – extracorporeal membrane oxygenation

EEA – European Economic Area

EU – European Union

EUCAST – European Committee On Antimicrobial Susceptibility Testing

ESBL – extended-spectrum-β-lactamases

GI – gastrointestinal

GN – gram-negative

HAI – healthcare-associated infection

ICU – intensive care unit

IQR – interquartile range

LOS – length of stay

MDR – multidrug resistant

MRGN – multidrug resistant gram-negative bacteria

MRSA – methicillin resistant staphylococcus aureus

NHSN – National Healthcare Safety Networks

NICU – neonatal intensive care unit

PDR – pandrug resistant

PICU – paediatric intensive care unit

RKI – Robert-Koch-Institute

SD – standard deviation

spp – species

TA – tracheal aspirate

TMP – trimethoprim

VAP – ventilator-associated pneumonia

VRE – Vancomycin-resistant *Enterococci*

XDR – extensively drug-resistant

1. Introduction

Infectious diseases have been a major global cause of morbidity and mortality for thousands of years. Control of infectious diseases has largely been influenced by the establishment of a new consciousness for hygiene by Semmelweiß in the 19th century. Public health education and vaccination programs were developed and environmental hygiene and pasteurization gained more and more importance. Apart from such prevention, effective treatment of infectious diseases became possible in the beginning of the 20th century with the discovery of Penicillin. The development of other antibiotic agents followed in the beginning of the 20th century (1). Nowadays, numerous potent antibiotic substances such as 4th and 5th generation cephalosporines, carbapenemes or glycylcyclines with broad spectrum of action are available (2).

Unfortunately, during the last decades these substances lost power: the problem of drug-resistant bacteria has emerged and is gaining importance. Whenever an antibiotic is used, it puts selective pressure on microorganisms, eradicating only the susceptible strains. Therefore, the emergence of antimicrobial resistance occurs as a direct consequence of the extensive use and particularly misuse of antibiotics. Globalization, international travel and trade allow resistant strains to pass borders and spread with an alarming speed. Prominent examples for resistant bacteria are methicillin resistant staphylococcus aureus (MRSA) or multidrug resistant gram negative bacteria (MRGN).

Infectious diseases, easily treated a few years ago, may now have no treatment option, if the causative organism does not respond to the antibiotic therapy anymore.

Environments with sick or immunocompromised patients and extensive antibiotic use such as hospitals or healthcare facilities are a source of multi-resistant bacteria (3).This leads to a higher risk of healthcare associated infections (HAI) for hospitalized patients, particularly for critically ill patients, patients of older age or children.

Critically ill children are at high risk for severe HAI due to invasive devices and procedures, secondary immunosuppression and underlying diseases. Consequently, nosocomial infections with drug-resistant organisms have become a serious concern for children admitted to the paediatric intensive care unit (PICU) and will supposedly increase in the next years (4,5).

1.1 Multidrug resistant gram-negative organisms

During the last decades, nosocomial infections caused by multidrug resistant organisms (MDR) have mainly been gram-positive, first of all MRSA. However, since the beginning of the 21st century, MDR gram-negative organisms have gained an increasing importance as a cause for nosocomial infections (6). Amongst these gram-negative bacteria, the family of Enterobacteriaceae is of particular concern. In hospitals, Enterobacteriaceae are amongst the most important causative organisms for various nosocomial infections such as bloodstream infections, pneumonia, urinary tract infections, surgical site infections, or rarely meningitis (7,8).

Enterobacteriaceae are facultative anaerobic organisms, including bacterial genera such as *Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter* and *Proteus*. These bacteria are a physiological part of the enteric system but can also cause different infections in healthy and especially in immunocompromised patients. Furthermore, *Yersinia* and *Salmonella* are part of the Enterobacteriaceae, those genera are not physiologically found in the human enteric system and are obligate pathogens (8).

1.2 Emerging antibiotic resistance

During the last years, a trend of increasing drug resistance in gram-negative organisms has been observed worldwide for several reasons: hospitals use antibiotics frequently and some complicated cases require a prolonged or even permanent antibiotic treatment. These therapies put bacteria under selective pressure and lead to the development of resistance (3,9). MDR organisms might as well have been brought into the hospitals from other environments: patients admitted from other hospitals, care facilities, or high prevalence countries might

be colonized with MDR bacteria (10). Furthermore, the resistant bacteria may be transmitted by doctors, nurses, from patient to patient or by visitors (11).

This worldwide trend is alarming, considering the fact that not only screening and prevention, but also the development of new antimicrobial drugs has focused on gram-positive organisms during the last years, leaving only few antibiotics to fight MDR gram-negative infections (6,12).

A striking example is the spread of carbapenem-resistant *Klebsiella pneumoniae* in Europe. Introduced in the 1980s, carbapenemes used to be the last-line antibiotics against MDR gram-negative infections. Their chemical structure led to a broad spectrum of activity and a very slow hydrolization by common bacterial beta-lactamases. However, the emergence of carbapenemases, beta-lactamases that hydrolyse carbapenemes, leads to inefficiency of these drugs against carbapenemase-producing bacteria (12). Treatment options for patients infected with carbapenem-resistant *K.pneumoniae* or other carbapenem-resistant bacteria are limited. Unfortunately, the European centre for disease control (ECDC) reports an increasing percentage of carbapenem-resistant *K.pneumoniae* in the European Union since 2009 (10).These data are similar to observations in the U.S. and other countries, that report spread and local outbreaks of carbapenemase-producing bacteria since the beginning of the century (10).

Apart from *K.pneumoniae,* especially *A.baumanii and P. aeruginosa* became resistant to several common antibiotic regimens (13). In consequence many first line-antibiotics are by now without effect against these organisms. Older drugs with wider spectrum and higher toxic potential have to be used to treat infections. Furthermore, empiric antibiotic treatment often fails when drug resistant organisms are the cause of infection. Therefore, infection with drug resistant organisms is potentially associated with higher mortality compared to infection caused by susceptible organisms (12).

1.3 Mechanisms of drug resistance

Resistances against antibiotics can be intrinsic i.e. the organism has a primary resistance against a class of antibiotics. An example is *Mycoplasma*, a bacillus without a cell wall. Consequently, it is intrinsically resistant to all β-lactam-antibiotics which inhibit the building of a cell-wall. Extrinsic resistances are caused by mutation of genes or by virus-plasmid transfer of resistance-genes from one bacillus to another, even between different species.

The most common mechanisms of gaining resistances in bacilli are:

1. **β-lactamases**: The most frequent mechanism is the expression of the enzyme β-lactamase that can inhibit several antibiotic agents by hydrolysing their β-lactam-structures. β-lactamases are a heterogeneous group of molecules, differing in genetics, molecular structure, substrates and possibilities to be inhibited. A highly important group are the **Extended-Spectrum-β-Lactamases** (ESBL) that cause resistances against penicillins, cephalosporins, monobactams and oxyimino-cephalosporins. *K.pneumoniae* and recently increasingly *E.coli* are the most frequently isolated ESBL-producers (14). Lately, more and more gram-negative organisms, especially *E.cloacae* express **Carbapenemases**, ß-lactamases that lead to resistance against carbapenemes (15). In *Citrobacter freundii* and *Morganella morganii* a higher expression of the intrinsic **AmpC-β-Lactamase** causes a resistance against cephalosporins and penicillins. In case of high concentrations of this enzyme, even special lactamase-inhibitors are not sufficient to inhibit the AmpC-β-Lactamase (16).

2. **Changes in the binding-side of antibiotics**: Mutations of the bacterial enzymes Gyrase and Topoisomerase IV impede the binding of antibiotics, e.g. fluorchinolones. These mutations are often transmitted by plasmids (17).

3. **Changes in the membrane**: Mutations in genes coding for certain membrane-proteins can lower the permeability of the bacterial cell-membrane for certain antibiotics. This mechanism is often found in ESBL-producing *E.coli* and *P. aeruginosa* (18).

4. **Active efflux**: Chromosomal mutations can lead to the production of transporting-proteins that remove antibiotics from the cell before they can unfold their effects. *P. aeruginosa* and *A. baumanii* are the most frequently reported bacteria with active efflux.

As mechanisms of resistance are highly variable, the diagnosis of drug-resistance and development of new antimicrobial drugs are difficult (19). Empiric antibiotic therapy without availability of cultures and sensitivities, or a therapy that is not adapted to the resistance-profile, can lead to selection of resistant organisms. Inefficency of antibiotic treatment against resistant clones of bacteria leads to overgrowth of the resistant clones, while the susceptible ones are suppressed by the treatment.

1.4 Risk of healthcare associated infections due to MDR organisms

Especially in PICUs, children are at a high risk for nosocomial infections. HAI are a particular threat due to physiological immaturity of the immune system in early stages of a child's life, severe underlying diseases, invasive procedures or secondary immuno-suppression (e.g. corticosteroid treatment, chemotherapy). In German PICUs gram-negative organisms are amongst the most frequently isolated organisms causing HAI onset (7,20). MDR organisms in particular are responsible for an increasing part of HAI on PICUs, differing between nations (16-31%) and hospitals (21,22).

Gram-negative bacteria elicit different types of nosocomial infections; bloodstream infections, respiratory infections, especially ventilator-associated pneumonia (VAP), surgical site infections and urinary tract infections are the most common ones. If these infections are caused by multi-resistant strains, treatment becomes challenging. Even though MDR organisms are not necessarily more pathogenic than susceptible ones, infections with resistant bacteria have a worse outcome. They lead to more complications, longer hospitalization, higher mortality and higher healthcare-costs (9). This worse outcome can be explained by the ineffective empiric therapy and the delay of appropriate antibiotic treatment (12).

The risk of infection with MDR gram-negative bacteria increases in several circumstances. Predisposing factors for MDR infections depend on the environment and the individual treatment of a patient. Immunosuppression, severe underlying disease, long hospitalization, catheterization and gastral intubation increase the risk of MDR infection just as previous antibiotic treatment, living in long term care facilities, staying in endemic areas or contaminated food (*E.coli*) (23-25). Robert E. et al reported an ESBL-rate on intensive care units (ICUs) twice as high as on normal wards (26). In children, premature birth, low age and female sex are additional risk factors for HAI due to gram-negative bacteria (21,27)

1.5 Screening and prevention of infection with multidrug resistant organisms

Children admitted to PICU from long term care facilities and with multiple previous hospitalizations should screened for bacteria and resistances, including MRSA and Vancomycin-resistant *Enterococci* (VRE) on admission. Particular screening guidelines for PICUs do not currently exist. However, screening can be based on the definition of multidrug resistant gram-negative bacteria recommended by the German Robert-Koch-Institute (RKI) and the RKI guidelines for NICUs (16,28). The RKI definition is based on four major classes of antimicrobial substances: acylureidopenicillins, $3^{rd}/4^{th}$ generation cephalosporins, carbapenemes and flourchinolones. Depending on the number of resistances, bacteria are defined as 3 or 4 MRGN. Taking into account that an empiric therapy with flourchinolones is contra-indicated in children, the RKI has subjoined the category 2 MRGN Neo-Päd for paediatrics and neonatology.

In case of a positive screening result, isolation precautions are warranted according to local/national hygiene guidelines or the RKI recommendation (16,29).

Contact precautions (single room, hand hygiene, single use gloves and coats) are mandatory in patients tested colonized with a 4 MRGN organism. In case of colonization of the respiratory tract, droplet precautions (surgical facemask) are necessary as well.

In a PICU setting, precautions for 3 MRGN and 2 MRGN Neo-Päd bacteria are the same as for 4 MRGN bacteria because the setting bears a higher risk for MRGN infection.

1.6 VAP on PICU

1.6.1 Ventilator-associated pneumonia

Ventilator-associated pneumonia is one of the most common nosocomial infections in critically ill children (7,30). VAP is a severe complication, occurring in 3-17% of ventilated PICU patients (31,32). The overall onset of VAP differs between 7.02-11.6/1000 ventilator-days (33,34). Children on mechanical ventilation are often critically ill, sedated and unable to communicate. Therefore, it can be difficult to diagnose a VAP, especially if younger children present with comorbidities or unspecific clinical symptoms. The American centre for disease control (CDC) has defined a set of criteria to diagnose VAP. The criteria include radiographic, clinical and microbiological findings (34). Even though the CDC definition for the diagnosis of VAP is commonly accepted, the criteria of VAP-diagnosis are not well established and differ between hospitals. The CDC definition is criticised for the requirement of radiographic evidence since the interpretation and language used to describe the findings is subjective and may differ between institutions. Furthermore, subjectivity of some clinical diagnostic criteria as well as variability in specimen collection and culturing practices may affect case-finding (35). However, the guidelines are widely established and can help to improve internal quality purposes (36). It is well known that VAP leads to prolonged ventilation, hospitalization and higher mortality (14%-76%) of the ventilated child (30,31,37,38).

1.6.2 Causative organisms for VAP

MDR Enterobacteriaceae have become a particular concern for mechanically ventilated patients (39). Placement of an endotracheal tube is followed rapidly by tracheal colonization with potentially pathogenic microorganisms from the oropharyngeal flora, including MDR organisms (40,41). During the last years, gram-negative bacteria were the most frequently isolated pathogens in VAP-PICU-patients in Germany and in the US (42-68%) followed by *S. aureus* (15-20%) and *Haemophilus influenza* (11%) (4,42). One third of all VAPs are caused not only by a single, but by different species at the same time (42).The high

prevalence of gram-negative bacteria in ventilated patients might be aggravated by the fact that in critically ill patients the oropharyngeal colonization can shift from gram-positive to gram-negative specimens (43).This shift could be caused by the use of antibiotics that suppress the normal intestinal and oropharyngeal microflora which is gradually replaced by *Klebsiella spp, Enterobacter spp, Serratia spp* and other gram-negative bacteria (44). Oropharyngeal bacteria can be transferred to the lower respiratory tract during intubation and cause severe infections thereafter.

In adults, gram-negative organisms, especially *Pseudomonas, Klebsiella* and *E.coli* are the most common pathogens causing VAP. Several studies report an increasing rate of drug resistance amongst these causative organisms: an Australian adult ICU reports 29% MDR of all isolates causing VAP, a Belgium adult ICU observed 21% of all gram-negative organisms causing VAP were MDR (45,46). From countries with lower medical and hygienic standards much higher MDR rates have been reported (47).

Gram-negative organisms are most likely to cause VAP in PICU patients (48). If MDR gram-negative organisms become more prevalent, it may well be that MDR-rates in PICU VAP rise as well. An analysis from a PICU in Shanghai supports this hypothesis by reporting that most of the gram-negative bacteria causing VAP were resistant (38).

1.6.3 Risk factors for VAP on PICU

Several different risk factors for VAP have been detected in various studies during the last years. The results partially differ or are even contradictory. Depending on the study, the PICU/NICU (neonatal intensive care unit) length of stay, duration of mechanical ventilation, previous antibiotic or steroid therapy, tracheostomy, genetic syndromes, bloodstream infection, emergency intubation, re-intubation, previous bronchoscopy, age <1 y, long term sedation, colonization of the upper respiratory tract, previous surgery, both enteral and parenteral nutrition, transfusion, female gender and discontinuous PICU-stay have been declared as risk factors (30,32,42,49-51).

In a meta-analysis from 2013, B. Liu et al. extracted significant and independent risk factors for VAP in ventilated children, which were genetic syndromes, re-intubation and accidental extubation, bloodstream infections, previous treatment with antibiotics or steroids and bronchoscopies (42). A factor that has not been mentioned in this analysis, but seems to be important too, is the duration of ventilation - prolonged ventilation also increases the risk of VAP (45,50).

1.6.4 Prevention, screening and treatment of VAP

Cooper et. al propose a bundle of prevention methods especially for VAP in children (52):

- Elevated head of the bed
- Hand hygiene before and after contact with patient or ventilator
- Providing oral hygiene according to the patients age
- Change ventilator circuit every 7 days or when circuit is visibly soiled or malfunctioning
- Suction endotracheal tube only when indicated by a clinical examination; do not instil physiological saline for suctioning
- Drain condensation from ventilator circuit every 2-4 hours

The current guidelines recommend quantitative cultures from material of the lower respiratory tract to diagnose a nosocomial pneumonia (53). Tracheal aspirate (TA) or bronchoalveolar lavage (BAL) can be used to screen patients for MDR-bacteria in the lower respiratory tract.
However, a positive screening result on MDR-bacteria in tracheal aspirate does not necessarily imply an infection. Infection rates in colonized patients are reported up to 33% (*E.coli*) and more than 50% (*Pseudomonas spp*) depending on species and patient population (16).

Furthermore, neither tracheal aspirate nor BAL are 100% sensitive or specific and can lead to false treatment and antibiotic overuse (54,55). A lung biopsy would provide the most accurate results to diagnose VAP. However, as lung biopsy is a highly invasive procedure, clinical CDC criteria in combination with tracheal aspirate or BAL are far more adequate in clinical practice.

Current treatment guidelines for VAP-prevention mostly refer to VAP in adults and recommend empirical coverage of gram-negative bacilli with a third or fourth generation cephalosporin, piperacillin-tazobactam or a carbapenem in combination with a fluoroquinolone or an aminoglycoside, based on the local patterns of susceptibility. In many cases colistin or tigecyclin are the only treatment options (56). However, tigecyclin is not licensed for the treatment of pneumonia and contra-indicated for children under 8 years and thus must be used off-label. The same limitations apply to flourqinolones; they are also contraindicated in children (57).

1.7 Aims of the study

Children admitted to a PICU have a high risk for severe HAI with all possible consequences. These may be elevated morbidity and mortality, longer hospitalization and higher healthcare costs. Amongst gram-negative organisms, MDR Enterobacteriaceae are becoming more dominant in intensive care medicine and rise concern in ventilated paediatric patients due to limited therapeutic options.

The present study aims:

1. (a) to determine the prevalence and spectrum of Enterobacteriaceae in tracheal aspirate of ventilated children on a PICU.
   (b) to determine the proportion of MDR Enterobacteriaceae in these isolates.
2. to identify risk factors for colonization or infection with MDR Enterobacteriaceae in ventilated patients
3. to identify risk factors for VAP due to Enterobacteriaceae in ventilated children
4. to compare the outcome of VAP due to multi-resistant bacteria to those due to sensitive bacteria

We hypothesize that the outcome (risk of acquiring VAP and risk of death) of patients colonized with sensitive Enterobacteriaceae is the same as in patients colonized with MDR Enterobacteriaceae.

2. Methods

2.1 Patient collective

The University Children's Hospital Tübingen has a 14-bed PICU. On the ward, children with general paediatric, general surgical, neurosurgical and cardio-surgical problems are cared for. Critically ill children and neonates on this PICU are aged between 0 and 18 years, the majority is younger than 5 years. Most of them live in Tübingen and surrounding areas, some are admitted from long term care facilities or from abroad, e.g. from eastern Europe and Senegal.

Eligibility criteria

The patients included in this study were identified from the database of the Institute of Medical Microbiology and Hygiene, University of Tübingen. Children from 0 to 18 years were included if they were intubated and ventilated through a chest tube. Children ventilated through a tracheostoma were excluded. Patients meeting the eligibility criteria were included in the study if they had a BAL or a tracheal aspirate positive for Enterobacteriaceae.

2.2 Data collection

All tracheal aspirates and BAL samples positive for Enterobacteriaceae were retrospectively collected from the database of the microbiology laboratory from 2005-2014 (HyBase Database, Cymed). For every specimen, the individual resistance profile was determined by the University Microbiology Laboratory according to the clinical breakpoints recommended by the European Committee On Antimicrobial Susceptibility Testing (EUCAST) to define susceptibility and resistance (58). Medical records of these patients were reviewed. Demographic, epidemiological and clinical data as well as treatment and procedures were collected from the medical records in the hospital database. All data were subsequently aggregated in Excel 2013.

2.3 Definitions

2.3.1 MDR Definition

International experts of the European Centre for Disease Prevention and Control (ECDC) and the CDC propose standardized definitions for multi- (MDR), extensive- (XDR), and pan- (PDR) drug resistance in common types of bacteria (59).

Criteria for defining MDR, XDR and PDR in Enterobacteriaceae*:*

- MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories.
- XDR: non-susceptible to ≥1 agent in all but ≤2 categories.
- PDR: non-susceptible to all antimicrobial agents listed.

In this study the following worksheet by Magiorakos et al. has been used to identify MDR organisms (59):

Antimicrobial categories and agents used to define MDR, XDR and PDR

Antimicrobial category	**Antimicrobial agent**	**Species with intrinsic resistance to antimicrobial agents or categories**
Aminoglycosides	Gentamicin	*Providencia rettgeri* (*P. rettgeri*), *Providencia stuartii* (*P. stuartii*)
	Tobramycin	*P. rettgeri*, *P. stuartii*
	Amikacin	
	Netilmicin	*P. rettgeri*, *P. stuartii*
Anti-MRSA cephalosporins	Ceftaroline (approved only for *Escherichia coli*,*Klebsiella pneumoniae*,*Klebsiella oxytoca*)	
Antipseudomonal penicillins + β-lactamase inhibitors	Ticarcillin-clavulanic acid	*Escherichia hermannii* (*E. hermanii*)
	Piperacillin-tazobactam	*E. hermanii*
Carbapenems	Ertapenem	
	Imipenem	

	Meropenem	
	Doripenem	
Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins	Cefazolin	*Citrobacter freundii* (*C. freundii*), *Enterobacter aerogenes* (*E.aerogenes*), *Enterobacter cloacae* (*E. cloacae*), *Hafnia alvei* (*H. alvei*), *Morganella morganii*(*M. morganii*), *Proteus penneri* (*P. penneri*), *Proteus vulgaris* (*P. vulgaris*), *P. rettgeri*, *P. stuartii*, *Serratia marcescens* (*S. marcescens*)
	Cefuroxime	*M. morganii*, *P. penneri*, *P. vulgaris*, *S. marcescens*
Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins	Cefotaxime or ceftriaxone	
	Ceftazidime	
	Cefepime	
Cephamycins	Cefoxitin	*C. freundii*, *E. aerogenes*, *E. cloacae*, *H. alvei*
	Cefotetan	*C. freundii*, *E. aerogenes*, *E. cloacae*, *H. alvei*
Fluoroquinolones	Ciprofloxacin	
Folate-pathway inhibitors	Trimethoprim-sulphamethoxazole	
Glycylcyclines	Tigecycline	*M. morganii*, *Proteus mirabilis* (*P. mirabilis*), *P. penneri*, *P. vulgaris*, *P. rettgeri*,*P. stuartii*
Monobactams	Aztreonam	
Penicillins	Ampicillin	*Citrobacter koseri* (*C. koseri*), *C. freundii*, *E. aerogenes*, *E. cloacae*, *E. hermanii*,*H. alvei*, *Klebsiellae spp*., *M. morganii*, *P. penneri*, *P. vulgaris*, *P. rettgeri*,*P. stuartii*, *S. marcescens*
Penicillins+ β-lactamase inhibitors	Amoxicillin-clavulanic acid	*C. freundii*, *E. aerogenes*, *E. cloacae*, *H. alvei*, *M. morganii*, *P. rettgeri*,*P. stuartii*, *S. marcescens*

	Ampicillin-sulbactam	*C. freundii*, *C. koseri*, *E. aerogenes*, *E. cloacae*, *H. alvei*, *P. rettgeri*,*S. marcescens*
Phenicols	Chloramphenicol	
Phosphonic acids	Fosfomycin	
Polymyxins	Colistin	*M. morganii*, *P. mirabilis*, *P. penneri*, *P. vulgaris*, *P. rettgeri*, *P. stuartii*,*S. marcescens*
Tetracyclines	Tetracycline	*M. morganii*, *P. mirabilis*, *P. penneri*, *P. vulgaris*, *P. rettgeri*, *P. stuartii*
	Doxycycline	*M. morganii*, *P. penneri*, *P. vulgaris*, *P. rettgeri*, *P. stuartii*
	Minocycline	*M. morganii*, *P. penneri*, *P. vulgaris*, *P. rettgeri*, *P. stuartii*

When a species has intrinsic resistance to an antimicrobial agent or to the whole category, that agent or category must be removed from the list in this table prior to applying the criteria for the definitions and should not be counted when calculating the number of agents or categories to which the bacterial isolate is non-susceptible (59).

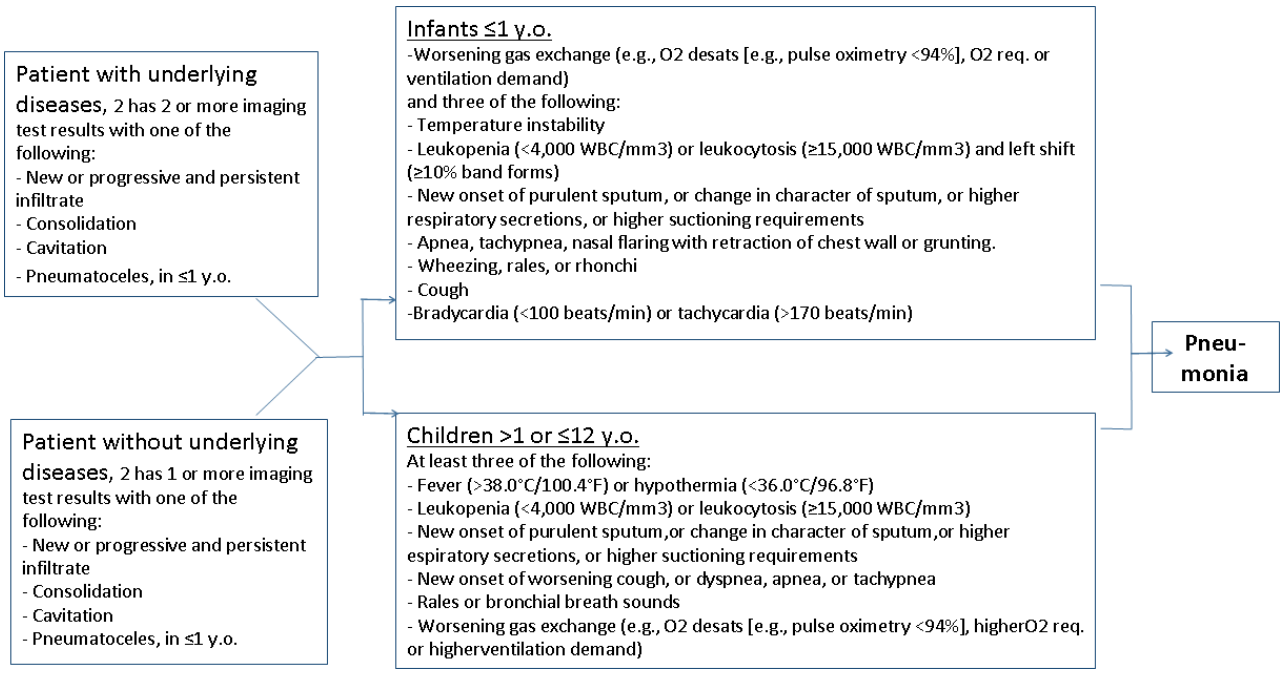
2.3.2 VAP Definition

Ventilator-associated pneumonia according to the CDC criteria is defined as: "A pneumonia where the patient is on mechanical ventilation for >2 calendar days on the date of event, with day of ventilator placement being Day 1, AND the ventilator was in place on the date of event or the day before. If the patient is admitted or transferred into a facility on a ventilator, the day of admission is considered Day 1."

A patient is defined as ventilated when a mechanical ventilation is performed through endotracheal tube or tracheostoma.

For the diagnosis of pneumonia, the CDC requires different laboratory, clinical and radiological criteria depending on the patient collective, based on the National Healthcare Safety Networks (NSHN) (34).

In this study the following flowchart from the CDC for the diagnosis of pneumonias in PICU patients was used.



y.o.=years old, O_2 req.=oxygen requirement, WBC=white blood cells

Source: CDC (34)

Furthermore, it can be distinguished between early- and late-onset VAP, depending on whether the infection occurs within the first 4 days of ventilation or afterwards.

2.4. Microbiology

Analysis of specimens

Sputum samples from the lower respiratory tract were collected through deep suctioning with the catheter passing beyond the endotracheal tube tip into the trachea or bronchi and sent to the University Microbiology Laboratory for routine analysis. Analysis was performed according to the local guidelines of the University Microbiology Laboratory, laboratory methods have been accredited according to DIN EN ISO 15189 and DIN EN ISO/IEC 17025. Organisms were grown on agar plates or with liquid culture technique and incubated at 37°C.

Monitoring was performed according to the local protocol and fast susceptibility testing was realized by rapid disc diffusion technique.

2.5. Statistics

Analysis of epidemiological data and tables was performed with Excel Version 2013 for Windows. Patient data were analysed using IBM SPSS Statistics Version 22 for Windows.

To avoid bias due to multiple isolates of one patient, we only used the first isolate per patient. Missing data points or data that were not applicable to the analysis were excluded.

Statistical analysis was performed in consultation with the Department of Statistics / Biometrics of the University of Tübingen. The categorical variables between groups were compared with the [chi]² test. For continuous variables that were normally distributed, the means of the two different groups were evaluated by two sample unpaired t-test. For continuous variables that were not normally distributed, the nonparametric Mann-Whitney-U-Test as well as the Wilcoxon-Test and the Kruskal-Wallis-Test for comparison between more than two groups were used to compare different groups. A p-value <0.05 was considered statistically significant. Results are presented as numbers for categorical variables. Normally and abnormally distributed quantitative variables are presented as mean ± standard deviation and median (minimum and maximum or interquartile range), respectively.

To identify the main risk factors for colonization with MDR Enterobacteriaceae, we used a classification and regression tree (CRT) approach and validated the findings with the common method of univariate and multivariate logistic regression. Many studies have been conducted to identify risk factors for colonization with MDR organisms (39,60,61). We selected 10 candidate risk factors available in the literature, that are of high relevance in the PICU setting. Namely, these risk factors were: patient age, duration of mechanical ventilation, duration of placement of a CVL or ECMO, length of stay on PICU prior to tracheal aspirate (TA), pre-existing GI-, cardiac or pulmonary disease, days of antibiotic pre-exposure, duration of catecholamine therapy. Furthermore the two most

prevalent species of Enterobacteriaceae in our study, *E.coli* and *Enterobacter* were included. All these criteria were analysed in a CRT-model to evaluate their impact on the risk of isolation an MDR-strain in the tracheal aspirate of ventilated children.

The CRT approach is a statistical tool to analyse specific subgroups and relationships in larger sets of predictor variables that might not be detected with more common methods, often used in similar analyses (e.g. multivariable regression equations).

The classification tree was built using SPSS CRT model (IBM SPSS Statistics Version 22 for Windows). The algorithm determines a set of predictor variables via binary partition, based on statistical significance to create a tree-based classification model to predict values of a target variable (dependent) based on independent predictor variables. The resulting classification model consists of a root node representing the dependent variable and several branches and nodes. Each node is assigned to one class representing the most appropriate target variable. Finally, the *if*-then spilt and stop conditions of the most relevant candidate predictors result in terminal nodes that provide an accurate prediction of the dependent target variable.

In consideration of the sample size, adequate default values of 19 cases for parent nodes and 9 cases for child nodes were chosen. We applied 25-fold cross-validation to avoid over-fitting and to increase the predictive accuracy of the model; maximum tree depth was 5, significance was set at 0.01. Since the CRT model is a relatively uncommon statistical method, we used a stepwise validation process to validate our findings with the common method of univariate and multivariate logistic regression.

3. Results

3.1 Patient characteristics

Between 2005 and 2014, we found 167 isolates of Enterobacteriaceae in lower respiratory tract material of 123 intubated patients on the PICU.
The patient population consisted of 74 boys (60.2%) and 49 girls, aged from 0 to 18 years. 17 patients died during their PICU stay or within 6 months after PICU admission. Characteristics of the population are shown in Table 1 below.

Table 1: Demographic and clinical characteristics of 123 intubated patients on the PICU.

IQR=interquartile range (minimum, maximum), PICU=Paediatric Intensive Care Unit, LOS=length of stay

	Median [IQR]
Age (years)	0.5 [0-17.9]
Gestational age (weeks)	37 [23-42]
Birthweight (kg)	2.8 [0.6–4.4]
PICU LOS (days)	14 [1-153]

Admission to the PICU had different reasons, 46% of the patients were hospitalized for cardiac surgery. Reasons for admission are shown in Figure 1.

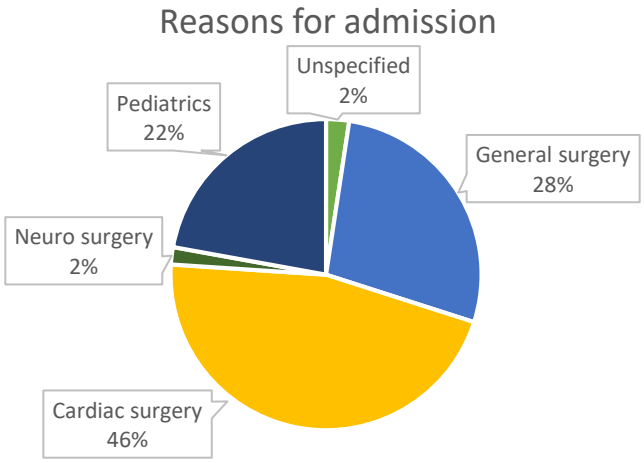


Figure 1: Reasons for admission to PICU.

During their stay on PICU, patients have been diagnosed with various infectious diseases, shown in Figure 2. Pneumonia was the most prevalent infectious diagnosis (n=64) followed by sepsis (n=40). Urinary tract infections and surgical site infections were rare cases.

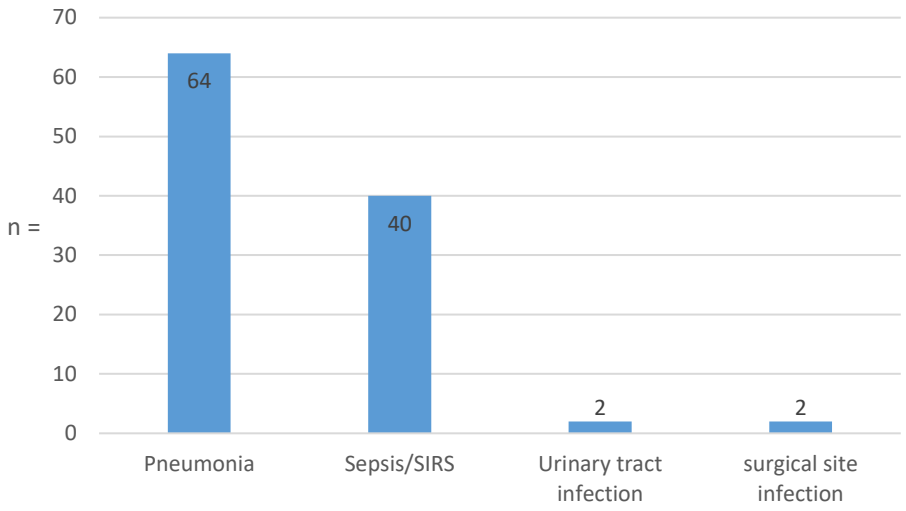


Figure 2: Count of diagnoses.
Frequency of infectious diseases in the paediatric intensive care unit diagnosed during the study period.
n=number, SIRS=Systemic Inflammatory Response Syndrome

3.2. Prevalence and spectrum of Enterobacteriaceae

During the 9-year study period, the number of isolated Enterobacteriaceae in lower respiratory tract materials has markedly increased. From 5 positive samples in 2005 their number has more than tripled to 17 positive samples in 2014 with a peak of 27 isolates in 2011. The proportion of MDR organisms amongst those Enterobacteriaceae has increased from 0% in 2005 up to 29% in 2014.

The incidence of both susceptible and drug resistant gram-negative bacteria in the tracheal aspirate of ventilated PICU patients has increased during the study period. Figure 3 shows the incidence of Enterobacteriaceae in the lower respiratory tract by year.

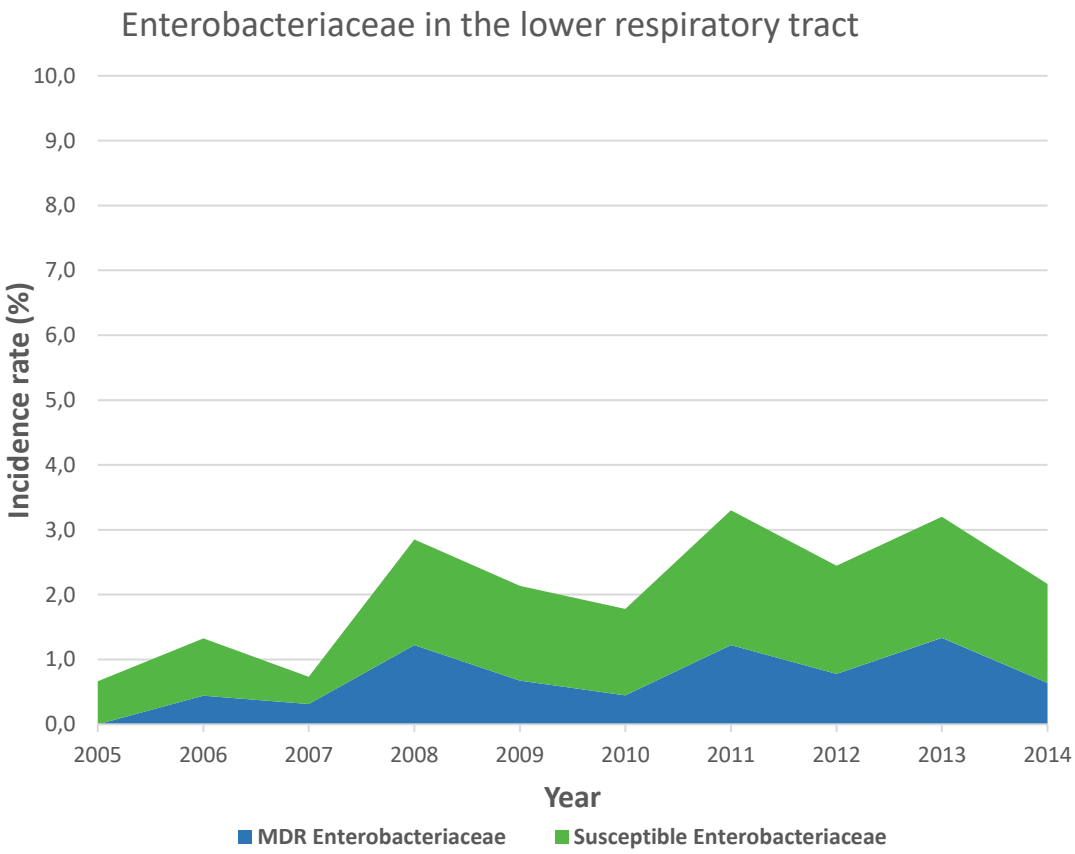


Figure 3: Incidence per year of susceptible and MDR Enterobacteriaceae in the lower respiratory tract material in PICU patients.

PICU=paediatric intensive care unit, MDR=multidrug resistant

The overall incidence rate of Enterobacteriaceae has increased from 0.66% in 2005 to 2.17% in 2014 with a peak of 3.3% in 2011. The MDR incidence rate has increased from 0% in 2005 to 0.64% in 2014 with a peak of 1.33% in 2013.

25 patients had more than one species of Enterobacteriaceae during the same hospital stay, (3 out of them had 3, 2 out of them 4 different species). 10 patients have either been admitted to PICU and tested positive twice or have been tested positive more than 30 days after the first testing, which was defined as a second period of infection. 5 Patients had 3 admissions or periods of disease.

Regarding the whole study period, the most frequently isolated species were *Enterobacter spp* (n=51) followed by *E.coli* (n=47) and *Klebsiella spp* (n=46). The distribution of all isolated Enterobacteriaceae is shown in Figure 4.

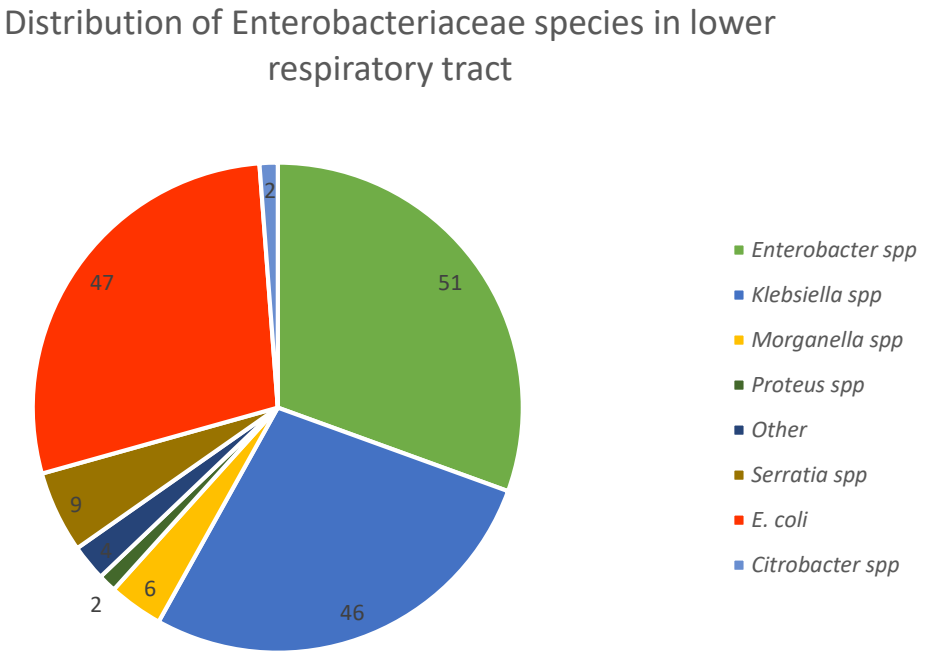


Figure 4: Species of all isolates 2005-2014.

Frequency of isolated Enterobacteriaceae species during the study period. spp=species

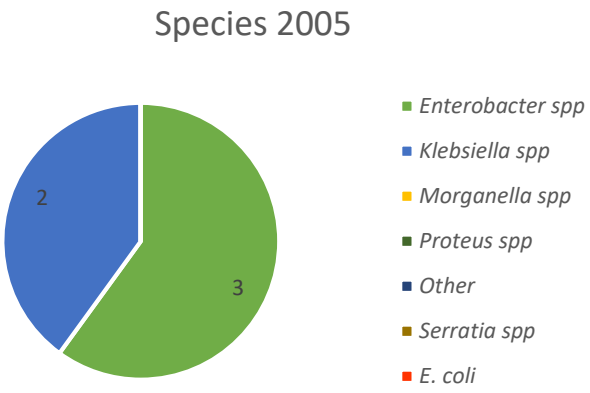


Figure 5: Species 2005.

Distribution of Enterobacteriaceae species in lower respiratory tract in 2005.

spp=species

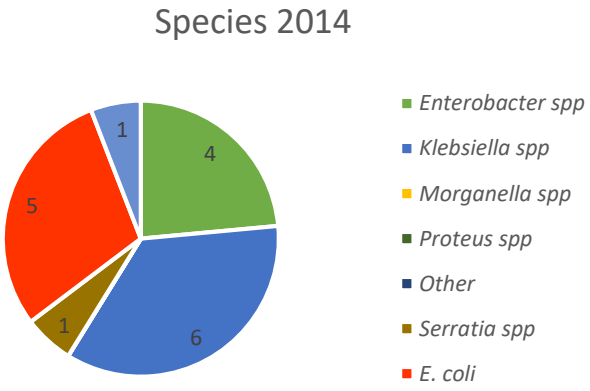


Figure 6: Species 2014.

Distribution of Enterobacteriaceae species in lower respiratory tract in 2014.

spp=species

Figure 5 and 6 show a notable shift in the spectrum of Enterobacteriaceae, especially *E.coli* has been isolated more frequently in 2014.

3.3 Proportion of drug resistant strains in different Enterobacteriaceae subspecies

All detected bacteria were tested for resistances against antibiotics and classified as MDR- and Non-MDR-organisms, following the CDC definition (59).

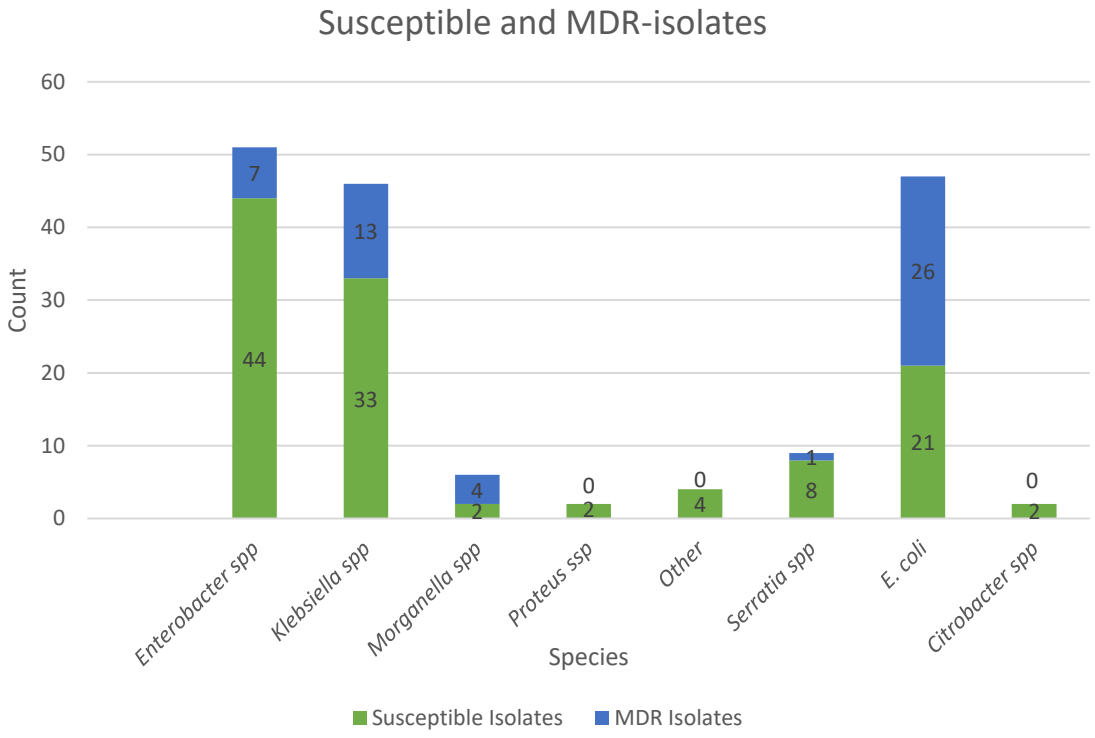


Figure 7: Susceptible and MDR-isolates.
Frequency of susceptible and MDR-strains in different Enterobacteriaceae species.
spp=species

Figure 7 shows the proportion of MDR Enterobacteriaceae species. *Morganella* was found to be the species with the most drug-resistant strains (67%) followed by *E.coli* (55.3%) and *Klebsiella spp* (28.3%). The total prevalence of MDR-organisms of all isolated Enterobacteriaceae was 30.5%.

3.4 ESBL-producing Enterobacteriaceae

The total proportion of ESBL producing organisms was 13 (7.8%) of all isolates. 7 out of 47 (14.9%) *E.coli* and 3 out of 46 (6.5%) *Klebsiella spp* were ESBL-producing isolates.

3.5 Resistance pattern

The resistance pattern of the 167 isolates revealed, that 70 % of all Enterobacteriaceae were resistant to penicillins and almost 50 % were resistant to cephalosporins. However, only 1.8 % of all Enterobacteriaceae were resistant to carbapenems.

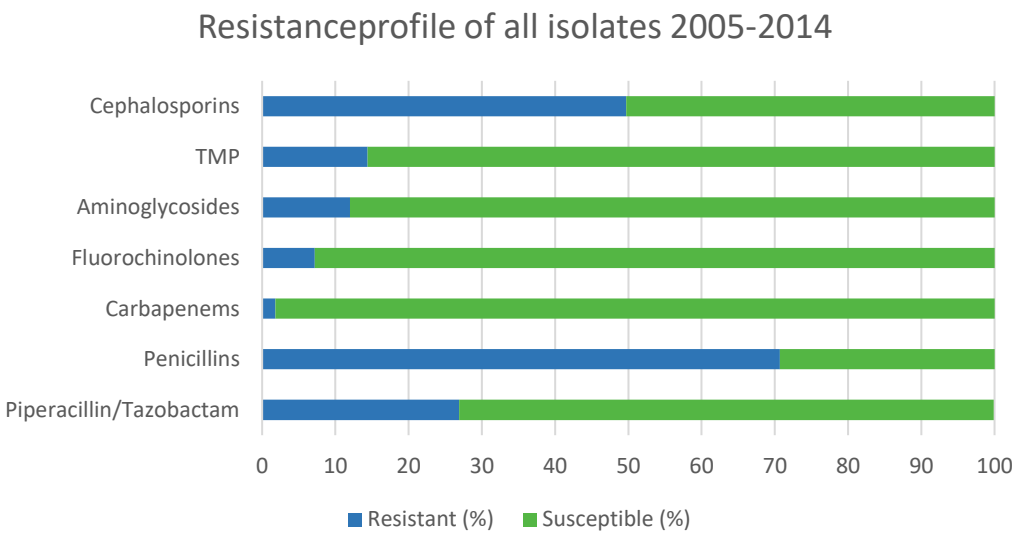


Figure 8: Resistance profile of all isolates 2005-2014.
Proportion of Enterobacteriaceae with resistance against different common antibiotic agents.
TMP= Trimethoprim.

The resistance profiles have changed over the years: while in 2005 and 2006 all isolates have been susceptible to carbapenems, in 2013 and 2014 4.9 % were resistant. A similar trend can be observed in resistances against fluoroquinolones, increasing from 5.9 % in the early study period to 17.1 % in the last two years of the study period (see Table 2). Interestingly, resistances against cephalosporins have decreased from 64.7 % to 56.1 % while resistances against Piperacillin/Tazobactam have increased from 29.4 % to 36.6 % as shown in Table 2.

Table 2: Resistant strains 2005-2006 and 2013-2014.
Proportion of resistant strains in isolated Enterobacteriaceae in 2005 and 2006 compared to those in 2013 and 2014.

Antibiotic agent	Resistant strains (%) 2005-2006	Resistant strains (%) 2013-2014
Carbapenems	0	4.9
Fluoroquinolones	5.9	17.1
Cephalosporins	64.7	56.1
Piperacillin/Tazobactam	29.4	36.6

3.6 Comparison of characteristics, risk factors and outcome in patients with MDR versus patients with susceptible bacteria

Patients who met the study criteria were younger than the entity of patients admitted to PICU. More than half of the study population were infants while only about 13% of all PICU patients were younger than one year old. In both collectives, boys counted for a little more than half of the population.

Table 3: Characteristics of the source population (all patients admitted to PICU during the study period) and the study population (a), comparison of patients colonized or infected with MDR vs susceptible Enterobacteriaceae (b).

(a) Characteristics of the source population and the study population
p=p-value, <0.05 considered significant, n=number, IQR=interquartile range, MDR=multidrug resistant, sus=susceptible, m=male, f=female

	Source population (n=7551)	Study population (n=123)		
		MDR (n=43)	Susceptible (n=80)	p-value (MDR vs sus)
Sex m/f	4129/3422	24/19	50/30	0.47
Infant/Non-infant	974/6577	25/18	53/27	0.37
Age in years (median, [IQR])	3.3 [0.5-10.7]	0.4 [0.1-2.5]	0.6 [0.2-2.0]	0.94

The comparison of the MDR vs the non-MDR patient group of the study population showed no significant differences for gender distribution or age.

(b) Comparison of patients colonized or infected with MDR vs susceptible Enterobacteriaceae

p=p-value, <0.05 considered significant, n=number, IQR=interquartile range, SD=standard deviation, MDR=multidrug resistant, BMI=body-mass-index, PICU=paediatric intensive care unit, LOS=length of stay, TA=tracheal aspirate, CVL=central venous line, VAP= ventilator-associated pneumonia

Demographic and clinical characteristics	**Sensitive Entero-bacteriaceae (n=80)**	**MDR Entero-bacteriaceae (n=43)**	**MDR vs Sensitive (p-value)**	**Total (n=123)**
Gestational age in weeks (median, [IQR])	37 [33-39]	37 [34-39]	0.76	37 [34-39]
Birthweight in kg (mean±SD)	2.67±0.87	2.52±1.05	0.49	2.62±0.93
BMI (mean±SD; kg/m²)	14.25±3.95	13.65±4.62	0.47	14.02±4.21
Underlying Diseases, n (%)				
Pulmonary	40 (50)	25 (58)	0.39	65 (53)
Cardiosurgical	53 (66)	27 (63)	0.70	80 (65)
Gastroenterological	35 (44)	26 (61)	0.08	61 (50)
Neurological	42 (53)	20 (47)	0.53	60 (50)
Hemato-oncological	7 (9)	3 (7)	0.73	10 (8)
Immunodeficiency	0 (0)	4 (9)	0.006	4 (3)
PICU LOS (median, [IQR])	14.5 [7-32.5]	14 [6-32]	0.89	14 [7-32]
Days of antibiotic therapy prior to TA (median, [IQR])	2 [0-5]	4 [0-9]	0.2	2 [0-7]
CVL in place, n (%)	38 (48)	24 (55)	0.48	62 (66)
CVL days (median, [IQR])	2 [0-7]	3 [0-9]	0.36	2.5 [0-8]

VAP incidence, n (%)	10 (13)	9 (21)	0.13	18 (15)
Days on ventilator (median, [IQR])	8 [2-16]	6 [3-19]	0.91	7 [3-18]
All-cause mortality, 6 months, n (%)	9 (11)	8 (20)	0.22	17 (14)

Source of Table 3 a and b: Renk et al (62)

Both patient collectives did not differ in gestational age, birthweight and length of stay on PICU before and after the testing. Patients with MDR organisms had significantly more often an immunodeficiency than the patient group with susceptible bacteria.
Other recorded criteria did not differ significantly between the two groups.

3.6.1 Risk factors for colonization or infection with MDR Enterobacteriaceae in the lower respiratory tract

We analysed 10 different risk factors for colonization of the lower respiratory tract with MDR gram-negative bacteria in a CRT model.
In our setting, the factors colonization or infection with *E.coli*, presence of gastrointestinal comorbidity and duration of antibiotic exposure prior to tracheal aspirate were the most important risk factors. Based on the CRT analysis a model to predict the chance of colonization with MDR Enterobacteriaceae was generated (Figure 9).

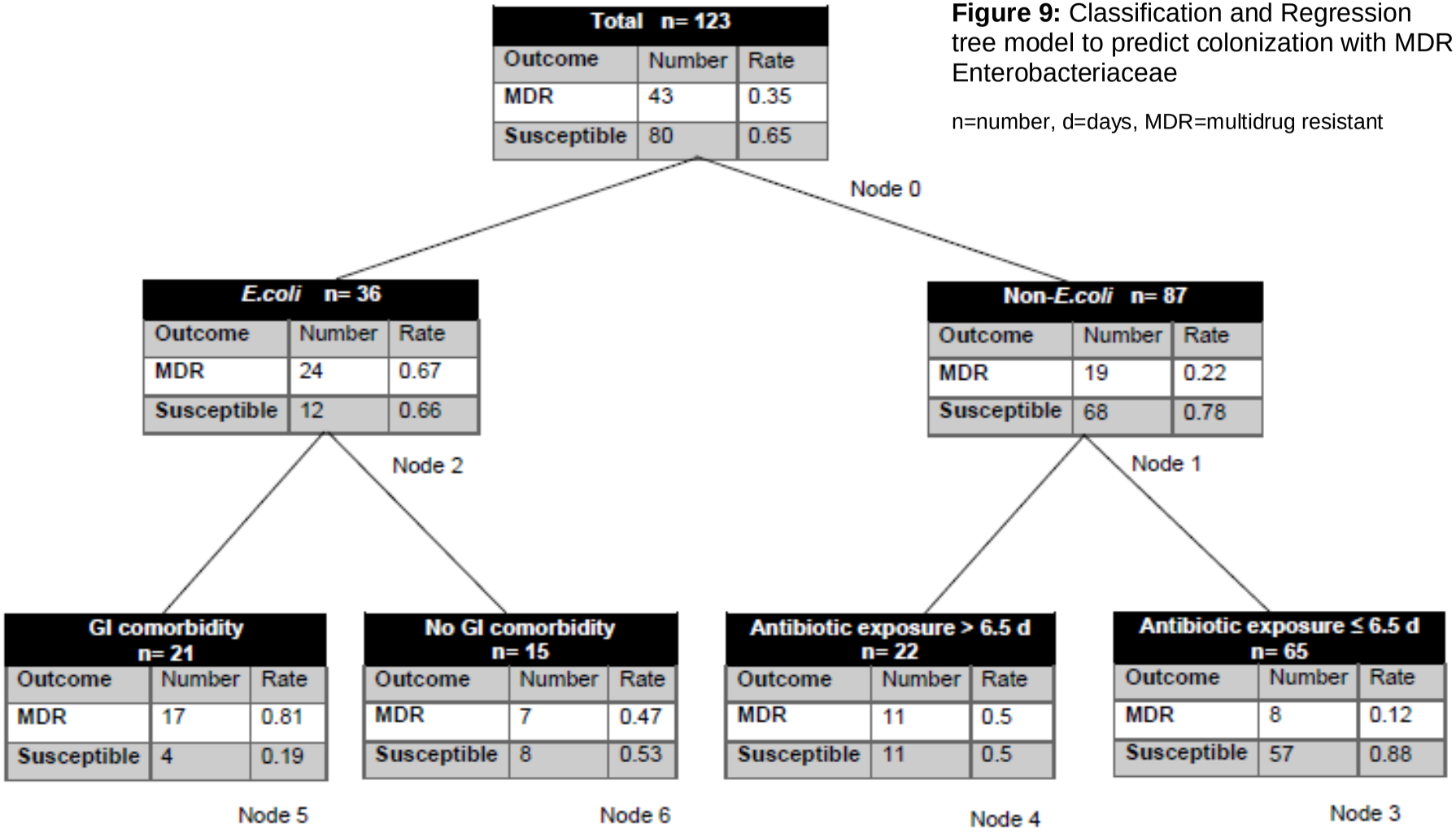


Figure 9: Classification and Regression tree model to predict colonization with MDR Enterobacteriaceae

n=number, d=days, MDR=multidrug resistant

Gastrointestinal (GI) comorbidity includes diagnoses such as gastritis, gastroenteritis, short bowel syndrome, ileus, liver cirrhosis or -steatosis and other liver diseases.

The CRT model reveals that in our patient population the MDR rate in *E.coli* bacteria is 67% compared to only 22% in non-E.coli bacteria (Node 1 and 2). Gastrointestinal comorbidity in combination with an *E.coli* leads to the highest risk (81%) for colonization or infection with an MDR strain (Node 3). On the other hand, infection with *E.coli* without a GI-comorbidity leads to a risk of only 47% for the isolation of an MDR organism from tracheal aspirate (Node 4). In non-*E.coli* bacteria, antibiotic exposure of less than 6.5 days leads to a high chance of 88% that bacterial isolates are susceptible to antibiotics (Node 6). On the other hand, a longer previous antibiotic treatment increases the chance of having a non-*E.coli* organism that is resistant to antibiotics up to 50% (Node 5). Conclusively, in our study, GI-comorbidity increased the risk of isolating an MDR *E.coli* strain in the lower respiratory tract, while previous antibiotic exposure increased the risk of isolating MDR Enterobacteriaceae other than *E.coli* .

Table 4: Risk estimate and classification of MDR and susceptible Enterobacteriaceae in tracheal aspirate. Growing method: CRT, dependent variable: MDR.

MDR=multidrug resistant, CRT=Classification and regression tree model

Observed	**Predicted**		
	Susceptible	**MDR**	**Percent correct**
Susceptible	76	4	95,0%
MDR	26	17	39,5%
Overall percentage	82.9%	17.7%	75.6%

As shown in Table 4, the model classifies 75.6% of cases correctly. The model is very accurate in predicting susceptible bacteria (95% of cases are predicted correctly) while the prediction of MDR strains is correct in only 39.5%.

3.6.2 Outcome of MDR infection or colonization vs. non-MDR infection or colonization

The clinical outcome of 123 intubated children colonized with Enterobacteriaceae is summarized in Table 5. No significant differences between the two groups were found in the duration of mechanical ventilation, the total length of stay on PICU or in the death rate.

Table 5: Clinical outcome of children colonized or infected with MDR vs susceptible Enterobacteriaceae.

MDR=multidrug resistant, PICU=paediatric intensive care unit, IQR=interquartile range, p-value < 0.05 considered significant

	MDR (n=43)	**Susceptible (n=80)**	**p-value**
Days on ventilator (median, [IQR])	6 [3-19]	8 [2-16]	0.91
PICU length of stay (median, [IQR])	14 [6-32]	14.5 [7-32.5]	0.89
All-cause mortality, 6 months (% of cases)	8 (n=20)	9 (n=11)	0.22

3.7 VAP caused by Enterobacteriaceae

During the study period, 12 girls and 7 boys out of 123 patients colonized by Enterobacteriaceae in their lower respiratory tract acquired a VAP during their PICU stay. The incidence rate was thus 15.4%.

Figure 10 shows the increase of VAPs in patients colonized with Enterobacteriaceae from 2005 (n=0) to 2014 (n=3) with a peak in 2013 (n=6).

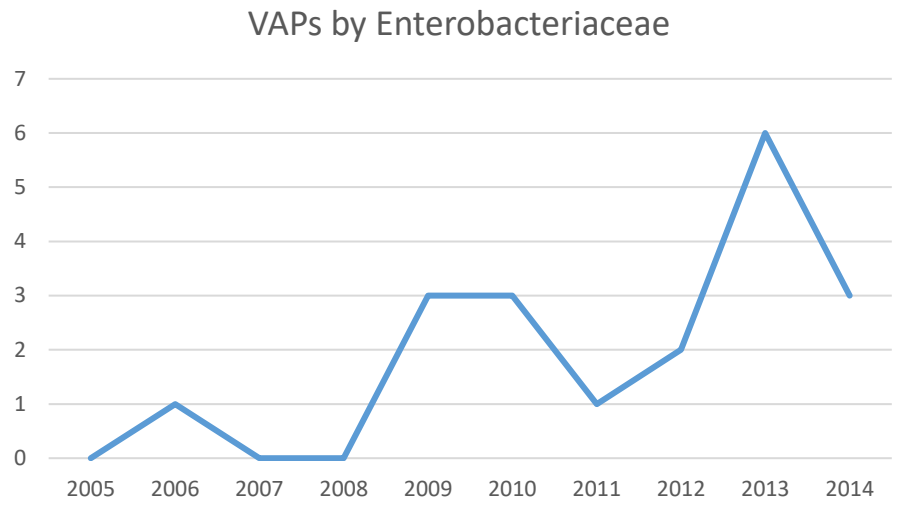


Figure 10 : Frequency of VAPs caused by Enterobacteriaceae.

VAP=ventilator-associated pneumonia

3.7.1 Distribution of Enterobacteriaceae causing VAP

As shown in Figure 11, episodes of VAP were mainly caused by *Enterobacter spp* (n=11), less often by *E.coli* (n=5) and *Klebsiella spp* (n=3).

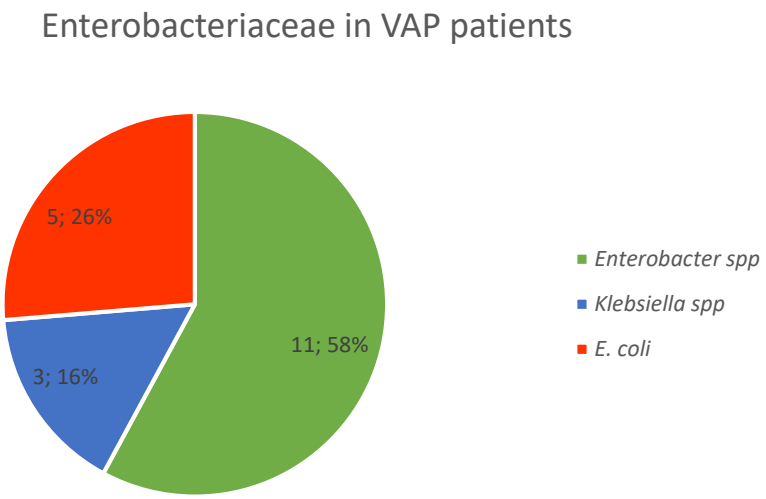


Figure 11: Enterobacteriaceae species isolated in patients with VAP.

VAP=ventilator-associated pneumonia, spp=species

Table 6: Comparison of the isolated Enterobacteriaceae species in colonized patients to those who actually acquired a VAP.

spp=species, MDR=multidrug resistant, p=p-value (<0.05 considered significant), sus=susceptible.

Bacterial subspecies n (% of all Enterobacteriaceae) n (% of bacterial subspecies)	**Colonization sus. (n=104) Colonization MDR (n=31)**	**VAP sus. (n=10) VAP MDR (n=9)**	**Colon vs. VAP p=**	**Total susceptible (Colon. + VAP) Total MDR (Colon. + VAP)**
E.coli	30 (24.4)	5 (4.1)	0.82	35 (28.5)
E.coli - MDR	18 (45.0)	4 (10.0)	0.47	22 (55.0)
Enterobacter	31 (25.2)	11 (8.9)	0.02	42 (34.1)
Enterobacter- MDR	2 (5.0)	4 (10.0)	0.005	6 (15.0)
Klebsiella	29 (23.6)	3 (2.4)	0.27	32 (26.0)
Kelbsiella-MDR	9 (22.5)	1 (2.5)	0.27	10 (25.0)
Morganella	3 (2.4)	0 (0)	0.45	3 (2.4)
Morganella – MDR	1 (2.5)	0 (0)	0.59	1 (2.5)
Proteus	1 (0.8)	0 (0)	0.67	1 (0.8)
Proteus – MDR	0 (0)	0 (0)	n.a.	0 (0)
Others	2 (1.6)	0 (0)	0.54	2 (1.6)
Others - MDR	0 (0)	0 (0)	n.a.	0 (0)
Serratia	6 (4.9)	0 (0)	0.28	6 (4.9)
Serratia – MDR	1 (2.5)	0 (0)	0.59	1 (2.5)
Citrobacter	2 (1.6)	0 (0)	0.54	2 (1.6)
Citrobacter – MDR	0 (0)	0 (0)	n.a.	0 (0)

Significant differences (p-value <0.05) were found for *Enterobacter spp*. *Enterobacter* was significantly more likely to cause a colonization than a VAP (p=0.02). MDR *Enterobacter* on the other hand, were more common in VAP patients, while susceptible strains were more often found in colonized patients (p=0.05). This trend is especially notable considering the fact that *Enterobacter spp* were the causative organisms for more than 50% of VAP cases in our patient population (compare Figure 11). The spectrum of the other Enterobacteriaceae did not differ between colonized and VAP-patients.

9 out of 19 bacterial stains causing VAP were MDR (47.4%), while the proportion of MDR organisms amongst non-VAP patients was only 29.8%. However, this difference did not reach significance.

3.7.2 Clinical aspects of VAP cases

Most of the patients who developed a VAP were girls (63%, n=12) and had an underlying cardiac (89.5%, n=17) and/or respiratory (57.9%, n=11) disease. Most patients were younger than one year old.

5 of these children who had a VAP died on the PICU or in the following 6 months after discharge, mostly after a long admission in hospital (median: 31 days, maximum: 244 d). Table 7 summarizes a comparison of patients colonized by Enterobacteriaceae who developed a VAP vs colonized patients who did not develop a VAP.

Table 7: Characteristics of patients colonized by Enterobacteriaceae and patients who developed a VAP caused by Enterobacteriaceae.

n=number, VAP=ventilator-associated pneumonia, IQR=interquartile range, BMI=body-mass-index, SD=standard deviation, CVL=central venous line, PICU=paediatric intensive care unit, LOS=length of stay, MDR=multidrug resistant, TA=tracheal aspirate, p=p-value (<0.05 considered significant).

Demographic and clinical characteristics	**Colonized (n=104)**	**VAP (n=19)**	**Colonized versus VAP (p)**	**Total (n=123)**
Age (median,[IQR])	0.5 [0-17.9]	0.4 [0.1-12.6]	0.09	0.45 [0-17.9]
Age <1 year (%)	59.6	84.2	0.04	63.4
Sex (female/male, n)	37/67	12/7	0.02	39.8
Gestational age in weeks (median,[IQR])	37 [23-42]	37 [27-42]	0.9	37 [23-42]
BMI (mean±SD; kg/m²)	14.3 ± 4.2	12.7 ± 3.8	0.13	14 ± 4.18
Underlying disease (% of all patients)				
Cardiosurgical	51.2	13.8	0.02	65.0
Gastroenterological	44.7	4.9	0.09	49.6
Neurological	45.5	4.9	0.07	50.4
Hemato-oncological	7.3	0.8	0.6	8.1

Antibiotic therapy 4 weeks before TA (% of all patients)	59.6	13.2	0.12	72.8
Duration of antibiotic treatment prior to TA (median,[IQR])	1 [0-105]	9 [0-73]	<0.001	2 [0-105]
Presence of CVL prior to TA (median,[IQR])	2 [0-105]	8 [2-111]	<0.001	3 [0-111]
Length of mechanical ventilation prior to TA (median,[IQR])	1 [0-38]	8 [2-34]	<0.001	1 [0-38]
Total length of mechanical ventilation (median,[IQR])	7 [0-93]	19 [5-54]	0.001	7 [0-93]
PICU LOS prior to TA (median,[IQR])	1 [0-105]	10 [2-73]	<0.001	2 [0-105]
Total Hospital LOS (median,[IQR])	29 [1-276]	40 [13-312]	0.06	32 [1-312]
MDR isolates (n)	31	9	0.13	40
Mortality rate (% of all patients)	9.9	4.1	0.09	14.0

VAP patients were significantly younger (p=0.04) with an obvious female predominance (p=0.02). VAP patients were more likely to have an underlying cardio-surgical disease compared to patients that were only colonized with Enterobacteriaceae (p=0.02). Furthermore, VAP patients had a longer stay on PICU (p=<0,001), duration of antibiotic therapy (p=<0.001), presence of a CVL (p=<0.001) and more days on mechanical ventilation (p=<0.001) prior to tracheal aspirate. During their stay on PICU, the VAP patients needed a longer time on mechanical ventilation (considering both before and after tracheal aspirate) (p=<0.001). However, differences in both groups in the total length of stay in the hospital, frequency of MDR isolates or in mortality rate did not reach significance.

3.7.3 Risk factors for developing a VAP caused by Enterobacteriaceae

We identified different aspects to be independent risk factors for acquiring a VAP. Results of the regression analysis are shown in Table 8.

Table 8: Simple logistic regression predicting VAP in colonized patients.
MDR=multidrug resistant, PICU=paediatric intensive care unit, LOS=length of stay, ECMO=extracorporeal membrane oxygenation, CVL=central venous line, CI=confidence interval, p-value <0.05 considered significant

Risk factor	Odds ratio	95% CI	p-value
Sex (female)	3.1	1.13-8.57	0.03
Birth weight	0.78	0.43-1.43	0.43
MDR isolate	2.1	0.79-5.73	0.14
Antibiotic treatment >7 days	10.35	3.03-35.32	<0.001
Cephalosporin pre-treatment	6.07	1.82-20.25	0.003
PICU LOS > 10 days	5.34	1.86-15.32	0.002
Mechanical ventilation >7 days	7.44	2.44-22.74	<0.001
ECMO	7.14	1.6-31.84	0.01
CVL >10 days	3.0	0.93-9.71	0.07

The simple logistic regression analysis revealed potential risk factors for the acquisition of VAP. Amongst these were antibiotic pre-treatment for more than 7 days, pre-treatment with cephalosporins, a duration of stay on PICU for more than 10 days, mechanical ventilation for more than 7 days and ECMO.

The isolation of an MDR organism from tracheal aspirate did not increase the risk for VAP. Interestingly, female patients were at higher risk for VAP.
Multiple regression analysis showed that pre-treatment with a cephalosporin was the risk factor with the highest impact in our study, followed by ECMO and mechanical ventilation for more than seven days. However, confidence intervals are wide. Results of multiple regression analysis are presented in Table 9.

Table 9: Multiple logistic regression predicting VAP in colonized patients. MDR=multidrug resistant, ECMO=extracorporeal membrane oxygenation, CI=confidence interval, p-value <0.05 considered significant

Risk factor	Adjusted Odds ratio	95% CI	p-value
Sex (female)	5.23	1.14-23.93	0.03
MDR isolate	2.61	0.61-11.12	0.19
Cephalosporin pre-treatment	11.38	2.07-62.7	0.005
Mechanical ventilation >7days	7.29	1.61-33.05	0.01
ECMO	10.6	0.86-130.83	0.07

3.7.4 Clinical course and outcome of VAPs due to MDR organisms vs VAPs due to susceptible organisms

We compared colonized patients, patients with a VAP caused by sensitive bacteria and patients with a VAP caused by an MDR strain. Table 10 summarizes the most important results.

Table 10: Comparison of colonized patients, drug-sensitive VAP patients and MDR VAP patients by Kruskal-Wallis test.
VAP=ventilator-associated pneumonia, MDR=multidrug resistant, CVL=central venous line, PICU= paediatric intensive care unit, LOS=length of stay, p-value <0.05 considered significant

	Colonized (n=104)	VAP sensitive (n=10)	VAP MDR (n=9)	p-value
Length of mechanical ventilation prior to tracheal aspirate (median,[IQR])	1 [0-38]	5 [2-34]	12 [2-26]	<0.001
Total length of mechanical ventilation (median,[IQR])	7 [0-93]	18.5 [8-54]	19 [5-45]	0.004
Presence of CVL prior to culture (median,[IQR])	2 [0-105]	4 [2-30]	12 [4-111]	0.001

Duration of antibiotic treatment prior to tracheal aspirate (median,[IQR])	1 [0-105]	6 [0-40]	11 [7-73]	0.001
PICU LOS prior to culture (median,[IQR])	1 [0-105]	8 [2-40]	12 [2-73]	<0.001
Hospital LOS (median,[IQR])	29.0 [1-276]	44.5 [13-244]	32 [21-312]	0.13

Children only colonized with Enterobacteriaceae had the shortest duration of mechanical ventilation, presence of a CVL, duration of stay on PICU and days of antibiotic treatment prior to detection of the gram-negative organism in tracheal aspirate. Patients with a VAP caused by susceptible Enterobacteriaceae had a significantly longer time on mechanical ventilation, CVL, days on PICU and antibiotic pre-exposure. The patient group with VAP caused by MDR Enterobacteriaceae had clearly the longest time of mechanical ventilation prior to the tracheal aspirate, more than twice as long as the non-MDR VAP group (12d vs 5d). Also the presence of a CVL, duration stay on PICU and antibiotic pre-treatment and were by far the longest in the MDR-VAP group.

The total length of mechanical ventilation (taking into account days on ventilation both before and after the tracheal aspirate) was shortest in the patient group colonized only. Both VAP groups had a similar total length of mechanical ventilation (non-MDR: 18.5 d, MDR: 19 d) that was a lot higher than in the colonized group (1 d).

No significant differences could be shown between all three groups (no VAP, sensitive VAP and MDR VAP) concerning the total length of stay in the hospital.

4. Discussion

This study aimed to determine the prevalence and spectrum of Enterobacteriaceae isolates in tracheal aspirate of ventilated PICU patients. 123 patients were included into the study and 167 isolates were analysed. The majority of the 123 patients were boys (60.2%), and most were infants (mean age 6 months). Demographic characteristics of the study population are presented in Table 1. Mean duration of PICU stay was 14 days. The main reason for admission to PICU was cardiac surgery (46%) or general surgery (28%). During the study period, 108 major infections were identified in 123 patients. Pneumonia was most prevalent, followed by sepsis. Both diseases are known to be common on PICUs. According to the literature, lower respiratory tract infections are responsible for 25-30% of nosocomial infections on PICUs (63-65).

4.1 Prevalence and spectrum of Enterobacteriaceae in tracheal aspirate of ventilated children

We analysed the spectrum of Enterobacteriaceae that was detected in the tracheal aspirate of intubated PICU patients between 2005 and 2014.

During the study period, the incidence rate of Enterobacteriaceae has increased from 0.66% in 2005 to 2.17% in 2014 with a peak of 3.3% in 2011. The MDR incidence rate has increased from 0% in 2005 to 0.64% in 2014 with a peak of 1.33% in 2013 (see Figure 3). However, these findings might be influenced by changing screening policies on the PICU and have therefore a limited value.

Enterobacter (30.5%), *E.coli* (28.1%) and *Klebsiella* (27.5%) were found to be the most frequently isolated pathogens in our patient population. The presence of these gram-negative bacteria in the lower respiratory tract is mainly explained by previous intubation: the placement of an endotracheal tube is quickly followed by tracheal colonization with potentially pathogenic organisms from the oropharyngeal flora, including MDR organisms (40,41).

The distribution of Enterobacteriaceae displayed in our study is consistent with data from recent research. Wilson et al took daily samples from intubated children and found *Klebsiella* and *E.coli* as the most common gram-negative isolates, followed by *Citrobacter freundii* and *Enterobacter cloacae* (40). A prospective

study of VAP in PICU patients found *Serratia, E.coli* and *Enterobacter* to be the most frequent gram-negative organisms cultured from patients with endotracheal colonization or VAP (42). Comparisons with data from the literature are difficult due to the scarcity of data and differences in study settings.

Our results show that *Enterobacter*, *E.coli* and *Klebsiella* are common causes for endotracheal bacterial colonization in intubated and ventilated PICU patients. Therefore, knowledge about resistance patterns of these organisms is crucial to adapt empiric treatment guidelines and ensure adequate prevention of PICU patients

4.2 Proportion of drug resistant Enterobacteriaceae

As drug resistant gram-negative pathogens are an emerging problem worldwide (11,16), we analysed the impact of drug resistant Enterobacteriaceae in our study population. The overall proportion of MDR organisms in this setting was 30.5% of all 167 bacterial isolates. *Morganella* was the species with the most drug-resistant strains (67%). However, the informative value of this proportion is limited by the small number of only 6 isolated *Morganella* strains. *E.coli* was MDR in 55.3% and *Klebsiella spp* in 28.3%. Another important observation was the increasing importance of MDR organisms, the incidence rate of MDR Enterobacteriaceae rose from 0% in 2005 up to 29% in 2014 (Figure 3).

This result is consistent with the global trend of emerging MDR gram-negative organisms. The ECDC reports in its “Antimicrobial resistance annual epidemiological report” a “significant increasing trend of combined resistance to multiple antibiotics in both *E.coli* and *Klebsiella pneumoniae* in more than one third of the EU/EEA countries” (66). Similar trends are being reported from the U.S. and worldwide (13,67).

Other studies found MDR rates that differed from our findings: Costa et al. reported 46.6% of all gram-negative infections on an oncologic PICU were due to MDR organisms between 2009 and 2012 (68). The MDR rate of gram-negative organisms causing HAI in a Thai PICU was even higher with 56% (21). Both these rates are markedly higher than in our setting. However, the settings are not

completely comparable. The differentiation between colonization and infection of the respiratory tract is not clearly defined. Our study included colonized patients, not just those with infection. Although our data from a single PICU cannot be extrapolated and compared to the above mentioned general data, our findings are consistent and might reflect these worldwide trends: MDR gram-negative organisms are gaining importance and should raise a special concern. Surveillance, adapted screening, treatment guidelines and new antibiotic agents are needed.

4.2.1 ESBL-producing Enterobacteriaceae

ESBL-production is one of the most important mechanisms of resistance in Enterobacteriaceae (14). We investigated the frequency of ESBL producing strains amongst all isolated bacteria. Our study displayed an overall proportion of ESBL-producing organisms of 7.8% in the tracheal aspirate of ventilated PICU patients. In *E.coli* 14.9% of the isolates were ESBL-builders and in *Klebsiella spp*, 6.5% of the isolates were ESBL-positive.

These findings in our setting are consistent with most recent research: studies from different settings displayed ESBL-rates between 6.2% and 8.4% for *E.coli* and between 7% and 13.3% for *Klebsiella spp* (14). A retrospective study from 2000-2004 reported an ESBL-rate of 7% for both *E.coli* and *Klebsiella* in tracheal aspirates of PICU-patients in Alabama (69). Other recent studies show higher rates of ESBL-building Enterobacteriaceae than our findings: Lee et al. described the microbiologic spectrum and susceptibility pattern of clinical isolates from a PICU and found a rate of 20% ESBL-positive *Klebsiella* in 2005 (70).

Comparability is limited by different patient characteristics and study settings. All our patients were mechanically ventilated but did not necessarily have an infection. Many other studies only included patients who were clearly diagnosed with an infectious disease.

Generally, the proportion of ESBL-producing Enterobacteriaceae in our study is comparable to the available general surveillance data in countries with comparable medical standards (14). Though, it is hard to find specific data about ESBL-building bacteria in the lower respiratory tract of ventilated children. As

ventilated children are especially vulnerable patients, knowledge about epidemiology and prevention of ESBL-building bacteria in children is highly important. Further research in this matter is needed, not only concerning ESBL-building bacteria but MDR gram-negative bacteria in general.

4.2.2 Carbapenemase-producing Enterobacteriaceae

Apart from ESBL-production, another important mechanism of drug resistance in gram-negative bacteria are carbapenemases. Lately, carbapeneme-resistant Enterobacteriaceae (CRE) have been emerging and raise a special concern. Both CDC and ECDC report rising rates of carbapenemase-producing *Klebsiella* (11% of HAIs in the US) and *E.coli* (2%)(11). This trend is especially disturbing, keeping in mind that carbapenems are the last line of antibiotics available to treat MDR infections. If gram-negative bacteria become resistant to these agents, treatment options are severely limited (56).

The findings in our study setting confirm the reports of CDC and ECDC concerning proportion and evolution of carbapenemase-building bacteria. In our setting, the overall proportion of carbapenem-resistant bacteria during the nine-year study period was 1.8% with a notable increase in the later study period (Figure 8, Table 2). In the last two years of the study period, 4.9% of all Enterobacteriaceae were resistant to carbapenems. This trend is alarming and illustrates the urgency for both optimizing prevention and developing new treatment strategies.

4.3 Risk factors for colonization with MDR Enterobacteriaceae in ventilated PICU patients

Knowledge about potential risk factors for colonization or infection with MDR Enterobacteriaceae allows the establishment of adequate prevention, screening and therapy. Therefore, we identified independent risk factors for isolation of MDR organisms in tracheal aspirate in our study population.

Comparison of patients with MDR bacteria to those with susceptible bacteria (Table 3) showed no significant differences in sex, age, gestational age, birth weight or body-mass-index (BMI). Regarding comorbidities, underlying

immunodeficiencies were more frequent in patients with MDR organisms. However, the number of patients suffering from immunodeficiency was very small in our study population, so the value of this finding is limited. Gastrointestinal comorbidities such as gastritis, gastroenteritis, short bowel syndrome, ileus, liver cirrhosis or -steatosis or other liver diseases occurred more often in MDR-patients, however this difference did not reach significance (p=0.08). Both groups showed no significant differences in the presence of other underlying diseases.

Concerning the clinical course and outcome, the groups showed no differences in the duration of antibiotic pre-treatment, days of mechanical ventilation, VAP incidence, CVL-days and all-cause mortality over 6 months.

We investigated several factors available in the literature that could potentially increase the risk of colonization with MDR Enterobacteriaceae (21,71). Out of these potential risk factors, three were finally identified by the decision and regression tree analysis as the most important ones in our setting: 1. colonization with *E.coli* 2. gastrointestinal comorbidity and 3. the duration of antibiotic exposure were most relevant for colonization with MDR Enterobacteriaceae (Figure 9).

1. MDR *E.coli* raise a special concern: in our setting 55.3% of all detected *E.coli* were drug resistant, the CRT model defined colonization with *E.coli* as independent risk factor for having an MDR organism and the number of isolated *E.coli* has increased over the last years.

These tendencies are consistent with a large, nationwide study of antibiotic drug use and bacterial resistance in the United States and a study from 53 German ICUs (72,73). In the latter, the most striking result was the ten-fold increase of 3rd generation cephalosporin-resistant *E.coli* from 2001 to 2008. Highly resistant *E.coli* are known to have a higher infection rate and a higher mortality compared to susceptible isolates (74). Keeping this in mind, MDR *E.coli* should raise increased awareness in PICUs.

2. The second risk factor for colonization or infection with an MDR organism in our study was the presence of an underlying gastrointestinal comorbidity. This correlation has been described in several publications (71,75). A recent case-control study from the US, reported that gastrointestinal comorbidities increase

the risk for infection at any site with both MDR and susceptible Enterobacteriaceae in children (71). It is well known that the stomach is a reservoir for gram-negative bacteria, especially in critically ill children or patients treated with H_2-antagonists that reduce the secretion of gastric acid (76).

A possible explanation might be the disruption of the physiological gastrointestinal flora caused by GI-diseases, surgical procedures or selection by antibiotic treatment. The disturbed bacterial flora can lead to an overgrowth of non-physiological, potentially drug-resistant bacteria. These bacteria might now be transported into the lower respiratory tract by gastroesophageal reflux and micro-aspiration or colonization of the upper pharynx and subsequent tracheal colonization after intubation (76).

Furthermore, health care workers might transport GI-bacteria into the respiratory tract e.g. when handling a PEG (percutaneous endoscopic gastrostomy) or during tracheal suctioning without sufficient hygiene measures.

3. The third risk factor detected by the CRT model was the duration of antibiotic treatment prior to the detection of drug resistant Enterobacteriaceae. It is well known that antibiotic treatment leads to a suppression of both physiological and pathogenic bacteria, favouring those strains that are resistant to the antibiotic agent and finally lead to overgrowth of these resistant bacteria (11,25). Therefore, it is not surprising that a longer duration of antibiotic treatment increases the risk of infection or colonization by MDR Enterobacteriaceae. In our study setting, the critical length of antibiotic treatment that increased the risk of infection with MDR bacteria was 6.5 days. Most studies agree that antibiotic exposure, especially pre-treatment with broad spectrum antibiotics, increases the risk of MDR-acquisition (21,23,25). A Chinese study reports, that administration of 3rd generation cephalosporins is an independent risk factor for infection with ESBL-producing bacteria (77). Unfortunately, mostly there is no specification concerning a cut-off or a critical length of the antibiotic treatment. Tamma et. al conducted a retrospective study to investigate the antibiotic treatment of ventilator-associated tracheitis in children. The patient group who received an antibiotic treatment for more than 7 days had a higher risk for an MDR organism in the lower respiratory tract than the patient group that was treated for a shorter

period (78). These findings correspond well with our cut-off of 6.5 days. More research is needed, to specify the critical length of antibiotic treatment that increases the risk of MDR acquisition.

The risk factors for isolation of MDR Enterobacteriaceae that we found in our study facilitate the identification of children at risk for colonization and infection with MDR organisms. This could potentially lead to adaption of empiric antibiotic treatment and consequently decrease the risk of MDR-acquisition. Mechanically ventilated children who have an *E.coli* in the tracheal aspirate and children who suffer from gastrointestinal comorbidities should be watched carefully for appearance of multidrug resistant bacteria. Antibiotic treatment should be kept as short as possible to avoid an increased risk for acquiring an MDR organism.

4.4 Outcome of colonization or infection with MDR organisms

Many studies show, that MDR organisms can worsen clinical outcomes compared to colonization or infection with susceptible strains. This might include longer duration of ventilation, longer duration of stay in the hospital or higher morbidity with consequently elevated healthcare costs (11,12,74,79). MDR organisms commonly do not feature higher pathogenicity than their more susceptible counterparts of the same genera. However, MDR organisms are known to increase mortality due to delayed or inappropriate antibiotic therapy (74,80).

In this study, we investigated whether patients colonized with MDR organisms had more ventilation days in total, a longer stay on PICU or a higher rate of death. In this setting, the overall outcome was similar in both groups (Table 5).

In our institution, susceptibility testing is rapidly available and empiric antibiotic therapy is rather aggressive. Rapid targeted therapy might be the explanation that differences in outcome and mortality have not been observed in our study. Additionally, differences in ventilation days, length of PICU stay and mortality may hardly be detected in our study, which was based on a low number of cases and included PICU patients with a vast variability in comorbidities and severity of diseases.

4.5 Enterobacteriaceae causing VAP on PICU

Ventilated PICU patients, as in our study setting, are at a special risk for acquiring VAP. Enterobacteriaceae are a major cause for these VAP (4,81) and MDR gram-negative organisms are becoming increasingly relevant in the PICU setting (4,42). Against this background, we investigated the distribution and proportion of drug-resistant Enterobacteriaceae in the patients who had a VAP. Furthermore, we determined risk factors and outcome of VAP in our patient population.

4.5.1 Distribution of Enterobacteriaceae subspecies causing VAP

Gram-negative bacteria, especially *P.aeruginosa*, are the main cause for VAP on PICUs. Gram-positive rods such as *S.aureus* are also common in VAP patients, but not as frequently isolated as gram-negative strains (4,42,82). As Enterobacteriaceae are a major part of the gram-negative bacteria, we took a closer look on the distribution of bacterial subspecies in VAP patients of our patient population.

Episodes of VAP were mainly caused by *Enterobacter*, less often by *E.coli* and *Klebsiella* (Figure 11). This is not surprising, keeping in mind that these three subspecies were by far the most common ones in our setting. A prospective study of VAP in children displayed a comparable spectrum of Enterobacteriaceae as causative organisms for VAP: Srinivasan et al. found gram-negative organisms to be the causative organisms for 42% of all VAPs with *E.coli* being the predominant gram-negative organism followed by *Enterobacter spp* and *Serratia spp* (42). In our setting, MDR organisms were found more often in VAP than in non-VAP patients. Almost half of the VAPs (47.4%) were caused by MDR Enterobacteriaceae (Table 6). However, this difference did not reach significance, possibly due to the small number of cases.

The high proportion of MDR organisms causing VAP that we observed, reflects the increasing relevance of MDR Enterobacteriaceae on PICUs (4,42). A multinational study displayed that 37,5% of all *Klebsiella* causing nosocomial infections on PICUs were MDR. Furthermore, the authors pointed out that the

amount of MDR organisms had increased towards the end of the study period (83).

In our study setting, the number of VAP cases caused by MDR Enterobacteriaceae is too small to draw conclusions about a general tendency whether MDR gram-negative bacteria are increasingly responsible for VAP on PICU. Although, given the high percentage of MDR-caused VAPs in our study and the reports from other PICUs, this question seems to be an important one and should be investigated in further research.

Furthermore, we investigated whether the distribution of bacterial subspecies and the proportion of drug resistant strains differed between patients who acquired a VAP and patients who were only colonized (Table 6).

We found that *Enterobacter spp* were significantly more likely to cause a colonization than a VAP (p=0.02). But interestingly, MDR *Enterobacter spp* were more common in VAP patients, while susceptible strains were more often found in colonized patients (p=0.05). Considering the fact that *Enterobacter spp* were the causative organisms for more than 50% of VAP cases in our patient population (Figure 11), these findings are especially notable. Whenever the diagnosis of a VAP seems probable, MDR *Enterobacter* should be kept in mind in matters of diagnosis and empiric therapy.

Finally, it has to be mentioned that all our findings concerning VAP caused by Enterobacteriaceae are based on isolation of these bacteria in tracheal aspirates. Some authors criticise, that this method is not accurate enough and can lead to over-diagnosis (40,55). Nevertheless, the S-3 guidelines for diagnosis of VAP in adults recommends non-invasive procedures such as tracheal aspirate instead of more invasive procedures like BAL or even lung biopsy (53). Summing up, isolation of specific pathogens in tracheal aspirate in combination with clinical findings are widely accepted for the diagnosis of VAP (34,84). In our study, we followed this approach and defined only children who fulfilled both microbiological and clinical criteria as VAP patients.

4.5.2 Characteristics of patients with VAP

19 out of 123 patients included in the study acquired a VAP during their hospital stay, the VAP rate during the study was thus 15.4%.

Most authors report a female predominance in VAP patients (32,85), our findings with a female/male ratio of 12:7 (coming from a patient population with 60% boys) and female sex being an independent risk factor for VAP are in accordance with these data.

VAP patients were significantly younger and more likely to have an underlying cardiac disease compared to patients who did not acquire a VAP. They had a longer stay on PICU and more days on mechanical ventilation before being diagnosed with VAP, with longer previous antibiotic therapy and previous presence of a CVL than non-VAP patients (Table 7).

Even though many studies show that VAP can lead to a longer hospital length of stay and higher mortality (30,42), VAP patients did not have a significantly longer total PICU length of stay or a higher mortality rate in our setting.

Interestingly the frequency of VAPs caused by Enterobacteriaceae increased over the study period while patient admissions stayed on a stable level (Figure 10). Keeping in mind the increasing amount of MDR organisms amongst Enterobacteriaceae (86,87), this trend should be observed closely.

4.5.3 Risk factors for acquiring VAP caused by Enterobacteriaceae on PICU

Knowledge about risk factors is important to optimize prevention and diagnosis of VAP. In our setting, antibiotic pre-treatment for more than 7 days, pre-treatment with cephalosporins, a stay on PICU for more than 10 days, mechanical ventilation for more than 7 days and being on ECMO have significantly increased the risk of VAP acquisition (Table 8 and 9). Most of the risk factors identified from our patient collective have been described as independent risk factors for VAP in studies from other settings.

The most important way of acquiring a VAP is the contamination of the lower respiratory tract by bacteria of the oro- or nasopharynx that happens during intubation (88). Antibiotic pre-treatment disturbs this endogenous oro- and nasopharyngeal flora and facilitates the colonization with nosocomial and

potentially drug resistant bacteria. Many studies have shown that antibiotic pre-treatment increases the risk of VAP in both children and adults (41,89,90).

When we tested all antibiotics independently on their impact on VAP-acquisition, only cephalosporins were found to be an independent risk factor for VAP. This finding might be due to the fact that cephalosporins were by far the most commonly used class of antibiotics in out setting and had thus a higher influence than the other antibiotics. Furthermore, cephalosporins are known to increase the risk of acquiring an MDR gram-negative organisms and can facilitate nosocomial infections as VAP (91).

A long stay on NICU/PICU and prolonged mechanical ventilation are further important risk factors described in many studies about VAP (49,51,89). This is reasonable, as a longer stay in the hospital means a longer exposure to nosocomial bacteria and a longer time of placement of the endotracheal tube means more chances of micro-aspirations and following VAP (51).

In the literature, many more risk factors for VAP such as re-intubation or enteral feeding are described (30,51). We could not investigate all these risk factors in our study.

Our study setting only includes ventilated PICU patients, and therefore a preselected patient population of ventilated children with Enterobacteriaceae in the tracheal aspirate. The findings about risk factors and outcome of VAPs consequently cannot be applied to all PICU patients.

General consequences can be drawn from these data. Antibiotic treatment, especially with cephalosporins, as well as mechanical ventilation should be used as restrictively as possible. If a child needs a prolonged treatment requiring the above mentioned risk factors, physicians should be aware of the risk for VAP caused by Enterobacteriaceae. This applies especially to girls, who are at a higher risk for VAP.

4.5.4 Clinical course and outcome of patients with MDR VAP

Finally, we analysed if the presence of an MDR organism had an impact on the clinical course of VAP (Table 10). We compared patients with VAP due to an MDR bacterium to both patients with VAP due to a susceptible organism and to patients colonized with Enterobacteriaceae without VAP. We evaluated different aspects that can be divided in (a) clinical course and interventions prior to the detection of Enterobacteriaceae in the tracheal aspirate and (b) clinical outcome, taking into account the time both before and after the diagnosis of VAP.

(a) Patients with VAP due to an MDR bacterium had a significantly longer duration of mechanical ventilation prior to the detection of a gram-negative organism in the tracheal aspirate than VAPs caused by a sensitive bacterial strain. Furthermore, MDR VAPs had a longer presence of a CVL, stay on PICU and more days on antibiotic treatment prior to the tracheal aspirate. Patients who did not have a VAP but were only colonized had a shorter duration of all the above-mentioned factors.

The three groups showed significant differences in aspects that are known to be risk factors for the acquisition of VAP: days on mechanical ventilation, presence of a CVL, days on PICU and previous antibiotic treatment. The two VAP groups (MDR and non-MDR) had a significantly longer exposure towards these risk factors than the non-VAP group. Furthermore, it seems that a prolonged exposure towards the risk factors increases the risk that the VAP is caused by MDR strains. This tendency appears to be important and should be investigated in further research.

(b) MDR organisms are known to be a cause for delayed adequate antibiotic treatment (24,68). For this reason, a worse outcome in patients with MDR organisms, including prolonged mechanical ventilation, a prolonged stay on PICU and a higher mortality could be expected. These tendencies have been reported in several studies (24,79,80), even though data from comparable study settings are rare. However, we could not confirm all of the mentioned expectations in our study: no significant differences could be shown between MDR VAPs, sensitive VAPs and colonized patients concerning the total length of stay in the hospital. The total length of mechanical ventilation was longer in the in VAP-patients than

in non-VAP patients, but the MDR-status of the bacteria that caused VAP did not have a significant influence.

Given that our setting included only 9 patients suffering from VAP due to MDR Enterobacteriaceae, these findings have to be treated with caution. Significant differences might not be detected in such small groups. Keeping in mind that VAPs are amongst the most important nosocomial infections on PICU and that MDR gram-negative organisms are becoming increasingly relevant as a cause for VAP, more research is needed to assess the impact of drug resistance on clinical course and outcome in PICU patients suffering from VAP.

Summary

MDR gram-negative bacteria are an emerging problem in Germany and worldwide. The family of Enterobacteriaceae in particular is a common cause for various nosocomial infections. One important way of acquiring nosocomial bacteria is endotracheal intubation, during which potentially drug resistant bacteria can be transported into the lower respiratory tract and eventually cause severe infections such as ventilator-associated pneumonia (VAP).

Against this background, we conducted a retrospective single-centre study to investigate the epidemiology of Enterobacteriaceae in tracheal aspirate of 123 ventilated PICU (paediatric intensive care unit) patients between 2005 and 2014. The study aimed to describe the frequency, distribution and resistance-status of Enterobacteriaceae in the lower respiratory tract of ventilated PICU patients; to identify risk factors and to analyse the clinical outcome of endotracheal colonization or infection with multidrug resistant Enterobacteriaceae. Furthermore, we identified patients who had a VAP due to Enterobacteriaceae and investigated risk factors and outcome with special attention to VAPs caused by MDR bacteria.

A total of 167 isolates of Enterobacteriaceae were detected in the tracheal aspirate of 123 patients. The most frequently isolated species were *Enterobacter spp, E.coli* and *Klebsiella spp* with an overall MDR-proportion of 30.5%. Both incidence of Enterobacteriaceae and proportion of MDR organisms have markedly increased over the study period. Analysis by a CRT model revealed, that colonization or infection with *E.coli*, presence of gastrointestinal comorbidity and antibiotic exposure of more than 6.5 days prior to tracheal aspirate were the most important independent risk factors for isolation of MDR Enterobacteriaceae in the tracheal aspirate. Clinical outcome did not differ between MDR and non-MDR patients.

19 of the patients included in the study acquired a VAP, mostly caused by *Enterobacter spp*. VAP patients were younger, with a higher proportion of females compared to males. The most important risk factors for VAP were prolonged stay on PICU, mechanical ventilation and antibiotic pre-treatment. 47.4% of the VAPs

were MDR, however, the MDR-status of the causative organism did not affect the clinical outcome of the VAP. The number of VAPs is too small to draw a final conclusion about risk factors and impact of MDR Enterobacteriaceae causing VAP on PICU.

The trend of emerging MDR gram-negative organisms in the PICU setting should raise increased awareness. Surveillance, adapted screening- and treatment guidelines as well as new antibiotic agents are needed. Furthermore, the possibility of a respiratory tract infection caused by potentially drug resistant Enterobacteriaceae should be kept in mind whenever a child is on mechanical ventilation.

The subject of VAP caused by MDR Enterobacteriaceae on PICU is not yet well investigated and more research is needed to assess epidemiology, risk factors and treatment options.

Zusammenfassung

Nosokomiale Infektionen werden häufig durch gramnegative Bakterien, insbesondere durch Enterobacteriaceae, ausgelöst. Leider zeichnete sich in den letzten Jahren in Deutschland und weltweit eine deutliche Zunahme an Antibiotikaresistenzen bei dieser Erregergruppe ab. Eine nosokomiale Infektion durch multiresistente Enterobacteriaceae birgt das Risiko vermehrter Komplikationen und eines schlechteren Outcomes, da die empirische Antibiotikatherapie nicht greift und eine adäquate antibiotische Therapie häufig erst verzögert eingesetzt wird. Da Kinder, insbesondere Säuglinge und Intensivpatienten, ein erhöhtes Risiko für nosokomiale Infektionen haben, wurde in dieser retrospektiven Studie die Epidemiologie von Enterobacteriaceae im Trachealsekret beatmeter Kinder auf der pädiatrischen Intensivstation der Universitätsklinik Tübingen untersucht.

Die Studie hatte folgende Ziele:

1. (a) Bestimmung der Prävalenz und des Spektrums von Enterobacteriaceae im Trachealsekret beatmeter Kinder
   (b) Bestimmung des Anteils an multiresistenten Bakterien in den Isolaten
2. Identifikation von Risikofaktoren für endotracheale Besiedlung oder Infektion durch multiresistente Enterobacteriaceae
3. Vergleich des Outcomes von Patienten mit sensiblen Keimen gegenüber Patienten mit multiresistenten Keimen
3. Identifikation von Risikofaktoren für beatmungsassoziierte Pneumonien bei Kindern mit endotrachealer Besiedlung durch Enterobacteriaceae

Ergebnisse: Es wurden retrospektiv alle Patienten identifiziert, in deren Trachealsekret zwischen 2005 und 2014 Enterobacteriaceae nachgewiesen wurden. Insgesamt wurden 167 Isolate von 123 Patienten ausgewertet. Die am häufigsten isolierten Gattungen waren dabei *Enterobacter spp*, *E.coli* und *Klebsiella spp* mit einem Anteil an multiresistenten Keimen von insgesamt 30.5%. Sowohl die Inzidenz von Enterobacteriaceae im Trachealsekret als auch der Anteil resistenter Keime stiegen im Verlauf der Studie deutlich an.

Die statistische Analyse mittels CRT Model ergab, dass endotracheale Besiedlung und Infektion durch *E.coli*, gastrointestinale Komorbiditäten sowie antibiotische Vorbehandlung von mehr als 6.5 Tagen vor der Entnahme des Trachealsekrets unabhängige Risikofaktoren für eine endotracheale Besiedlung oder Infektion durch multiresistente Enterobacteriaceae darstellten. In diesem Setting unterschied sich das klinische Outcome von Patienten mit resistenten Erregern nicht signifikant vom Outcome der Patienten mit sensiblen Erregern.

19 Patienten erfüllten die Kriterien einer beatmungsassoziierten Pneumonie (ventilator-associated pneumonia, VAP). 58% dieser VAPs wurden durch *Enterobacter spp* hervorgerufen. Die VAP-Patienten waren signifikant jünger und häufiger weiblich als die Gesamtheit der Patienten. Unabhängige Risikofaktoren für eine VAP bei Kindern mit Enterobacteriaceae im Trachealsekret waren ein langer Aufenthalt auf der Intensivstation, eine lange Beatmungsdauer und eine lange antibiotische Vorbehandlung. 47% der beatmungsassoziierten Pneumonien wurden durch multiresistente Erreger ausgelöst, das klinische Outcome änderte sich dadurch jedoch nicht. Aufgrund der geringen Zahl an VAPs sind diese Ergebnisse allerdings nur eingeschränkt verwertbar.

Fazit: Die in vielen Studien beschriebene steigende Inzidenz multiresistenter gramnegativer Erreger ließ sich auch in unserem Setting beobachten. Diese Entwicklung ist besorgniserregend und erfordert adäquate Screening- und Behandlungsschemata. Im klinischen Alltag sollte bei allen intubiert beatmeten Kindern die Möglichkeit einer endotrachealen Besiedlung durch multiresistente Enterobacteriaceae berücksichtigt werden. Dies gilt insbesondere bei Vorhandensein von entsprechenden Risikofaktoren, wie endotrachealer Besiedlung durch *E.coli* oder gastrointestinaler Komorbiditäten. Antibiotika sollten so restriktiv wie möglich eingesetzt werden, um einer Entwicklung von Resistenzen bei endotrachealer bakterieller Besiedlung vorzubeugen.
Die Datenlage zu beatmungsassoziierte Pneumonien durch multiresistente Enterobacteriaceae im pädiatrischen Setting ist derzeit nicht ausreichend und erfordert weitere Forschung.

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Declaration of authorship

This dissertation has been realized at the interdisciplinary centre for infection medicine of the Universitary Hospital Tübingen under the supervision of Prof. Dr. med. D. Hartl. The study has been designed and conducted in cooperation with Dr. med. H. Renk from the paediatric department. I independently collected all required data. The following statistical analyse was realized by myself after consultation of the institute for biometrics and statistics and instruction by Dr. Renk. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work. At the present time, none of this work has been published.

Publications

1. L. Stoll, H. Renk, F. Neunhoeffer, M. Kumpf, M. Hofbeck, D. Hartl **Besiedelung von beatmeten pädiatrischen Intensivpatienten mit multiresistenten Enterobakterien: epidemiologische Daten und Risikofaktoren**, FV 44. Abstracts Monatsschrift Kinderheilkunde, May 2015, Volume 163, Supplement 1, pp 1-85. gnpi Jahrestagung 2015, Stuttgart. Oral presentation.

2. Renk H, Stoll L, Neunhoeffer F, Kumpf M, Hofbeck M, Hartl D. **Multidrug-resistant Enterobacteriaceae in PICU patients: Epidemiological data and risk factors for colonization**, Poster dgpi 2015, Freiburg. Poster presentation.

3. Renk H., Stoll L., Neunhoeffer F. Hölzl F., Kumpf M., Hofbeck M., Hartl D. **Suspicion of respiratory tract infection with multidrug-resistant Enterobacteriaceae: Epidemiology and Risk factors from a Paediatric Intensive Care Unit,** BMC Infectious Diseases, 2017, Volume 17 (1). 163 ff.

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