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ORIGINAL PAPER

Secular trends in pediatric bloodstream infections over a 20-year period at a tertiary care hospital in Germany

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Abstract
Objective Over the last 20 years, a number of medical innovations with impact on the incidence of bacterial and fungal bloodstream infections (BSIs) in children have been developed and implemented. Although appropriate empirical antimicrobial therapy is a prerequisite to the successful treatment of BSIs, to date, epidemiological data on long-term microbiological trends in BSIs of hospitalized children have not been available.
Methods Two cohorts of pediatric patients who were hospitalized in a single-center tertiary care hospital in Germany over a 20-year time span (period A from 1985 to 1995 vs. period B from 1997 to 2006) were retrospectively analyzed and compared with respect to the epidemiology and microbiology of BSIs.
Results A total of 1,646 cases of monomicrobial BSIs were detected. The rate of positive blood culture results dropped from 4.5% in period A to 2.0% in period B. The proportion of gram-positive vs. gram-negative pathogens recovered from blood cultures remained stable. Among gram-positive pathogens, an increase in enterococci (3.3% vs. 8.2%) and in coagulase-negative staphylococci (CoNS) (22.9 vs. 28.2%) was observed. In contrast, BSIs caused by *Staphylococcus aureus* (16.4% vs. 11.7%), *Streptococcus agalactiae* (4.9% vs. 2.1%), *Haemophilus influenzae* (7.3% vs. 0.7%), and *Neisseria meningitidis* (1.9% vs. 0.5%) diminished. In analyzing subgroups, an increase of enterococcal and CoNS infections was noted among patients with immunosuppression and neonatal early-onset sepsis (EOS), while a decrease was found among late-onset sepsis (LOS) cases with *S. viridans*. Notably, aminopenicillin-resistant enterococci and aminopenicillin- and fluoroquinolone-resistant *Enterobacteriaceae* all increased over time, while the overall resistance pattern was still favorable. The overall mortality rate of BSIs decreased (5.2% vs. 2.6%).
Conclusions Over the 20-year study period, the spectrum of specific microorganisms among BSIs shifted, with opportunistic pathogens becoming predominant. Despite an increase in the proportion of antibiotic-resistant organisms, however, the mortality rate decreased.

Keywords Bloodstream infections · Pediatric patients · Epidemiological trends · Microbiological trends

Abbreviations

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
BSI	Blood stream infection
CoNS	Coagulase-negative staphylococci
CVC	Central intravascular catheter
EOS	Early-onset sepsis
ESBL	Extended spectrum beta-lactamase
GBS	Group B streptococci
Hib	Haemophilus influenzae type B
JMML	Juvenile monomyelocytic leukemia
LOS	Late-onset sepsis
MCV	Meningococcus C conjugate vaccine
MDS	Myelodysplastic syndrome

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NBSIs	Nosocomial bloodstream infections
NICU	Neonatal intensive care unit
n.a.	Not available
n.d.	Not determined
n.s.	Non-significant
PICU	Pediatric intensive care unit
PCV	Pneumococcal conjugate vaccine
RR	Relative risk
spp.	Species
vs.	Versus
95%-CI	95% confidence interval

Introduction

A major cause of morbidity and mortality in hospitalized children worldwide [1, 31], bloodstream infections (BSIs) are either community- or hospital-acquired. A recent prospective single pediatric center study has shown that nearly half of all BSIs are contracted in hospitals [15]. However, the distinction between hospital- and community-acquired BSIs is unlikely to have a major influence, because the majority of BSIs currently occur in patients who have serious underlying illnesses and undergo repeat hospital visits [11, 28]. In cases where a patient contracts a BSI without having been hospitalized in the previous 48 hours, his or her BSI would be classified as community-acquired, even though the responsible pathogen most likely emerged from the hospital environment. When occurring 48 hours after hospital admission, BSIs are defined as nosocomial. As such, they are the most common cause of nosocomial infections in children [33]. Recent studies on BSIs in pediatric patients have focused on individual pathogens [5, 7, 8, 12, 17, 21], on patient categories [2, 16, 18, 29], and on specific age groups [20, 27]. Studies relating to the general pediatric population are rarely found in the literature [4, 15, 24]. General trends in BSIs over time, such as the increasing importance of gram-positive pathogens [25] and the rise in antibiotic resistance [32], are mainly derived from epidemiological studies in adult patients. In a general pediatric population, BSIs' long-term epidemiological trends have not been reported to date. Knowledge of the local epidemiology of BSIs is, nonetheless, critical to guiding appropriate empiric antibiotic treatment and infection control procedures.

Our study retrospectively compared epidemiological trends in two cohorts totaling 1,646 BSI cases in children hospitalized at a single tertiary care center over a 20-year period. The aim of the study was to ascertain general trends in the pattern of BSIs in a general pediatric population over an extended time period, in order to reevaluate antibiotic prescription and infection control policies.

Patients and methods

Setting

Freiburg University Medical Center is a 1,800-bed, tertiary care facility in southwestern Germany. The Center of Pediatrics and Adolescent Medicine is a 145-bed pediatric tertiary care center, including a neonatal and a pediatric intensive care unit, and an oncology division with a bone marrow transplant unit. The hospital serves a combined urban and rural population of circa 400,000 inhabitants. Approximately 7,500 patients, including 400 to 500 newborns, are admitted to the hospital annually.

Study design

All reports on blood cultures taken between July 1997 and December 2006 (period B) at the Freiburg Center of Pediatrics and Adolescent Medicine were retrospectively analyzed and compared to blood cultures taken between January 1985 and December 1995 (period A), for which clinical data have been previously published [4]. For the current study, patients' medical charts were reviewed retrospectively. Data routinely collected included: the collection date of the pathogen; the patient's age, sex, and predisposing clinical conditions; the presence of implanted foreign body material, such as central intravascular catheters (CVCs); the clinical outcome from hospitalization; and the microbiological characteristics (species differentiation and antimicrobial susceptibility) of the cultured pathogen.

The diagnosis of a bacteremia or BSI was made when at least one blood culture grew a pathogen. Only monomicrobial bloodstream infections were included in the analysis. If the bloodstream isolate was determined to be a potential skin contaminant (e.g., diphtheroids, spore-forming rods, coagulase-negative staphylococci, or micrococci), the episode was considered to be a true bacteremia only if: (a) the pathogen was isolated in two or more blood cultures; (b) the pathogen was isolated in one blood culture, as well as from another infection site; or (c) the patient showed clinical signs of infection in the presence of an intravascular catheter, in the absence of an alternative explanation for the clinical findings, and also when clinical signs and symptoms resolved after appropriate antibiotic therapy and/or removal of the device. Detection of the same pathogen in the same patient within a four-week period was considered as a relapse and was, subsequently, excluded from analysis. A bacteremic episode occurring during the first week of life was defined as early-onset sepsis (EOS), while that between the second and twelfth weeks of life was noted as late-onset sepsis (LOS) [23]. Mortality was defined as BSI-related when death occurred within seven days after the detection of the pathogen in at least one blood culture, and when

other potential causes of death were eliminated by the physicians in charge.

Microbiological methods

Blood cultures were processed in the microbiology laboratory of the Freiburg Center of Pediatrics and Adolescent Medicine. Under normal circumstances, 1 ml of venous blood was drawn from newborns and infants, 2–5 ml from children aged two to six years, and 5–10 ml from children aged six and over. During period A (1985 to 1995), equal parts of blood volume were inoculated in aerobic and anaerobic blood culture bottles. During period B (1997 to 2006), anaerobic blood culture bottles were inoculated only if the patient: (a) had a dental focus or chronic sinusitis, (b) presented with signs or symptoms of mucositis, (c) had an abdominal focus, (d) suffered from a bite wound, or (e) was neutropenic. The identification and classification of bacteria and fungi were performed by standard microbiology methods. Antimicrobial susceptibility testing was done using the agar diffusion method, following the current recommendations of the National Committee for Clinical Laboratory Standards (NCCLS).

Statistical analysis

For statistical analysis, Microsoft Excel and GraphPad Prism V.3 were used. The results were expressed as a percentage of the total number of isolates or the total numbers of patients. Proportional differences were compared using either a Chi-square test or Fisher's exact test, as appropriate for the context. All statistical tests were performed as two-tailed and were considered significant if the p-value was <0.05.

Results

Study population

During period A (1985 to 1996), a total of 22,940 blood cultures were taken, while during period B (1997 to 2006), a total of 30,369 blood cultures were drawn. In period A, 1,695 (7.4%) blood cultures showed bacterial or fungal growth, compared to 812 (2.7%) cultures in period B. After the exclusion of polymicrobial growth, presumed skin contaminants, and relapses, 1,037 BSIs were determined to have occurred in period A (4.5%) and 609 BSIs in period B (2.0%). Thus, as shown, the rate of positive blood cultures declined significantly between the earlier and later time periods ($p<0.0001$). The number of patients admitted to our hospital has increased from ≈6,500 per year in period A [4] to 8,000 per year in period B. Although exact numbers documenting annual admissions were not available from the hospital for period A, we instead employed population figures from the Freiburg census to calculate the BSI incidence rate (Fig. 1). This showed that BSI incidence decreased from 331 per 100,000 children below 18 years of age in period A to 200 per 100,000 in period B ($p<0.0001$, 95%-CI 1.389–1.972).

Microbiological features

During the combined study period, the relative contribution of pathogen classes (i.e., gram-positive bacteria, gram-negative bacteria, anaerobic bacteria, and yeasts) to all BSIs remained unchanged (Table 1). However, among gram-positive bacteria, an increase of coagulase-negative staphylococci (CoNS, 22.9% vs. 28.2%) and enterococci (3.3% vs. 8.2%), as well as a decrease of *Staphylococcus aureus*

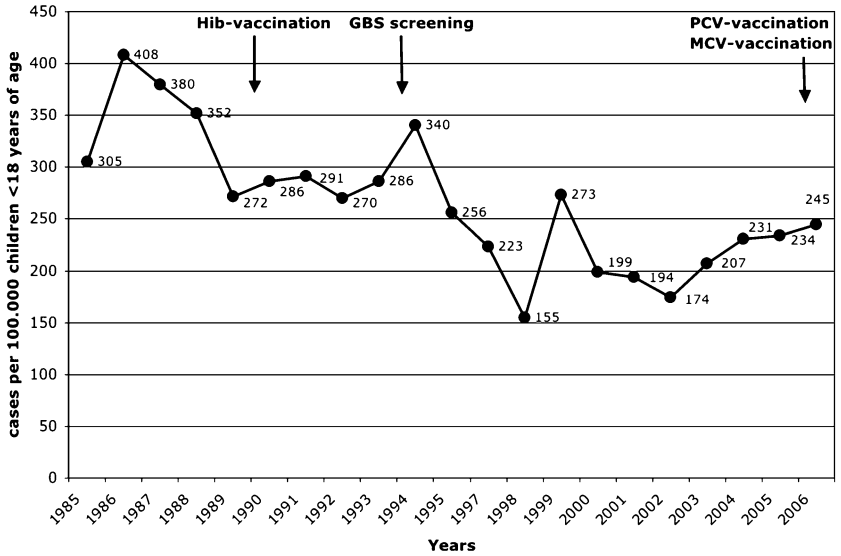

Fig. 1 Bloodstream infection (BSI) incidence rates per 100,000 children below the age of 18 years charted on an annual basis over time

Table 1 Comparison of the distribution of pathogens isolated from monomicrobial bloodstream infections (BSIs) in hospitalized pediatric patients during the time period January 1985 to December 1995 vs. July 1997 to June 2006

Pathogen	Total and percentage, 1985–1995 (n=1,037)	Total and percentage, 1997–2006 (n=609)	p (Fisher's exact test) and 95%-CI
Gram-positive pathogens	719 (69.3%)	428 (70.3%)	n.s. (0.949–1.082)
CoNS	237 (22.9%)	172 (28.2%)	0.016 (1.044–1.463)
S. aureus	170 (16.4%)	71 (11.7%)	0.009 (0.549–0.921)
S. pneumoniae	88 (8.5%)	55 (9.0%)	n.s. (0.771–1.468)
S. viridans	112 (10.8%)	54 (8.9%)	n.s. (0.605–1.122)
Enterococcus species	34 (3.3%)	50 (8.2%)	<0.0001 (1.639–3.827)
S. agalactiae	51 (4.9%)	13 (2.1%)	0.005 (0.238–0.792)
S. pyogenes	14 (1.4%)	7 (1.1%)	n.s. (0.326–1.938)
Others	13 (1.3%)	5 (0.8%)	n.s. (0.235–1.829)
Gram-negative pathogens	303 (29.2%)	174 (28.6%)	n.s. (0.836–1.144)
E. coli	75 (7.2%)	58 (9.5%)	n.s. (0.949–1.828)
Klebsiella species	29 (2.8%)	22 (3.6%)	n.s. (0.749–2.228)
Pseudomonas species	20 (1.9%)	19 (3.1%)	n.s. (0.870–3.007)
Acinetobacter species	25 (2.4%)	19 (3.1%)	n.s. (0.719–2.330)
Enterobacter species	19 (1.8%)	14 (2.3%)	n.s. (0.634–2.485)
Salmonella species	25 (2.4%)	12 (2.0%)	n.s. (0.414–1.615)
Serratia species	1 (0.3%)	6 (1.0%)	0.012 (1.232–84.71)
H. influenzae	76 (7.3%)	4 (0.7%)	<0.0001 (0.033–0.244)
N. meningitidis	20 (1.9%)	3 (0.5%)	0.016 (0.076–0.856)
Others	13 (1.3%)	17 (2.8%)	0.034 (1.089–4.553)
Anaerobes	12 (1.2%)	5 (0.8%)	n.s. (0.251–2.005)
Yeasts	16 (1.5%)	7 (1.1%)	n.s. (0.308–1.801)

n.s.=non-significant; CoNS=coagulase-negative staphylococci

(16.4% vs. 11.7%) and *S. agalactiae* (4.9% vs. 2.1%), was observed. Among gram-negative bacteria, *Serratia* species were more frequently isolated during period B (0.3% vs. 1.0%), whereas *Haemophilus influenzae* and *Neisseria meningitidis* were isolated less often (7.3% vs. 0.7%, and 1.9% vs. 0.5%, respectively) in the same period. The rates for anaerobic bacteria and yeasts remained stable across the two periods.

Predisposing underlying conditions

BSIs in patients with underlying medical conditions increased markedly between study periods A and B (Table 2). In period A (1985 and 1995), only 49.9% of patients had a predisposing medical condition, whereas in period B (1997 to 2006), 79.8% of patients had a predisposing condition. Among malignancies, the proportion of patients with leukemia and lymphomas (6.6% vs. 23.2%) and solid tumors (4.3% vs. 8.9%) increased. Similarly, the proportion of patients with underlying immunodeficiency syndromes rose over time (0.6% vs. 2.3%). In contrast, the rate of newborns with bloodstream infections decreased (12.7% vs. 6.4%), although the proportion of preterm infants remained the same over the 20-year period.

When the distribution of specific pathogens in patients with underlying immunosuppressive conditions (i.e., prematurity, malignancies, solid organ transplants, and primary immunodeficiency syndromes) were compared over time, an increase in the proportion of CoNS (28.3% vs. 39.6%) and enterococci (0.9% vs. 11.5%), and a reduction of *S. viridans* bacteremia (23.9% vs. 5.8%), were evident (Table 3). In children without immmunosuppression, neither CoNS nor enterococci showed an increase (Table 4).

BSIs among infants were classified as either early-onset sepsis (EOS, within the first week of life) or late-onset sepsis (LOS, between the second and twelfth weeks of life). Between the two study periods, LOS increased (60.8% vs. 70.5%) and EOS decreased (40.2% vs. 29.5%). In the period 1997 to 2006, 4,014 newborn and preterm infants were admitted to the Freiburg hospital and the annual incidence of neonatal bacteremia was 30.1 per 1,000 admissions. The overall ratio of gram-positive or gram-negative pathogens remained the same between the two time periods, but the relative significance of specific pathogens within the two groups shifted. Specifically, in EOS, enterococci was found more often in the recent period (5.7% vs. 27.9%, Table 5), while no increase in the percentage of *E. coli* BSI was observed. In LOS, the percentage of bacteremia caused by CoNS rose (29.8% vs.

Table 2 Comparison of predisposing medical conditions among hospitalized pediatric patients with BSIs during the period January 1985 to December 1995 vs. July 1997 to June 2006

Predisposing condition	Total and percentage, 1985–1995 (n=1,037)	Total and percentage, 1997–2006 (n=609)	p (Fisher's exact test) and 95%-CI
Any predisposing condition	517 (49.9%)	486 (79.8%)	<0.0001 (1.488–1.722)
Newborn/preterm infants	271 (26.1%)	121 (19.9%)	0.004 (0.629–0.919)
Newborn infants	132 (12.7%)	39 (6.4%)	<0.0001 (0.357–0.709)
Preterm infants	139 (13.4%)	82 (13.5%)	n.s. (0.779–1.295)
Malignancies	113 (10.9%)	195 (32.0%)	<0.0001 (2.384–3.622)
Leukemia/Lymphoma	68 (6.6%)	141 (23.2%)	<0.0001 (2.691–4.633)
ALL	n.a.	64 (10.5%)	n.d.
AML	n.a.	32 (5.3%)	n.d.
MDS	n.a.	19 (3.1%)	n.d.
JMML	n.a.	10 (1.6%)	n.d.
Lymphoma	n.a.	16 (2.6%)	n.d.
Solid tumors	45 (4.3%)	54 (8.9%)	0.0003 (1.393–2.996)
Solid organ transplant	0	7 (1.1%)	0.0009 (no 95%-CI)
Immunodeficiencies	6 (0.6%)	14 (2.3%)	0.004 (1.535–10.29)
Hematologic	n.a.	17 (2.8%)	n.d.
Gastrointestinal	n.a.	39 (6.4%)	n.d.
Cardiac	12 (1.2%)	24 (3.9%)	<0.0001 (18.18–65.71)
Pulmonary	2 (0.2%)	4 (0.7%)	n.s. (0.625–18.55)
Others	113 (10.9%)	65 (10.7%)	n.s. (0.734–1.307)
No predisposing condition	520 (50.1%)	123 (20.2%)	<0.0001 (0.340–0.477)

n.s.=non-significant; ALL=acute lymphoblastic leukemia; n.a.=not available; n.d.=not determined; AML=acute myeloid leukemia; MDS=myelodysplastic syndrome; JMML=juvenile monomyelocytic leukemia

Table 3 Comparison of the distribution of pathogens isolated from BSIs of pediatric patients with underlying immunosuppression* during the time period January 1985 to December 1995 vs. July 1997 to June 2006

Pathogens	Total and percentage, 1985–1995 (n=113)	Total and percentage, 1997–2006 (n=278)	p (Fisher's exact test) and 95%-CI
Gram-positive pathogens	69 (61.1%)	175 (62.9%)	n.s. (0.867–1.225)
CoNS	32 (28.3%)	110 (39.6%)	0.038 (1.007–1.939)
S. aureus	4 (3.5%)	9 (3.2%)	n.s. (0.287–2.910)
S. pneumoniae	5 (4.4%)	3 (1.1%)	0.048 (0.059–1.004)
S. viridans	27 (23.9%)	16 (5.8%)	<0.0001 (0.135–0.430)
Enterococcus species	1 (0.9%)	32 (11.5%)	0.0002 (1.798–94.09)
S. agalactiae	0	4 (1.4%)	n.s. (no 95%-CI)
S. pyogenes	0	0	n.s. (no 95%-CI)
Others	0	1 (0.4%)	n.s. (no 95%-CI)
Gram-negative pathogens	33 (29.2%)	99 (35.6%)	n.s. (0.879–1.692)
E. coli	9 (7.9%)	30 (10.8%)	n.s. (0.665–2.762)
Klebsiella species	6 (5.3%)	13 (4.7%)	n.s. (0.343–2.260)
Pseudomonas species	7 (6.2%)	15 (5.4%)	n.s. (0.365–2.080)
Acinetobacter species	10 (8.8%)	7 (2.5%)	0.011 (0.111–0.729)
Enterobacter species	n.a.	13 (4.7%)	n.d.
Salmonella species	0	0	n.d.
Serratia species	1 (0.9%)	6 (2.1%)	n.s. (0.297–20.04)
H. influenzae	0	0	n.d.
N. meningitidis	0	0	n.d.
Others	10 (8.8%)	15 (5.4%)	n.s. (0.282–1.317)
Anaerobes	n.a.	4 (1.4%)	n.d.
Yeasts	n.a.	5 (1.8%)	n.d.

*i.e., prematurity, malignancies, solid organ transplants, and primary immunodeficiencies; CoNS=coagulase-negative staphylococci; n.s.=non-significant; n.a.=not available; n.d.=not determined

Table 4 Comparison of the distribution of pathogens isolated from BSIs of pediatric patients without immunosuppression during the time period January 1985 to December 1995 vs. July 1997 to June 2006

Pathogens	Total and percentage, 1985–1995 (n=909)	Total and percentage, 1997–2006 (n=331)	p (Fisher's exact test) and 95%-CI
Gram-positive pathogens	638 (70.2%)	253 (76.4%)	0.032 (1.012–1.172)
CoNS	205 (22.6%)	62 (18.7%)	n.s. (0.644–1.072)
S. aureus	166 (18.3%)	62 (18.7%)	n.s. (0.788–1.335)
S. pneumoniae	83 (9.1%)	52 (15.7%)	0.001 (1.246–2.377)
S. viridans	85 (9.4%)	38 (11.5%)	n.s. (0.865–1.762)
Enterococcus species	33 (3.6%)	18 (5.4%)	n.s. (0.855–2.624)
S. agalactiae	51 (5.6%)	9 (2.7%)	0.036 (0.241–0.974)
S. pyogenes	15 (1.7%)	7 (2.1%)	n.s. (0.527–3.116)
Others	0	5 (1.5%)	0.001 (no 95%-CI)
Gram-negative pathogens	237 (26.1%)	75 (19.3%)	n.s. (0.692–1.091)
E. coli	66 (7.2%)	28 (8.5%)	n.s. (0.763–1.780)
Klebsiella species	23 (2.5%)	9 (2.7%)	n.s. (0.502–2.299)
Pseudomonas species	13 (1.4%)	4 (1.2%)	n.s. (0.277–2.574)
Acinetobacter species	15 (1.7%)	11 (3.3%)	n.s. (0.934–4.341)
Enterobacter species	n.a.	2 (0.6%)	n.d.
Salmonella species	24 (2.6%)	12 (3.6%)	n.s. (0.695–2.715)
Serratia species	0	0	n.d.
H. influenzae	76 (8.4%)	4 (1.2%)	<0.0001 (0.053–0.392)
N. meningitidis	20 (2.2%)	3 (0.9%)	n.s. (0.123–1.378)
Others	34 (3.7%)	2 (0.6%)	0.019 (0.039–0.669)
Anaerobes	n.a.	1 (0.3%)	n.d.
Yeasts	n.a.	2 (0.6%)	n.d.

44.1%, Table 4), whereas the rate of *S. viridans* bacteremias dropped (4.2% vs. 0%, Table 5). In addition, there was a trend (although it was not statistically significant) towards fewer *S. aureus* and *S. agalactiae* BSIs in period B.

Outcome

In patients with monomicrobial BSI, crude mortality decreased over the 20-year period (Table 6). During the first period, 5.2% of the patients died of bacteremia within seven days of a positive blood culture. In contrast, only 2.6% of the bacteremic patients had a lethal outcome in the second period.

Crude mortality among patients with gram-positive pathogens remained unchanged, whereas mortality among patients with gram-negative bacteremia declined (9.9% vs. 4.0%). This decline was primarily noted with BSIs caused by *E. coli* (9.3% vs. 1.7%; without statistical significance) and *Pseudomonas* species (40.0% vs. 5.2%). Furthermore, none of our patients with bacteremias caused by *Salmonella* spp., other *Enterobacteriaceae* species, *S. pyogenes*, or *S. agalactiae* died during period B.

Antibiotic susceptibility

Antibiotic resistance patterns changed significantly during the 20-year period (Table 7). Among enterococcal species, no vancomycin resistance was found, but the resistance rate to aminopenicillins (9.5% vs. 34.7%) increased. The rate of aminoglycoside-resistant enterococci remained stable over the two-decade period, with no isolates showing high-level aminoglycoside resistance. Methicillin resistance in *S. aureus* was not observed. None of the *S. pneumoniae* isolates were resistant to penicillins. The antibiotic resistance pattern among gram-negative pathogens also shifted, particularly the percentage of *Enterobacteriaceae* with resistance to aminopenicillins, which increased from 28.1% to 61.7% (Fig. 2). A similar rise occurred in the rate of *Enterobacteriaceae* resistant to fluoroquinolones (0.4% vs. 4.2%). In contrast, the rate of extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* remained unchanged from 1997 to 2006.

Discussion

BSIs are among the most serious and potentially life-threatening infectious diseases in pediatric patients. Early diagnosis and appropriate empiric antimicrobial therapy are essential for the prevention of morbidity and mortality [14, 31]. Crucial to successful therapy is knowledge of the most likely pathogen(s) and the associated antimicrobial susceptibility pattern. The BSI pattern is dependent upon the type

Table 5 Comparison of the distribution of pathogens isolated from BSIs of pediatric patients with early-onset sepsis (EOS) and late-onset sepsis (LOS) in the time period January 1985 to December 1995 vs. July 1997 to June 2006

Pathogens	Early-onset sepsis			Late-onset sepsis		
	Total and percentage, 1985–1995 (n=140)	Total and percentage, 1997–2006 (n=43)	p (Fisher's exact test) and 95%-CI	Total and percentage, 1985–1995 (n=141)	Total and percentage, 1997–2006 (n=103)	p (Fisher's exact test) and 95%-CI
Gram-positive pathogens	113 (80.7%)	35 (81.4%)	n.s. (0.856–1.188)	100 (70.9%)	72 (70.6%)	n.s. (0.836–1.163)
CoNS	21 (15.0%)	9 (20.9%)	n.s. (0.691–2.816)	42 (29.8%)	45 (44.1%)	0.030 (1.049–2.051)
S. aureus	32 (22.9%)	6 (13.9%)	n.s. (0.274–1.362)	25 (17.7%)	9 (8.8%)	n.s. (0.240–1.011)
S. pneumoniae	1 (0.7%)	0	n.s. (no 95%-CI)	0	0	n.d.
S. viridans	11 (7.9%)	1 (2.3%)	n.s. (0.039–2.229)	6 (4.2%)	0	n.s. (no 95%-CI)
Enterococcus spp.	8 (5.7%)	12 (27.9%)	0.0002 (2.136–11.17)	8 (5.7%)	12 (11.8%)	n.s. (0.871–4.843)
S. agalactiae	35 (25.0%)	7 (16.2%)	n.s. (0.312–1.360)	15 (10.6%)	5 (4.9%)	n.s. (0.171–1.216)
S. pyogenes	0	0	n.d.	4 (2.8%)	1 (1.0%)	n.s. (0.039–3.019)
Others	5 (3.6%)	0	n.s. (no 95%-CI)	0	0	n.d.
Gram-negative pathogens	23 (16.4%)	7 (16.3%)	n.s. (0.457–2.149)	37 (26.2%)	29 (28.4%)	n.s. (0.709–1.624)
E. coli	18 (12.9%)	4 (9.3%)	n.s. (0.259–2.023)	15 (10.6%)	19 (18.6%)	n.s. (0.926–3.248)
Klebsiella spp.	0	0	n.d.	n.a.	0	n.d.
Pseudomonas spp.	0	0	n.d.	9 (6.4%)	4 (3.9%)	n.s. (0.193–1.922)
Acinetobacter spp.	0	1 (1.4%)	n.s. (no 95%-CI)	n.a.	1 (1.0%)	n.d.
Enterobacter spp.	0	0	n.d.	n.a.	5 (4.9%)	n.d.
Salmonella spp.	0	0	n.d.	n.a.	0	n.d.
Serratia spp.	0	0	n.d.	n.a.	0	n.d.
H. influenzae	1 (0.7%)	0	n.s. (no 95%-CI)	1 (0.7%)	0	n.s. (no 95%-CI)
N. meningitidis	0	0	n.d.	0	0	n.d.
Others	4 (2.9%)	2 (4.7%)	n.s. (0.309–8.588)	11 (7.8%)	0	n.s. (no 95%-CI)
Anaerobes	3 (2.3%)	0	n.s. (no 95%-CI)	0	1 (1.0%)	n.s. (no 95%-CI)
Yeasts	1 (0.7%)	1 (2.3%)	n.s. (0.208–51.00)	4 (2.8%)	1 (1.0%)	n.s. (0.039–3.019)

CoNS=coagulase-negative staphylococci; n.s.=non-significant; n.d.=not determined; spp.=species

Table 6 Comparison of the outcome among hospitalized pediatric patients with BSIs during the time period January 1985 to December 1995 vs. July 1997 to June 2006

Outcome	Total and percentage, 1985–1995 (n=1,037)	Total and percentage, 1997–2006 (n=609)	p (Fisher's exact test) and 95%-CI
Cure	982 (94.7%)	593 (97.4%)	0.011 (0.291–0.874)
Death caused by bacteremia	54 (5.2%)	16 (2.6%)	0.012 (1.008–1.047)
Death caused by gram-positive pathogen	21 of 719 (2.9%)	7 of 428 (1.6%)	n.s. (0.240–1.306)
CoNS	5 of 237 (2.1%)	1 of 172 (0.5%)	n.s. (0.032–2.339)
S. aureus	5 of 170 (2.9%)	1 of 71 (1.4%)	n.s. (0.057–4.028)
S. pneumoniae	1 of 88 (1.1%)	1 of 55 (1.8%)	n.s. (0.102–25.08)
S. viridans	5 of 112 (4.5%)	1 of 54 (1.9%)	n.s. (0.050–3.466)
Enterococci species	1 of 34 (2.9%)	3 of 50 (6.0%)	n.s. (0.221–18.81)
S. agalactiae	3 of 51 (5.9%)	0 of 13	n.s. (no 95%-CI)
S. pyogenes	1 of 14 (7.1%)	0 of 7	n.s. (no 95%-CI)
Deaths caused by gram-negative pathogen	30 of 303 (9.9%)	7 of 174 (4.0%)	0.021 (0.182–0.906)
E. coli	7 of 75 (9.3%)	1 of 58 (1.7%)	n.s. (0.023–1.460)
Klebsiella species	0 of 29	3 of 22 (13.6%)	n.s. (no 95%-CI)
Pseudomonas species	8 of 20 (40.0%)	1 of 19 (5.2%)	0.020 (0.018–0.955)
Citrobacter species	0 of 1	1 of 3 (33.3%)	n.s. (no 95%-CI)
Serratia species	0 of 1	1 of 6 (16.7%)	n.s. (no 95%-CI)
Salmonella species	1 of 25 (4.0%)	0 of 12	n.s. (no 95%-CI)
Other Enterobacteriaceae	10 of 52 (15.4%)	0 of 17	n.s. (no 95%-CI)
H. influenzae	1 of 76 (1.3%)	0 of 4	n.s. (no 95%-CI)
N. meningitidis	3 of 20 (15.0%)	0 of 3	n.s. (no 95%-CI)
Death caused by anaerobes	2 of 12 (16.7%)	0 of 5	n.s. (no 95%-CI)
Death caused by yeasts	1 of 16 (6.3%)	2 of 7 (28.6%)	n.s. (0.491–42.54)

n.s.=non-significant; CoNS=coagulase-negative staphylococci

of patients treated at a hospital and the antimicrobial regimens in use there.

To our knowledge, the current study covers a longer time period than any previously published investigation of BSIs in patients admitted to a general pediatric hospital, and is, therefore, more robust against short-term effects. Over the combined 20-year study period, the rate of positive blood cultures dropped from 4.5% in the first decade to 2.0% in the following period. The corresponding BSI incidence rates decreased from 331 to 200 per 100,000 for children below 18 years of age. Unfortunately, the incidence rates for children with a similar geographic background do not

Table 7 Comparison of the antibiotic susceptibility pattern of bloodstream isolates from hospitalized pediatric patients during the time period January 1985 to December 1995 vs. July 1997 to June 2006

Antibiotic resistance	Total and percentage, 1985–1995 (n=1,037)	Total and percentage, 1997–2006 (n=609)	p (Fisher's exact test) and 95%-CI
Methicillin-resistant *S. aureus*	0 of 170	0 of 71	n.d.
Methicillin-resistant CoNS	n.a.	108 of 172 (62.8%)	n.d.
Vancomycin-resistant enterococci	0 of 34	0 of 50	n.d.
Penicillin-resistant			
S. pneumoniae	0 of 88	0 of 55	n.s. (no 95%-CI)
S. viridans	8 of 112 (7.1%)	8 of 54 (14.8%)	n.s. (0.823–5.230)
N. meningitidis	n.a.	0 of 3	n.d.
Ampicillin-resistant enterococci	2 of 21 (9.5%)	17 of 49 (34.7%)	0.040 (0.923–14.39)
Aminoglycoside-resistant enterococci	12 of 20 (60.0%)	22 of 49 (44.9%)	n.s. (0.466–1.202)
Ampicillin-resistant *Enterobacteriaceae*	64 of 228 (28.1%)	74 of 120 (61.7%)	<0.0001 (1.709–2.824)
Fluoroquinolone-resistant *Enterobacteriaceae*	1 of 228 (0.4%)	5 of 120 (4.2%)	0.020 (1.122–80.43)
Carbapenem-resistant *Enterobacteriaceae*	1 of 228 (0.4%)	0 of 120	n.s. (no 95%-CI)
Extended spectrum beta-lactamase producing *Enterobacteriaceae*	n.a.	13 of 120 (10.8%)	n.d.

n.d.=not determined; CoNS=coagulase-negative staphylococci; n.a.=not available; n.s.=non-significant

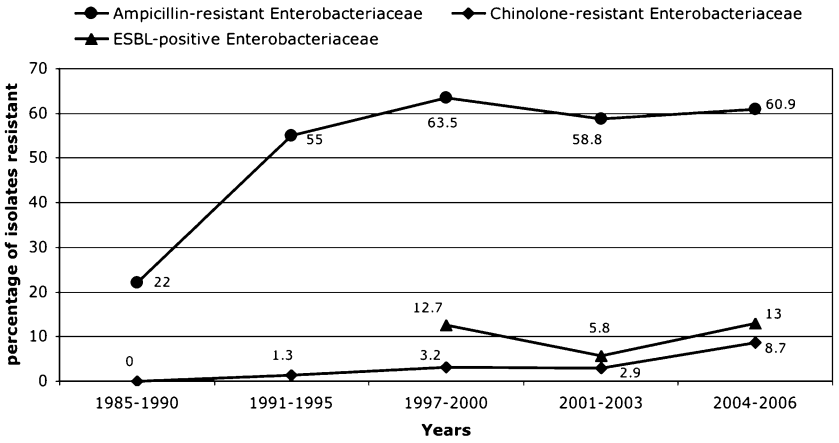

Fig. 2 Rates of antimicrobial resistance among gram-negative isolates recovered in 338 hospitalized pediatric patients with *Enterobacteriaceae* BSIs over the time period January 1985 to December 1995 vs. July 1997 to June 2006

exist in the literature. Most published BSI incidence rates are based on the number of hospital admissions. However, because we did not have exact information regarding the total hospital admissions during period A, BSI incidence rates—based on annual admission rates—could not be calculated for the whole study period, but, rather, only for period B. During period B, the BSI incidence was 9.6 per 1,000 admissions (data not shown), a rate which is lower than the reported 14.7 per 1,000 admissions in a cohort from Birmingham, UK [15]. Based on a documented increase in admissions to our hospital over the 20-year period, we conclude that the BSI incidence rates have decreased over the 20-year period, but have, nevertheless, remained stable over the last 10 years. For newborn infants, we were able to calculate the BSI incidence as 30.1 per 1,000 hospital admissions for period B. This rate is lower than the incidence of nosocomial BSI reported from a prospective multi-center NICU study performed [6], but is similar to the rate shown from a two-year retrospective single-center PICU study [2]. In general, the BSI incidence is considered to have increased over time in pediatric [15, 33] as well as in adult patients [3, 25].

In our study, the decrease in the rate of positive blood cultures is most likely explained by a change in the patient spectrum. During the second study period, an increasing number of patients with malignancies were treated. Between 2002 and 2003, for example, a 33% increase in the admissions of patients with malignancies was noted, and most of these patients received central venous catheters. Whenever these patients developed fever, a set of blood cultures (aerobic and anaerobic) per catheter lumen was drawn. Thus, a fever episode resulted in the inoculation of four blood cultures. Consequently, the total number of blood cultures, and, therefore, the denominator for the calculation of BSI rates, increased, which may have resulted in a documentation of lower BSI rates.

The proportion of gram-positive vs. gram-negative pathogens recovered from blood cultures remained stable over time. Approximately 70% of BSIs were caused by gram-positive organisms, while gram-negative pathogens accounted for 29%. These rates are in accordance with findings from other studies with a similar pediatric population [15, 16, 24, 29, 33]. The rate of cultured anaerobic bacteria and yeasts was also similar to that found by other European investigators [15, 24]. In contrast, fungal growth has been more commonly detected in blood cultures in the United States (e.g., 11% [32]). The low yield of positive fungal cultures in our cohort is most likely explained by our hospital having used standard bacterial and fungal culture systems which had not been specifically adjusted for the improved detection of fungal growth [26].

In our study, among specific pathogens, coagulase-negative staphylococci and enterococci became more prevalent over time—a trend which has also been observed by other investigators [5, 15, 33]. This increase in CoNS and enterococcal BSIs was found mainly in patients with malignancies, immunodeficiency syndromes, and in neonatal patients. Both organisms are considered as opportunistic pathogens with increased virulence in immunocompromised patients. This increase in the rate of CoNS and enterococcal BSI was also associated with a rise in the hospital's use of central venous catheters (CVCs) for patients with severe underlying conditions. From 1985 to 1995, only 4.4% of patients had a CVC in place, whereas in 1997 to 2006, 64.9% of patients had a central line. Comparable high rates of CVC use in pediatric patients have been previously reported [15, 33]. Both CoNS and enterococci produce biofilm after the colonization of plastic material. By dispersal, they then cause BSIs [19]. This phenomenon provides a probable explanation for why CVCs constitute important risk factors for the development of BSIs [6, 18, 29, 30, 33].

The decline of *H. influenzae* BSIs began in 1990, coinciding with the introduction of a general Hib vaccination in Germany in that year (data not shown). Similar effects have been found in other countries where a general

Hib vaccination was introduced. The decline of meningococcal BSI cannot be attributed to the introduction of the Meningococcus C conjugate vaccine, however, because this vaccine—together with the pneumococcal conjugate vaccine—was not incorporated into the general vaccination plan in Germany until mid-2006, which was before the study period ended. The decline in GBS BSI rates started in 1994, coinciding with the introduction of a selective intrapartal chemoprophylaxis strategy in Germany during that same year (data not shown).

Despite the increased number with severe underlying diseases in our patient population, crude mortality significantly decreased over time. In the first period, 5.2% of patients died of BSI, whereas in the second period, the rate was 2.6%. This latter rate is similar to that reported by Gray [15]. A trend in reduced crude mortality has been documented in an adult cohort with BSI [25], but has not been previously reported in a pediatric population. Importantly, although a reduction in mortality over time occurred in BSIs with both gram-positive and gram-negative bacteria, this trend was more marked in the case of gram-negative bacteria. We noticed a decline in mortality related to BSIs caused by various pathogens (*S. agalactiae*, *S. pyogenes*, *E. coli*, *Pseudomonas* spp., *Salmonella* spp., and other *Enterobacteriaceae*), but this tendency did not achieve statistical significance. As a trend noted among so many diverse pathogens, this most likely cannot be attributed to pathogen-specific aspects, but, rather, to broader changes, such as the improvement of supportive treatment.

The development of antibiotic resistance in our study cohort remains favorable in comparison to other countries [14, 15, 22, 24, 33]. We found no vancomycin- or high-level aminoglycoside-resistant enterococci, no penicillin-resistant pneumococci, and no methicillin-resistant *S. aureus*. The rate of aminopenicillin-resistant enterococci increased over time, but the more recent figure of 34.7% is still lower than the rates reported in other studies [10, 15]. The percentage of aminopenicillin- and fluoroquinolone-resistant *Enterobacteriaceae* also rose in our study. The more recent rates of 61.7% aminopenicillin-resistant *Enterobacteriaceae* and 4.2% fluoroquinolone-resistant *Enterobacteriaceae* remain lower than the rates published by Gray [15]. The overall susceptibility of bacteria to antibiotics at the Freiburg hospital recalls the situation of other health care settings in the early 1990s. At that time, the development of antibiotic resistance among *Enterobacteriaceae* preceded the development of antibiotic resistance among gram-positive pathogens [9]. The most important reason for the favorable antibiotic resistance pattern in Freiburg is most likely related to a policy restricting the use of antibiotics overall. This policy is supported by a well-established infectious disease consultation system, as well as by ongoing topical training for the medical staff. The resistance situation in Freiburg is similar to that found in countries with known restrictive antibiotic use policies, such as the Netherlands or Scandinavian countries [13]. It is Freiburg's hospital policy to particularly restrict the use of broad-spectrum cephalosporins, carbapenems, glycopeptides, and fluoroquinolones. Standard antibiotic treatment for bacteremias is still a combination of an ureidopenicillin, together with aminoglycosides. Due to the close collaboration between the Freiburg hospital and local physicians, restrictive antibiotic use has also been adopted as a common policy by the Freiburg area pediatricians in private practice.

The perspective gained from our experience now affords us the opportunity to make more prudent decisions regarding antibiotic use, and, hopefully, will allow us to avoid the kind of mistakes that have led to multidrug-resistant pathogens in the past.

In conclusion, our study provides insight into the influence of medical practices on invasive bacterial and fungal infections. Further, our data offer guidance for adjusting empirical antimicrobial treatment and implementing more effective infection control procedures in hospital settings.

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