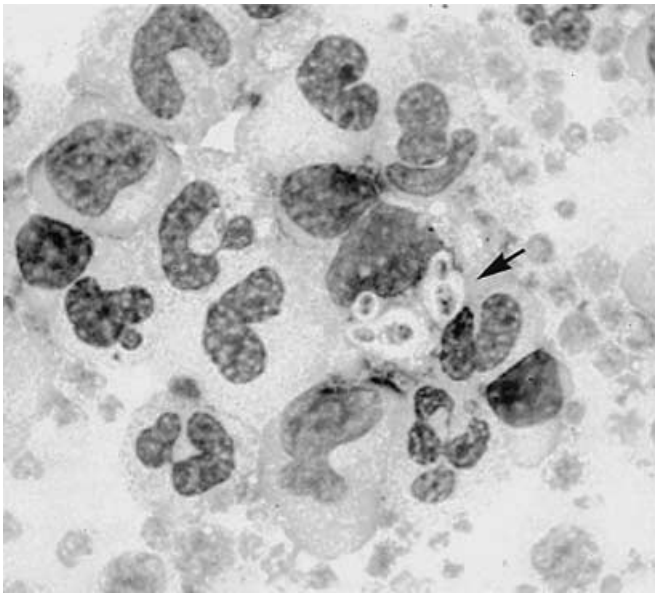

Figure 1 Peripheral blood buffy-coat smear from an AIDS patient, demonstrating encapsulated yeast forms of *Cryptococcus neoformans* (arrow) (magnification × 1000)

This result was confirmed by a serum cryptococcal antigen titer of 1:3,400. In addition, bronchoalveolar lavage, blood, and cerebrospinal fluid cultures subsequently all grew *Cryptococcus neoformans*. A serum test for *Histoplasma* antigen was negative. Following 2 weeks of treatment with intravenous amphotericin B, the patient's clinical condition improved, and he was discharged to complete therapy for cryptococcosis with fluconazole.

This case emphasizes the importance of considering disseminated fungal infection in critically ill AIDS patients. A peripheral blood buffy-coat smear examination is a simple, rapid test that can assist in the diagnosis of disseminated fungal infections in patients with AIDS.

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Antimicrobial Susceptibility Among Nosocomial Pathogens Isolated in Intensive Care Units in Germany

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More than 20% of all patients admitted to European intensive care units (ICUs) develop a nosocomial infection [1, 2]. The risk and site of infection may vary according to the type of ICU, but the frequency with which specific pathogens are isolated varies by infection site [3–5]. Nosocomial infections are life-threatening complications, especially in ICU patients, and the increasing incidence of infections caused by antibiotic-resistant pathogens contributes to the seriousness of this problem. Knowledge of current trends in the development of resistance against available and new antimicrobial agents is an important prerequisite for the effective administration of empirical antimicrobial therapy in this hospital setting. In view of the increasing frequency of antimicrobial resistance seen in ICUs, it is imperative that new antibiotics be developed. In this study, various new antimicrobial agents were tested against commonly isolated pathogens causing nosocomial infections in ICUs throughout Germany.

Between September 1996 and October 1997, bacterial isolates causing nosocomial infections throughout Germany were supplied by 19 laboratories. More than 90% of the bacteria studied were obtained from 12 laboratories in nine different cities. Among the microorganisms investigated, 53% were isolated from tracheal secretions, 22% from wounds, 10% each from

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Table 1 Comparative in vitro activity of different antimicrobial agents against gram-positive bacteria

Organism (no. tested)	MIC (μg/ml)	VAN	TCP	LY-333328	ROX	AZI	CLA	PNU-100766	RP-59500
MSSA (52)	Range	0.5–1	0.06–0.5	0.25–2	0.06–>64	0.06–>64	0.06–>64	0.25–1	0.03–0.25
	MIC50	0.5	0.25	1	0.25	0.25	0.125	0.5	0.125
	MIC90	0.5	0.25	2	0.5	0.5	0.25	0.5	0.25
MRSA (53)	Range	0.25–1	0.06–1	0.5–2	–	–	–	0.25–1	0.03–0.25
	MIC50	0.5	0.25	1	–	–	–	1	0.125
	MIC90	0.5	0.5	2	–	–	–	1	0.25
CNS (57)	Range	0.5–4	0.06–8	0.125–1	0.03–>64	0.03–>64	0.03–>64	0.25–0.5	0.03–0.5
	MIC50	1	2	1	>64	16	64	0.25	0.03
	MIC90	1	8	1	>64	>64	>64	0.5	0.125
E. faecium (53)	Range	0.125–64	0.06–64	0.125–1	0.06–>64	1–>64	0.03–>64	0.25–1	0.03–16
	MIC50	0.5	0.125	0.5	>64	>64	>64	1	0.25
	MIC90	64	16	0.5	>64	>64	>64	1	2
E. faecalis (53)	Range	0.25–64	0.06–8	0.06–4	0.25–>64	0.5–>64	0.06–>64	0.25–1	0.5–16
	MIC50	1	0.06	1	16	16	4	1	4
	MIC90	2	0.125	2	>64	>64	>64	1	8

MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; CNS, coagulase-negative staphylococci; VAN, vancomycin; TCP, teicoplanin; ROX, roxithromycin; AZI, azithromycin; CLA, clarithromycin

Table 2 Comparative in vitro activity of different antimicrobial agents against gram-negative bacteria

Organism (no. tested)	MIC (μg/ml)	CFP	CFT	IMP	MRP	MK-826	CIP	OFX	LFX	CFX	TFX
E. coli (53)	Range	0.03–64	0.03–64	0.03–0.5	0.03–0.125	0.03–0.25	0.015–32	0.015–32	0.015–16	0.015–2	0.015–8
	MIC50	0.03	0.06	0.06	0.03	0.03	0.015	0.03	0.03	0.015	0.06
	MIC90	0.06	2	0.06	0.03	0.03	0.125	0.5	0.5	0.06	0.25
E. cloacae (53)	Range	0.03–4	0.06–64	0.06–1	0.03–0.5	0.03–2	0.015–0.25	0.015–0.5	0.03–0.25	0.015–0.125	0.06–0.5
	MIC50	0.06	1	0.25	0.06	0.125	0.015	0.06	0.06	0.015	0.06
	MIC90	1	64	0.5	0.125	0.5	0.03	0.125	0.125	0.03	0.125
K. pneumoniae (54)	Range	0.03–1	0.03–64	0.031–2	0.03	0.03–0.06	0.015–32	0.03–32	0.03–16	0.015–8	0.06–8
	MIC50	0.03	0.06	0.06	0.03	0.03	0.015	0.06	0.06	0.03	0.06
	MIC90	0.125	0.5	0.125	0.03	0.03	0.25	1	0.5	0.125	1
C. freundii (52)	Range	0.03–1	0.06–64	0.06–1	0.03–0.125	0.03–0.25	0.015–2	0.015–4	0.015–2	0.015–0.5	0.06–4
	MIC50	0.03	0.25	0.25	0.03	0.03	0.015	0.06	0.03	0.015	0.06
	MIC90	1	64	0.5	0.06	0.25	0.125	0.5	0.25	0.06	2
P. vulgaris (52)	Range	0.03–4	0.03–8	0.03–2	0.03–0.125	0.03	0.015–0.06	0.03–0.125	0.03–0.125	0.015–0.03	0.06–0.5
	MIC50	0.06	0.03	0.5	0.06	0.03	0.015	0.06	0.03	0.03	0.25
	MIC90	0.125	0.06	1	0.125	0.03	0.03	0.06	0.06	0.03	0.5
S. marcescens (52)	Range	0.03–2	0.06–16	0.125–64	0.03–0.125	0.03–0.5	0.03–4	0.06–8	0.06–4	0.03–1	0.125–8
	MIC50	0.06	0.125	0.25	0.06	0.03	0.06	0.25	0.125	0.03	0.25
	MIC90	0.25	0.5	0.5	0.06	0.125	0.5	2	1	0.25	2
M. morganii (50)	Range	0.03–0.125	0.03–32	0.5–64	0.06–0.25	0.03–0.06	0.015–0.25	0.015–0.125	0.015–0.125	0.015–0.03	0.06–0.5
	MIC50	0.03	0.5	1	0.125	0.03	0.015	0.06	0.03	0.015	0.125
	MIC90	0.125	8	2	0.125	0.03	0.06	0.125	0.06	0.03	0.25
P. aeruginosa (53)	Range	0.5–32	0.5–64	0.03–16	0.06–64	0.25–32	0.06–32	0.25–32	0.25–16	0.06–16	0.25–8
	MIC50	2	1	0.5	0.5	8	0.125	1	0.5	0.25	0.5
	MIC90	8	4	4	4	32	4	16	8	8	8
A. baumannii (53)	Range	0.5–64	0.5–64	0.03–4	0.125–2	1–16	0.06–32	0.06–32	0.06–16	0.03–16	0.03–8
	MIC50	2	4	0.125	0.5	2	0.25	0.25	0.25	0.06	0.06
	MIC90	8	32	0.25	1	4	32	16	8	4	4
S. maltophilia (53)	Range	2–64	0.5–64	0.5–64	0.25–64	1–32	0.5–16	0.5–16	0.25–8	0.06–1	0.125–4
	MIC50	32	4	64	64	32	2	2	1	0.25	0.5
	MIC90	64	32	64	64	32	4	4	2	0.5	2

CFP, cefepime; CFT, ceftazidime; IMP, imipenem; MRP, meropenem; CIP, ciprofloxacin; OFX, ofloxacin; LFX, levofloxacin; CFX, clinafloxacin; TFX, trovafloxacin

urine and blood samples and 7% from other specimens (puncture fluids and biopsies). All of the microorganisms were identified using the MicroScan Aerobic Gram-Positive and Gram-Negative Bacilli Biotype Codebook (DADE Diagnostika, Germany). A total of 268 gram-positive and 525 gram-negative microorganisms were tested for susceptibility to novel antimicrobial agents (Tables 1 and 2).

The minimum inhibitory concentrations (MICs) were determined according to the guidelines of the National Committee for Clinical Laboratory Standards

Table 3 Percentage of susceptible *Enterobacteriaceae* isolates

Organism (no. tested)	AMX/ CLA	PIP	PIP/ TZB	CRX	CFT	CFP	IMP	MRP	CIP	LFX	TOB	NET
E. coli (53)	89	66	96	89	94	98	100	100	91	96	96	98
E. cloacae (54)	4	65	78	26	69	100	100	100	100	100	96	98
C. freundii (52)	0	60	73	56	67	100	100	100	98	100	98	98
K. pneumoniae (52)	94	85	96	88	94	100	100	100	94	94	96	96
P. vulgaris (52)	98	75	100	2	100	100	100	100	100	100	100	100
S. marcescens (52)	2	79	92	0	96	100	98	100	94	94	100	100
M. morganii (50)	0	84	100	4	96	100	98	100	100	100	84	84

AMX/CLA, amoxicillin/clavulanate; PIP, piperacillin; TZB, tazobactam; CRX, cefuroxime; CFT, ceftazidime; CFP, cefepime; IMP, imipenem; MRP, meropenem; CIP, ciprofloxacin; LFX, levofloxacin; TOB, tobramycin; NET, netilmicin

Table 4 Percentage of susceptible isolates of gram-negative nonfermenting bacteria

Organism (no. tested)	PIP	PIP/TZB	CFT	CFP	IMP	MRP	CIP	LFX	TOB	NET
P. aeruginosa (53)	89	91	94	92	94	91	81	80	89	85
A. baumannii (53)	77	89	87	91	100	100	74	77	81	74
S. maltophilia (52)	8	13	64	15	4	4	43	91	6	8

PIP, piperacillin; TZB, tazobactam; CFT, ceftazidime; CFP, cefepime; IMP, imipenem; MRP, meropenem; CIP, ciprofloxacin; LFX, levofloxacin; TOB, tobramycin; NET, netilmicin

(NCCLS) [6] using a microdilution broth technique (Micronaut system; Merlin, Germany). The MIC90 and MIC50 values were defined as the MICs at which 90% and 50% of the strains, respectively, were inhibited. The percentage of susceptible strains was also calculated according to the NCCLS guidelines.

All of the staphylococcal isolates examined were susceptible to vancomycin, teicoplanin and LY-333328; they were also highly susceptible to PNU-100766 (linezolid) and RP-59500 (quinupristin/dalfopristin) (Table 1). Methicillin-susceptible *Staphylococcus aureus* isolates were susceptible to the macrolide antibiotics roxithromycin, azithromycin and clarithromycin, with MIC90 values of 0.5 and 0.25 μg/ml, respectively; coagulase-negative staphylococci, however, were resistant with MIC90 values of >64 μg/ml. Macrolide antibiotics also had low in vitro activity against enterococcal isolates. To some extent, *Enterococcus faecium* showed resistance to the two glycopeptide antibiotics tested, vancomycin and teicoplanin, with MIC90 values of 64 and 16 μg/ml, respectively. *Enterococcus faecalis* was noticeably susceptible to teicoplanin, and both *Enterococcus faecalis* and *Enterococcus faecium* isolates were susceptible to PNU-100766.

All of the *Enterobacteriaceae* isolates examined were susceptible to imipenem, meropenem and MK-826, with the exception of two imipenem-resistant isolates, one of *Morganella morganii* and one of *Serratia marcescens* (Table 2). Eighty-nine percent of the *Escherichia coli* isolates were susceptible to amoxicillin/clavulanate and cefuroxime (Table 3). With the exception of one *Escherichia coli* isolate, all of the *Enterobacteriaceae* isolates were susceptible to the fourth-generation cephalosporin cefepime. Extended-spectrum beta-lactamase activity was found in none of the *Escherichia coli* isolates and in three of the *Klebsiella pneumoniae* isolates tested. Susceptibility to ceftazidime was found in only 69% of the *Enterobacter cloacae* and 67% of the *Citrobacter freundii* isolates; this was in contrast with *Proteus vulgaris*, *Serratia marcescens* and *Morganella morganii*, all of which were highly susceptible. The *Enterobacter cloacae* isolates were fully susceptible to all of the quinolones tested.

The number of quinolone-resistant isolates among nonfermenting bacteria was strikingly high (Table 4). A considerable number of *Pseudomonas aeruginosa* isolates showed no susceptibility to the fluoroquinolones. However, the majority of *Stenotrophomonas maltophilia* isolates (91%) were susceptible to levofloxacin. *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii* were 94%, 64% and 87% susceptible to ceftazidime, respectively. Eighty-nine percent of the *Pseudomonas aeruginosa* isolates were susceptible to piperacillin, and more than 90% of them were susceptible to the carbapenems. Although recent reports of *Acinetobacter baumannii* outbreaks involving strains that are resistant to imipenem, ceftazidime and other routinely tested antibiotics are alarming [7, 8], the *Acinetobacter baumannii* strains isolated in this study were highly susceptible to imipenem, meropenem, MK-826, clinafloxacin and trovafloxacin, but not to ciprofloxacin and ceftazidime.

In summary, PNU-100766, RP-59500 and LY-333328 displayed the highest level of antimicrobial efficacy against gram-positive microorganisms causing nosocomial infections in ICU patients in Germany, whereas clinafloxacin, meropenem and carbapenem MK-826 displayed the highest level of antimicrobial efficacy against gram-negative pathogens. These new drugs show great promise, especially for use in empirical therapy, but the danger of organisms developing resistance to them should serve as a strong incentive for their responsible and judicious use.

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Comparison of the E Test and Agar Dilution Methods for Susceptibility Testing of *Arcanobacterium haemolyticum*

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The clinical importance of *Arcanobacterium haemolyticum,* an aerobic, fastidious, slowly growing, gram-positive rod, has been recognized since the mid-1940s [1]. Recently, this organism has been isolated not only from patients with tonsillitis but also from patients with other infections [1]. The susceptibility testing of individual clinical isolates of *Arcanobacterium haemolyticum* is problematic because no standardized routine disk diffusion method is currently available. Although the E test (AB Biodisk, Sweden) has been previously used in minimum inhibitory concentration (MIC) determinations for *Arcanobacterium haemolyticum*, this method has never been validated [2, 3]. In the present study, the MIC of 12 antimicrobial agents for 70 clinical isolates of *Arcanobacterium haemolyticum* were determined by the E test and by the National Committee for Clinical Laboratory Standards (NCCLS) agar dilution method [4, 5].

Of the 70 *Arcanobacterium haemolyticum* strains studied, 66 were collected by clinical microbiology laboratories in Finland between 1989 and 1999. Of these isolates, 29 were from wound cultures, 30 from throat, one from a maxillary sinus, and six from blood. Three blood isolates were from the Culture Collection of the University of Gothenburg, Gothenburg, Sweden (accession numbers CCUG 30325, CCUG 38122, and CCUG 39796). One blood isolate was kindly provided by Dr. R. Skov, Statens Serum Institut, Copenhagen, Denmark. The following antimicrobial agents were tested: benzylpenicillin (Orion Pharmaceuticals, Finland); cefotaxime (Hoechst Marion Roussel, Sweden); cefuroxime (Glaxo Wellcome, UK); ciprofloxacin (Bayer, Germany); clindamycin (Sigma Chemicals, USA); erythromycin (Sigma); imipenem (Merck Sharp & Dohme, Netherlands); levofloxacin (Hoechst Marion Roussel); ofloxacin (Hoechst Marion Roussel); rifampin (Sigma); tetracycline (Sigma), and vancomycin (Dumex-Alpharma, Denmark).

The strains were cultured on horse-blood agar plates for 48 h at 35 °C in a humidified atmosphere of 5% CO_2 in air. Identification was performed as previously described [6] using Gram stain, catalase, reverse CAMP, DNase, and α-mannosidase tests. API Coryne (bioMérieux, France) biochemical profiles were also obtained. *Arcanobacterium haemolyticum* ATCC 9345 was used as a control strain.

The agar dilution MIC determinations were performed according to NCCLS recommendations [4, 5] on Mueller-Hinton II agar (BBL Microbiology Systems, USA) supplemented with 5% defibrinated sheep blood. Bacteria harvested from horse-blood agar plates were suspended in 0.9% saline and adjusted to a density of approximately 0.5 on the McFarland turbidity scale. The suspension was further diluted 1 to 10 in saline. With a multipoint inoculator (Mast Laboratories, UK), a final inoculum of approximately 10^4 cfu was delivered onto Mueller-Hinton plates. The

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