Bakterien: Resistenz und Empfindlichkeitsprüfung

Resistenz bei Bakterien

Wirkstoff	Testmethodik	Standard	Spezies / Bemerkung	Abstract
Enrofloxacin	MBD	k.A.	*Mycoplasma gallisepticum*	C1-1963
Erythromycin	k. A.	k.A.	*Str. pneumoniae* – erhöhte Resistenz nach Vakzinierung	G1-2104
Imipenem	MBD	k.A.	*Ps. aeruginosa;* GN *Klebsiella pneumoniae*	C1-1952; C2-202; C2-209; C2-3896; K-3506
Linezolid			MRSA	C2-1087a
Tetracyclin	ET		MRSA	C1-3713
Tetracyclin	ET	--	Bifidobakterien	C1-3908
Tigecyclin	AGD; ET	k.A.	*A. baumannii*	C1-1036
Trimethoprim	Gen-Detektion	--	MRSA	C1-173
Vancomycin		k.A.	*Enterococcus faecium*	C2-2003

MBD = Mikrobouillondilution; AGF = Agardiffusion; ET = Etest; AGD = Agardilution; k.A. = keine Angabe
GN = Gram-negative Bakterien

Empfindlichkeitsprüfung: Bakterien

Wirkstoff	Testmethodik	Standard	Spezies / Bemerkung	Abstract
Ceftarolin	MBD	CLSI	Gram(+/-) Bakterien	D-2232
Daptomycin	MBD	CLSI	MRSA	C1-137
Diverse	MBD	CLSI	*Nocardia* spp.	C1-3854
Oritavancin	MBD	CLSI	MRSA	C1-3842
Polymyxine	AGD, AGF, ET	CLSI	*Pseudomonas aeruginosa*	D-2236
Teicoplanin	MBD vs. MicroScan	CLSI	85-99% Korrelation der Methoden	D-2240
MICE	Teststreifenevaluierung	ISO	Oxoid-Teststreifen + ISO MBD	D-2243

MBD = Mikrobouillondilution; AGF = Agardiffusion; ET = Etest; AGD = Agardilution;
GP = Gram-positiv; GN = Gram-negativ

C1-137

Comparative Activity of Daptomycin Against Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus*

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Background: Daptomycin is a novel lipopeptide agent with rapid *in vitro* bactericidal activity against gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA). The objective of this study was to determine the *in vitro* activity of daptomycin and other agents against MRSA isolates.
Methods: We tested 755 non-duplicate clinical isolates collected as part of a multicenter surveillance of antimicrobial resistance, the VIRA study, from 40 medical centers throughout Spain between 2001 and 2006. Comparator agents included vancomycin, teicoplanin, linezolid, quinupristin-dalfopristin, trimethoprim-sulfamethoxazole, tetracycline, clindamycin, erythromycin, ciprofloxacin, rifampin, gentamicin, and chloramphenicol. MICs were determined according to the CLSI guidelines using the broth microdilution method. The test medium was Mueller-Hinton broth adjusted to contain physiological levels of calcium (50 µg/ml) when testing daptomycin.
Results: The majority of isolates were resistant to ciprofloxacin (96%) and to erythromycin (79.7%). Quinupristin-dalfopristin showed good activity (0.5% resistance). Rifampin was active against all but 16 isolates. The resistance rate to gentamicin was 27.9%. We detected one isolate with a linezolid MIC of >8 µg/ml and another that exhibited intermediate susceptibility to vancomycin; both were susceptible to daptomycin (MICs of 0.5 and 1 µg/ml, respectively). Daptomycin yielded an MIC_{50}/MIC_{90} of 0.5/0.5 µg/ml, compared with 1/2 µg/ml for linezolid, vancomycin, and teicoplanin. Daptomycin MICs were in the range of £0.125-2 µg/ml. Only 1 isolate had a reproducible daptomycin MIC of 2 µg/ml.
Conclusions: Daptomycin was highly active against the MRSA isolates tested. The results of the present study indicate that this new agent represents a therapeutic option for infections caused by MRSA.

Tabelle

C1-173

Molecular Analysis of a Novel Trimethoprim Resistance Gene, dfrK, Identified in a Porcine Methicillin Resistant Staphylococcus aureus Isolate

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Background: During screening of methicillin resistant *Staphylococcus aureus* (MRSA) isolates from the BfT-GermVet monitoring study for the resistance genes present, a porcine MRSA strain of sequence type ST398 was shown to harbour the *mecA* gene as part of a type V SCC*mec* cassette and a plasmid-borne *tet*(L) gene in addition to a chromosomal *tet*(M) gene. This MRSA strain also exhibited trimethoprim (Tmp) resistance by a so far unknown gene. The aim of this study was to identify this Tmp resistance gene and to determine its location.
Methods: Plasmid isolation was done by alkaline lysis and transferred to *S. aureus* RN4220 by protoplast transformation using tetracycline or Tmp for selection. The transformed plasmid was mapped by restriction analysis. HindIII and BglII fragments were cloned into pBluescript II SK+ and used for sequence analysis.
Results: The tetracycline resistance gene *tet*(L) was located in this MRSA isolate on the 30-kb plasmid pSTS15 which also mediated Tmp resistance. Sequence analysis revealed a novel *dfr* gene, tentatively designated *dfrK*, 283 bp downstream of the *tet*(L) gene. The *dfrK* nucleotide sequence showed 86 % identity to the *dfrG* from *S. aureus* SAV0404. The DfrK protein consists of 163 amino acids (aa) and shows 90 % identity to the next closely related 165-aa DfrG protein. The *dfrK* flanking regions - about 300 bp upstream until the stop codon of *tet*(L) and about 600 bp downstream - did not show homology to sequences deposited in the databases. In contrast, the region upstream of the *tet*(L) gene showed homology to other *tet*(L)-carrying plasmids and included the plasmid replication gene *repU* and an insertion sequence of type IS*257*.
Conclusions: In this study, a novel Tmp resistance gene, *dfrK*, was identified on a *tet*(L)-carrying plasmid from a porcine MRSA isolate. The co-localization of *dfrK* and *tet*(L) on the same plasmid points towards co-selection of both resistance genes under a selective pressure imposed by the use of either Tmp or tetracyclines.

Tabelle

C1-1036

Differences in Expression of *adeABC* in Two Epidemic UK Clones of *Acinetobacter baumannii* in Relation to Tigecycline MICs

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Background: Two epidemic clones of *A. baumannii*, OXA-23 clone 1 and the South East (SE) clone, are major public health concerns in the UK; each has been found in >50 centres in London and south-east England. Both are multi-resistant, including to carbapenems. Tigecycline and polymyxins remain active against most isolates of these clones, but tigecycline MIC distributions differ significantly between them, with the modal MIC for the SE clone 4-fold higher than for OXA-23 clone 1. The RND efflux pump AdeABC affects tigecycline susceptibility in *A. baumannii* and we investigated its expression in multiple representatives of both clones.
Methods: Isolates were identified by PCR; MICs were determined by BSAC agar dilution and Etest on IsoSensitest agar. PFGE was used to assign clones. Expression of *adeABC* was examined by real-time reverse transcriptase (RT)-PCR with primers specific for *adeB*, and quantified relative to the RNA polymerase β subunit gene, *rpoB* (reference expression level **= 1.0).**
Results: Five isolates of each of OXA-23 clone 1 and the SE clone required tigecycline MICs of £0.25 and 1 mg/L, respectively. Real-time RT-PCR identified a mean 6-fold higher level of *adeABC* expression in the SE clone.
Conclusions: The widespread OXA-23 clone 1 and SE clone lineages display different tigecycline MIC distributions. Higher MICs for the SE clone correlate with higher expression of *adeABC*.

	adeABC* expression relative to *rpoB	
	OXA-23 clone 1	**SE clone**
Range	0.004 to 0.011	0.034 to 0.082
Lower 95% CI of mean	0.007	0.044
Upper 95% CI of mean	0.009	0.061

Tabelle

C1-1952

In Vitro Antimicrobial Activities of Sitafloxacin in Combination with Cefepime and Cefozopran Against Imipenem-Aesistant *Pseudomonas aeruginosa*

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Background: Imipenem-resistant *Pseudomonas aeruginosa* (IRPA) have become increasingly problematic in hospitals all over the world. Some IRPA strains are resistant to almost all clinically used antimicrobial agents. We presented Sitafloxacin (STFX) demonstrates stronger antimicrobial activity for IRPA than some other fluoroquinolones (47th ICAAC, Chicago, IL, 2007). In this paper, we present the in vitro activities of the combinations STFX and 4th generation cephalosporines, cefepime (CFPM) and cefozopran (CZOP).
Methods: 20 strains of metallo-beta-lactamase producing (MB-IRPA) and non producing IRPA (NMB-IRPA) were used for each experiment. The susceptibilities of IRPA to STFX, CFPM and CZOP were assessed using broth microdilution method. Studies of effects of two-drug combinations were performed by the checkerboard broth dilution and, for some strains, time-kill assay.
Results: STFX exhibited excellent activity for NMB-IRPA (MIC range = 0.016-4 mg/L / MIC_{50} = 0.125 mg/L / MIC_{90} = 1 mg/L) as compared with CFPM (2-64 / 16 / 32) and CZOP (1-128 / 16 / 32). For MB-IRPA the MICs of STFX were relatively low (0.5-32 / 4 / 16) while those of CFPM and CZOP were extremely high (>64). STFX and CFPM or CZOP combinations were more effective for some strains of IRPA than single challenges. MICs of CFPM and CZOP alone for NMB-IRPA became 2 to 3 tubes lower, CFPM (0.25-8 / 2 / 8) and CZOP (0.25-32 / 2 / 8), by the addition of 1/2 MICs of STFX.
Conclusions: MICs of STFX for most of tested IRPA were lower than the concentration compatible to clinical serum and tissue levels. STFX and 4th generation cephalosporines combinations showed synergic effects for some NMB-IRPA strains. These combinations may be useful in patients with infections caused by IRPA that are difficult to cure.

Tabelle

C1-1963

Characterization of Quinolone Resistance-Determination Regions in DNA Gyrase and Topoisomerase IV of *Mycoplasma gallisepticum* Clinical Isolates

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Background: For more than a decade enrofloxacin and other fluoroquinolones have been widely used in commercial poultry flocks. Recently, enrofloxacin-resistant clinical isolates of *M. gallisepticum* were reported by our group and others, but molecular characterization of chromosomal mutations in genes encoding enzymes targeted by the drug has not been carried out. In this study, the Quinolone Resistance-Determination Regions (QRDRs) of DNA Gyrase subunits GyrA and GyrB and Topoisomerase IV subunits ParC and ParE were characterized in 25 *M. gallisepticum* strains with different levels of susceptibility, isolated from commercial poultry flocks during 1997-2007.
Methods: Amino acid substitutions in the QRDRs were identified and compared in enrofloxacin-susceptible and resistant strains, as determined by the microbroth dilution method.
Results: Sequence analysis revealed that all ten enrofloxacin-resistant isolates harbored amino acid substitutions in the QRDRs of each of three genes: GyrA (87 Glu to Lys or, less frequently, 83 Ser to Ile), GyrB (437 Asp to Asn), and ParC (80 Ser to Leu) but not in ParE. Susceptible strains had amino acids substitutions in the QRDR of only one gene (usually GyrB), with a single exception containing substitutions in QRDRs of both GyrB and ParC. Some of these mutations and resulting amino acid substitutions have been described previously in fluoroquinolone-resistant mutants selected *in vitro*. Molecular typing by Randomly Amplified Polymorphic DNA (RAPD) analysis revealed three different major patterns, with the predominant type present in seven out of ten isolates. This RAPD pattern was previously identified in a susceptible strain isolated in 1997, suggesting that acquisition of resistance may have occurred by selection in flocks treated with fluoroquinolones.
Conclusions: This is the first molecular characterization of QRDRs in enrofloxacin-resistant clinical isolates of *M. gallisepticum*.

Tabelle

C1-3713

Minocycline, not Doxycycline, to Treat Tetracycline-Resistant Community-Acquired Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) Skin and Skin Structure Infections (SSSI)?

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Background: Doxycycline (Doxy) and minocycline (Mino) are often avoided in the treatment of tetracycline (Tet)-resistant CA-MRSA SSSI. We examined cross-resistance for tetracycline class antimicrobials among CA-MRSA.
Methods: Clinical MRSA isolates were genotyped and screened by PCR for *tetK* and *tetM* and the multi-drug resistant plasmid, pUSA03. MICs were determined by E-test. Isolates with *tetK* where incubated in sub-therapeutic concentrations of Doxy, Mino, tigecycline (Tige) and MIC testing was repeated.
Results: 60 (33%) of 183 MRSA isolates were Tet-resistant. 58 (97%) of the Tet-resistant isolates were USA300. 18 (31%) of the USA300 carried pUSA03. 52 (94.5%) had *tetK*, 3 (5.5%) had *tetM*, and 1 (1.8%) had both *tetK* and *tetM*. Isolates containing only *tetK* had mean Doxy, Mino, Tige MICs of 2.12 mcg/ml (range 1.5-4), 0.19 mcg/ml (0.094-0.25), and 0.16 mcg/ml (0.094-0.125) respectively. After incubation in Doxy (0.5 mcg/ml), mean Doxy MICs of *tetK* isolates increased to 8.72 mcg/ml (range 8-12; $p < 0.001$) (CLSI breakpoint for susceptibility £ 4 mcg/ml). Mino and Tige MICs were unchanged following incubation in Mino or Tige (both 0.01 mcg/ml). Isolates containing *tetM* had mean Doxy, Mino, Tige MICs of 29.01 mcg/ml (range 24-32), 18.31 mcg/ml (8-32), and 0.10 mcg/ml (0.094-0.125) respectively.
Conclusions: Tetracycline resistance among USA300 is due primarily to *tetK*. There is cross-resistance between Tet and Doxy, but not between Tet and Mino. Mino may be the preferred tetracycline for the treatment of SSSI due to Tet-resistant CA-MRSA.

Tabelle

C1-3842

In Vitro Activity of Cethromycin (CER) Against Toxigenic *Clostridium difficile* Clinical

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Background: *C. difficile* is the major cause of antibiotic-associated diarrhea. CER, is a novel ketolide antibiotic with activity against gram-positive bacteria resistant to penicillin, macrolides and quinolones, and is in development for treatment of community acquired pneumonia. Ketolide antibiotics are less often associated with alteration of the intestinal microflora or with *C. difficile* colonization. With only vancomycin (VAN) and metronidazole (MTZ) as first line therapy, the search for new agents with activity against *C. difficile* is warranted. We investigated the *in vitro* activity of CER against *C. difficile* isolates.
Methods: 110 unique toxigenic *C. difficile* isolates of differing REA type known to have caused CDI were utilized for susceptibility testing. Inoculum preparation and agar dilution were performed according to the CLSI method for anaerobes (M11-A7). ATCC 700057 strain was included as the control and CER was assayed alongside VAN and MTZ.
Results: Cethromycin demonstrated *in vitro* activity with a geometric mean MIC and MIC_{50} in between those of MTZ and VAN. 80% of the tested isolates had cethromycin MICs of 0.03 - 4 µg/mL with the MIC_{90} being 128 µg/mL.
Conclusions: CER had good *in vitro* activity against a majority of *C. difficile* isolates tested. If confirmed in clinical trials, this activity, combined with the preservation of normal intestinal flora of ketolide antibiotics including CER, may lessen the likelihood of developing CDI while being treated with CER and reduce recurrence of CDI if it is treated with CER compared with other agents.

	MIC (µg/mL)			
	Range	**Geometric Mean**	**50%**	**90%**
CER	0.03 - 128	0.527	0.125	128
VAN	0.5 - 4	1.045	1	2
MTZ	0.125 - 2	0.248	0.25	0.25

Tabelle

C1-3854

Antibiotic Sensitivity of *Nocardia* Species in Argentina

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Background: *Nocardia spp.* are agents of systemic, respiratory and skin infections particularly in immunocompromised.Therapeutic efficacy in patients may depend on species identity and on in vitro susceptibility studies.The aim of this study was to assess the species distribution of Nocardia isolates in Argentina and to investigate whether the different species identified differed or antimicrobial susceptibility.
Methods: Between 01/01/2000 and 30/3/2008, we considered 56 isolated of *Nocardia species* from 33 sputum, 11 skins and soft tissue, 4 blood culture, 3 pleural fluid, 3 ganglionar biopsy, 1 bone and 1 LCR. The strains were identified by conventional biochemical tests: 26 *N. asteroides complex*, 9 *N. farcinica*, 9 *N. nova*, 8 *N. brasiliensis*, 2 *N. pseudobrasiliensis, 1 N. cyriacigeorgica* and *1 N. abscessus*. MIC were determine by E Test for: trimethoprim-sulfamethoxazole (TMP/SMX), cefotaxime (CTX), ciprofloxacin (CIP), imipenem (IMP), clarithromycin (CLA), amikacin (AK), minocycline (MC) y amoxicillin-clavulanic acid (AMC), in Müeller Hinton agar 0.5 de McFarland, incubated a 37+/-2º C for 48-72 Hs. Result were interpreted with CLSI M24-A.
Results: Table Show Range, MIC50 and MIC90.
Conclusions: The most active drugs were AK (100%) and MC (98.2%). The overall sensitivity for TMP/SMX was 78.5%, being more active against *N. brasiliensis*, *N. pseudobrasiliensis* and *N. nova. Same activity was detected by IMP.* CIP, CLA and CTX showed less activity, with sensitivity 69.8%, 62.5% and 58.9%, respectively. AMC reveal high activity with all the species, 83.9% except *N. nova* ($CIM_{90} \geq 64$) and *N. asteroides complex* ($CIM_{90} \geq 256$). *N. abscessus* was sensitive for the all drug tested. *N. cyriacigeorgica was* only resistant to CIP.

ATB, Range, MIC50 and 90 (ug/ml)

TMP/SMX	0.002/32	0.19	>32
CTX	0.023/256	4	>256
CIP	0.006/32	1	>32
IMI	0.008/32	0.75	>32
CLA	0.004/256	2	>256
AK	0.016/2	0.25	1
MIN	0.016/256	0.38	4
AMC	0.016/256	2	64

Tabelle

C2-202

Are Isolates from Patients who Acquire Colonization with Imipenem-Resistant *Pseudomonas aeruginosa* and had a Previous Susceptible Strain Clonal*?*

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Background: The incidence of imipenem- resistant *Pseudomonas aeruginosa* (IRPA) is increasing. The aim of this study was to study the molecular epidemiology of patients who had a previous surveillance culture that was imipenem-sensitive *P. aeruginosa* (ISPA) and acquired IRPA while in the ICU. To our knowledge, this has not been done previously and not for a large number of patients.
Methods: We performed a prospective cohort study of patients admitted between September 1, 2001 and September 1, 2006 to the medical and surgical ICUs at the University of Maryland Medical Center. Patients had peri-anal cultures on admission, weekly and upon discharge. Within this cohort we analyzed the subset of patients who had a previous surveillance culture with an ISPA and subsequently acquired IRPA while in the ICU. We used pulsed-field gel electrophoresis (PFGE) to determine the strain relatedness of both the ISPA and IRPA strains.
Results: Out of 156 patients that acquired an IRPA only 43 patients were colonized with an ISPA on a prior culture. Of these 43 patients, 33 (77%) had ISPA and IRPA isolates with identical pulsed-field types and 10 (23%) had strains with different pulsed-field types.
Conclusions: The majority (72%) of ICU patients who acquire IRPA did not have prior colonization by ISPA. Among the 28% who were initially colonized with ISPA, 77% had an IRPA that was genetically identical to the initial isolate. We hypothesize that these patients are more likely to have acquired the resistant organism from the patient's endogenous flora possibly by antibiotic selection.

Tabelle

C2-209

Impact of Ertapenem Use on *Pseudomonas aeruginosa* Susceptibility to Imipenem

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Background: Overuse of group 2 carbapenems may impact *P. aeruginosa* resistance. We evaluated the effect of replacing imipenem with the group 1 carbapenem ertapenem in patients who did not require *P. aeruginosa* coverage.
Methods: Our tertiary-level 200-bed hospital mandated use of ertapenem for infections caused by ESBL-producing Enterobacteriaceae in the absence of co-infection with non-fermenting gram-negative bacilli. Imipenem was restricted to non-fermenting gram-negative bacilli infections sensitive only to carbapenems and suspected gram-negative infections in unstable patients not responding to other agents. Susceptibility to ertapenem was extrapolated from imipenem results (Clinical and Laboratory Standards Institute (CLSI) M100-S16). Antibiograms were obtained for all *P. aeruginosa* isolated as the cause of infection for the 12 months before and after ertapenem introduction in March 2006. Strains were identified by Vitek 1 automated microbial identification system. Enterobacteriaceae susceptibility tests used the GNS 655 card. Susceptibility tests for non-fermenting bacteria done by disk diffusion (CLSI M100-S16). Consumption of ertapenem and imipenem were monitored.
Results: The defined daily doses (DDD) of imipenem declined from 58.4 to 23.8 DDD/1000 patient days one year after ertapenem was introduced. The median consumption of ertapenem was 37.0 DDD/1000 patient days. Twenty *P. aeruginosa* strains were isolated as causative pathogens during the 12 months prior to ertapenem introduction; four (20%) were imipenem resistant. None of the 18 *P. aeruginosa* strains isolated over the 12-months following ertapenem were imipenem resistant, although this trend in reduced number of resistant isolates was not significant.
Conclusions: Increased use of ertapenem, a pseudomonal-sparing carbapanem, combined with restricted use of imipenem, had a positive impact on *P. aeruginosa* susceptibility to imipenem

Tabelle

C2-1087a

Linezolid Non-Susceptible *Staphylococcus aureus* in Three Patients with Cystic Fibrosis Receiving Prolonged Linezolid Therapy

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Background: Resistance to linezolid (LZD), while uncommon, has emerged in *Staphylococci* and *Enterococci* and presents challenges to both antibiotic stewardship and infection control practices. Development of resistance is thought to be difficult and has not been widely reported due to the requirement of mutations in multiple copies of genes that encode the 23S rRNA. We report a cluster of LZD non-susceptible MRSA strains in the cystic fibrosis (CF) population at Children's Medical Center Dallas (CMCD) between April and June of 2008.
Methods: Three patients had sputum specimens submitted for routine culture and susceptibility. Each grew MRSA with atypical morphology on blood agar and normal morphology on mannitol salt agar. The organisms were not viable for routine susceptibility testing; therefore, susceptibilities were tested using blood Meuller Hinton agar incubated in 5% CO2. In addition to the phenotypic analysis of the susceptibilities, sequence analysis of domain V of the 23S rRNA gene was performed as well as pulse field gel electrophoresis (PFGE) to determine whether these strains were related. Finally, LZD exposure was compared to 10 patients within the same CF clinic with LZD exposure and LZD-susceptible MRSA.
Results: Sequence analysis of domain V of the 23S rRNA demonstrated previously unreported changes in one of the isolates (2447 G>T & 2234 G>A; MIC 8 ug/ml), while the other two isolates had the 2576 G>T sequence (MICs 8 & 16 ug/ml). PFGE results showed 3 distinct and unrelated strains. Patients with LZD non-susceptible isolates received a mean of 302 (126-592) days of therapy prior to developing resistance compared to a mean of 61 (24-142) in LZD susceptible patients.
Conclusions: These unrelated isolates had unusual morphologies and did not grow in routine susceptibility conditions. One of the isolates has previously unreported point mutations. Intermittent, extended courses of LZD in CF patients may have dire consequences. Sub-therapeutic exposure may also play a role in the development of resistance. This report highlights the importance of screening for difficult to detect LZD resistant isolates in patients who have seen significant LZD exposure. Each of these strains would have gone undetected using routine laboratory methods.

Tabelle

C2-2003

Driving Forces of Vancomycin-Resistant *E. faecium*

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Background: The use of selected antibiotics (AM) such as vancomycin (VAN), broad-spectrum cephalosporins (BSC) and AM with potent activity against anaerobic bacteria, have been associated with increasing rates of VAN resistant *E. faecium* (VRE). We evaluated AM use and rates of solid organ (SOT) and bone marrow transplan (BMT), before and after the implementaion of an Antimicrobial Stewardship Program (ASP) and the reemergence of VRE at a tertiary pediatric hospital.
Methods: In 2004 active AM surveillance was implemented at AIDHC. Indications for AM use were incorporated as mandatory fields using the computerized information system. A real-time report of AM prescribed, doses, patient demographic and microbiology data was reviewed by a pharmacist and an infectious disease physician. AM use, defined by the number of doses administered / 1000 patient days (DA/1000PD), was measured before and after implementation of ASP. Nosocomial transmission was assessed by PFGE. Data was analyzed with SPSS 14.
Results: The use of AM declined from 2088 DA/1000 PD to 1880 DA/1000 PD ($P<0.01$) while rates of invasive VRE increased from no cases to 6 in 2003-2004 and 2007-2008, respectively. 83% of VRE developed in SOT and BMT patients. Use of VAN declined from 378 DA/1000 PD/year to 252 DA/1000 PD/year ($P<0.001$). The use of BSC decreased from 475 DA/1000 PD/year to 391 DA/1000 PD/year ($P<0.001$). The use of metronidazole dropped from 206 DA/1000 PD/year to 61 DA/1000 PD/year ($P<0.001$). The use of piperacillin-tazobactam (PIPT) decreased over the last two years ($P<0.001$). Statistical correlation was found between VRE rates and PIPT (r .8, P: 0.01) and BMT (r .5, P:0.05). PFGE identified the same strain in 4/6 patients.
Conclusions: In our experience, reemergence of VRE did not correlate with use of VAN, BSC, or other AM except PIPT. BMT and nosocomial transmission were the most important associated factors.
Controlling the forces driving the emergence of invasive VRE infection requires an interdisciplinary approach and commitment

Tabelle

C2-3896

Development of Imipenem Resistance in *Klebsiella pneumoniae* among Patients with Prolonged Hospitalization and Antibiotic Exposure

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Background: The present study was conducted to investigate the recent increase of imipenem-resistant *Klebsiella pneumoniae* infections in our hospital.
Methods: Between 2004 and 2006, 11 patients with imipenem-resistant *K. pneumoniae* infections were retrospectively studied. Medical records of the patients were reviewed. Twenty-two isolates with various imipenem susceptibilities were subjected for laboratory investigation.
Results: All patients had various underlying diseases and were admitted with respiratory problems. Multiple antibiotics, including imipenem, were prescribed before the imipenem-resistant isolates were found. Three patients were recovered, while the remaining 8 were expired due to various causes that were mostly related to the original underlying diseases. Although various combinations of extended-spectrum cephalosporinases were found among the isolates, only AmpC enzymes (DHA-1 or CMY-2) were produced by all imipenem-resistant isolates. No known carbapenemases were identified. OmpK35 lost was observed in all isolates, while OmpK36 was further lost in imipenem-resistant isolates. OmpK36 lost was mainly due to insertional interruption by IS*Pa13* or IS*5*. These IS elements could be found in some imipenem-susceptible isolates, suggesting that an intrinsic insertion mechanism may occur, probably under the selection pressure of imipenem therapy, and lead to the resistance phenotype. In 3 patients, imipenem-susceptible and -resistant isolates were not clonally related, implicating an unknown exogenous origin.
Conclusions: Patients with respiratory tract diseases and other underlying diseases usually required long-term hospitalization and multiple antimicrobial therapy, all of which are factors predisposing to infections with imipenem-resistant pathogens. The resistance may occur endogenously from the originally presented susceptible bacteria or exogenously from other sources.

Tabelle

D-2232

Effects of In Vitro Test Method Variables on Ceftaroline Activity Against Aerobic Gram-Positive and -Negative Pathogens

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Background: Ceftaroline (CPT) is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including methicillin-resistant *Staphylococcus. aureus* (MRSA) and multidrug-resistant *Streptococcus pneumoniae* (MDRSP), as well as common gram-negative pathogens. CPT is currently in phase 3 development. This study determined the effects of different *in vitro* test conditions on the MICs of 30 strains representing 10 species of clinically important organisms.
Methods: The strains were clinical or ATCC quality control (QC) strains of *E. coli*, *K. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, *E. faecalis*, *S. pyogenes*, and *S. pneumoniae*. The CLSI broth microdilution method in cation adjusted Mueller Hinton broth was used with the following respective modifications: 50 mg/L Ca++, 5% NaCl, pH 6, pH 8, inoculum at 10^4 cfu/ml and 10^6 cfu/ml, 10% and 50% human serum, 2.5% laked horse blood (LHB), HTM broth, incubation in 5% CO_2 and incubation anaerobically. In addition, agar dilution MICs in plain MHA, MHA with LHB, and HTM with 1.5% agar were determined.
Results: MICs for the QC strains were within their acceptable ranges. 5% NaCl inhibited growth and/or reduced MICs for *E. coli* and *K. pneumoniae* and completely inhibited growth of *M. catarrhalis,* and *H. influenzae* and all streptococci. The 10^6 cfu/ml inoculum increased the MIC five-fold for 1 of 3 *E. coli* and 1 of 3 *K. pneumoniae* strains. The higher inoculum also increased MICs 3 to 5 fold for *M. catarrhalis*, while the addition of blood or serum enhanced their growth without changing the MIC. All other variables had minimal effect and the MICs were generally within one dilution of the reference method.
Conclusions: The *in vitro* antibacterial activity of CPT was not adversely affected by most modifications in testing methods.

Tabelle

D-2236

Comparison of Disk Diffusion, Etest and Agar Dilution for Testing the Susceptibility of Metallo-β-Lactamase Producing *Pseudomonas aeruginosa* to Polymyxins

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Background: The emergence of infections caused by metallo-β-lactamase (MBL) producing *P.aeruginosa* has revived interest in polymyxin B(PL-B) and colistin(CL). On the other hand, it has been known that CL-resistant mutants could be easily selected *in vitro* and the resistant strains were isolated from the patient treated with polymyxins. These findings suggest that susceptibility test for the isolates, especially MBL producing ones, to polymyxins is very important. We evaluated differences of susceptibility results using disk diffusion, Etest and agar dilution tests of MBL producing *P.aeruginosa* strains, which were clinically isolated, to PL-B and CL.
Methods: A total of 75 clinical isolates of *P. aeruginosa* bla_{IMP}-type gene that were detected by PCR were used in this study. Susceptibility tests of PL-B and CL using the agar dilution method were carried out in accordance with the guidelines of the CLSI. The disk diffusion susceptibility test and Etest for PL-B and CL were performed according to the protocol provided by the manufacturers.
Results: All 75 strains were susceptible to both polymyxins using the disk diffusion test although only 34 (45.3%) and 26 (34.7%) susceptible strains to PL-B and CL, respectively, were observed with the agar dilution tests. Additionally, 2 (2.7%) and 8 (10.7%) strains were defined as intermediate to PL-B and CL respectively and all the others were susceptible to polymyxins by Etest. Breakpoint cathegorical concordance between Etest and agar dilution methods was respectively 50.7% for PL-B and 37.3% for CL and agreement within 1 twofold dilution were 84% for PL-B and 45.3% for CL.
Conclusions: Although Etest and disk diffusion methods are easy and convenient test for determination of susceptibility to polymixins, it should be recognized that there was poor concordance among those three methods in MBL producing *P.aeruginosa*.

Tabelle

D-2240

Multicenter Evaluation of a MicroScan Synergies plus Panel for Susceptibility Testing with Teicoplanin Against *Staphylococci* and *Enterococci*

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Background: A multi-site evaluation assessed the accuracy of teicoplanin susceptibility testing with a MicroScan Synergies plus test panel. This panel combines early (4.5-5.5 hours) MIC results with the capability to read panels incrementally up to 18 hours.
Methods: Teicoplanin was evaluated with clinical Enterococcus and Staphylococcus species. Test panels were processed in the WalkAway system and the results were compared to a CLSI broth reference panel. All panels were read visually after overnight incubation.
Results: Three results were obtained for each MicroScan test panel: rapid read (less than or equal to 12 hours), overnight instrument read (16-18 hours), and overnight manual read (16-18 hours). Essential agreement (EA) and categorical agreement (CA) for the various read methods when compared to reference results were rapid read, 97.6% (540/553) EA and 99.5% (550/553) CA; overnight instrument read, 98.3% (566/576) EA and 99.7% (574/576) CA; overnight manual read, 97.6% (562/576) EA and 99.1% (571/576) CA. For the Rapid read method, greater than 85.6% of all results were available by 8 hours. Results were similar for enterococci and staphylococci with 86.7% (246/286) and 84.5% (245/290) of results available by 8 hours, respectively.
Conclusion: These data showed that the MicroScan Synergies plus panel gave excellent correlation with the CLSI reference method for antimicrobial susceptibility testing of teicoplanin.

Tabelle

D-2243

Validation of the MIC Results of MIC Evaluator Strips Following ISO Guidelines

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Background: The M.I.C.Evaluator (M.I.C.E.) test strips (Oxoid Ltd) is a new product for determining the MIC. These strips provide a gradient of antibiotic stabilized on a polymer strip covering 15 doubling dilutions. The aim of the study was to evaluate the MIC values of the M.I.C.E. products vs the MIC reference method (ISO 20776-1) according to ISO guidelines (ISO 20776-2).
Methods: Following the ISO protocol, during daily clinical laboratory work 362 consecutive gram negative strains (fresh isolates) were collected and identified. From each strain, the MICs were determined following ISO guidelines (ISO 20776-1) and instructions of the M.I.C.E. manufacturer, respectively. The MIC values were read following instruction by two independent lab technicians. The following M.I.C.E. were evaluated: gentamicin 256-0.016 mg/L (CN 256), gentamicin 1024-0.064 mg/L (CN 1024), amoxicillin 256-0.016 mg/L (AML), amoxicillin-clavulanic acid 256-0.016 mg/L (AMC), cefotaxime 32-0.002 mg/L (CTX 32), cefotaxime 256-0.016 mg/L (CTX 256), levofloxacin 32-0.002 mg/L (LEV), imipenem 32-0.002 mg/L (IPM), ciprofloxacin 32-0.002 mg/L (CIP). Discrepancy analysis was performed following the ISO guideline.
Results: The isolates collected were: *Citrobacter* spp (n=20), *Enterobacter* spp (n=47), *Escherichia coli* (n=99), *Hafnia alveii* (n=1), *Klebsiella* spp (n=98), *Morganella morganii* (n=12), *Pantoea agglomerans* (n=1), *Proteus* spp (n=74), *Providencia rettgeri* (n=1), *Serratia marcescens* (n=9). Overall, 71% of the strains were susceptible for the different antimicrobial agents. The essential agreement after discrepancy analysis was: CN 256 97%, CN 1024 97%, AML 99%, AMC 100%, CTX 32 97%, CTX 256 97%, LEV 99%, IPM 92% and CIP 99%.
Conclusions: The M.I.C.Evaluator strips demonstrated an excellent performance, essential agreement 92 - 100%, to determine the MIC value for the antimicrobial agents tested and can be used during routine practice.

Tabelle

G1-2104

Antibiotic Pressure Drives Nasopharyngeal Carriage of Erythromycin-Resistant *Streptococcus pneumoniae* Among Heptavalent Pneumococcal Conjugate Vaccine Recipients in Central Greece

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Background: In Greece, a high prevalence of erythromycin-resistant (Ery-R) *Streptococcus pneumoniae* (Pnc) has been observed. In our country, the heptavalent pneumococcal conjugate vaccine (PCV7) became available in Oct. 2004 and it was officially incorporated into the national immunization schedule in Jan. 2006.

Methods: In Feb. 2005, our group began yearly surveillance of the nasopharyngeal (NP) carriage of Pnc among children attending day-care centers in Central Greece.

Results: Between Feb. 2005 and May 2007, NP cultures were obtained from 1829 children aged 13-76 mos (median age 47 mos). The proportion of attendees vaccinated with one or more doses of PCV7 increased from 13% (2005) to 33% (2006) and to 70% (2007); 98% had been immunized on toddler catch-up schedules. Among age-appropriately vaccinated carriers, the proportion of Ery-R isolates was 33% in 2005, 31% in 2006, and 26% in 2007 (chi square for trend; P=0.350), whereas among unvaccinated carriers the proportion of Ery-R Pnc was 30% in 2005, 37% in 2006, and 34% in 2007 (P=0.238). Among vaccinated carriers, Ery-R Pnc belonged to serotypes 19F, 6A, nontypeable, 23F, 14, 6B, 10A, 7F, 15B, and 35A (in order of decreasing frequency). A significant relationship existed between carriage of Ery-R Pnc and recent use of one or more courses of macrolides in both the PCV7 vaccinated (P=0.001) and unvaccinated populations (P=0.009) (Table).

Conclusion: The study indicates the necessity of a judicious use of macrolides in PCV7 vaccinated children, in order to sustain a reduced colonization rate with Ery-R Pnc.

Characteristic	Age-appropriately PCV7 vaccinated children		Unvaccinated children	
	Rx in the preceding 3 mos		Rx in the preceding 3 mos	
	Macrolides (n=70)	None (n=285)	Macrolides (n=126)	None (n=527)
Pnc carriers	31 (44)[a]	145 (51)	59 (47)	285 (54)
Ery-S	12 (17)	114 (40)	29 (23)	212 (40)
Ery-R	19 (27)	31 (11)	30 (24)	73 (14)
OR (95%CI)	3.05 (1.60-5.80)		1.94 (1.20-3.14)	

[a]percent

Tabelle

K-3506

Risk Factors for the Emergence of Carbapenem Resistant Gram Negative Organisms in Necrotising Pancreatitis

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Background: Carbepenems are commonly used in severe acute necrotising pancreatitis (NP). The data on the emergence of carbapenem resistant gram negative (CRGN) infections in NP is sparse.
Methods: A retrospective cohort study of 13 cases of CRGN (11 *pseudomonas aeruginosa*, 1 *proteus mirabilis*, 1 *acenitobacter baumani*) organisms compared to 12 controls in acute NP from 1/05-2/08 was conducted. NP was diagnosed by CT scan. Bacteriologic status of pancreas were determined by CT or Ultrasound guided fine needle aspirate or surgical drainage. We collected data on demographic characteristics, comorbid conditions, duration of antibiotic usage, length of hospitalization, number of washouts, mortality and adverse events. Emergence of CRGN organisms from any source was included. Statistics were analysed with chi square and univariate analysis.
Results: The incidence of CRGN organisms in patients with NP on carbapenem was found to be 7.9% (13/165). Main factors are in Table 1. Risk factors significantly associated with the emergence of CRGN were prolonged use of carbapenem (median duration of 17 days, $p<0.05$), prolonged length of hospital stay ($p<0.006$) and the number of washouts($p<0.025$). Renal toxicity, bacteremia, and *clostridium diffcile* were noted in 7 (54%), 5(38%) and 6(46%) patients respectively in case group. 3(23%) deaths were noted in the case group.
Conclusions: The main risk factors associated with the emergence of CRGN infections in NP were prolonged use of carbapenem, prolonged length of hospital stay and the number of washouts. This is important to guide empiric therapy for NP, for better infection control strategies and for epidemiological purposes.

Demographics

Characteristics	Cases N=13	Controls N=12	P value
Median Age (yrs)	55	56	
Male Sex	9	7	0.6
Alcohol related	6	1	0.4
Gall Stone	5	5	0.9
Diabetes mellitus	2	4	0.3
Median LOS (days)	55	11	<0.001
Median Days of Carbapenem(Imipenem) Use	17.5	8	0.045

Tabelle