originally have been transferred to *C. difficile* from unrelated gram positive bacteria such as staphylococci or enterococci, species which might have come into close contact with *C. difficile*.

In vitro beta-lactamase induction in *E. coli* by ceftriaxon

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In a broth culture of *E. coli* K12 921 the concentration of ceftriaxon was increased stepwise during several passages. This leads to a selection of substrains with MIC's increasing from 0.06 µg/ml to 2.5 µg/ml. The reduced susceptibility of these newly obtained strains is accompanied by the occurrence of a chromosomally encoded β-lactamase (pI 8.6). Similar results were obtained using ampicillin. The MIC increased from 2.5 µg/ml to > 250 µg/ml. However, this strain also showed an increased resistance to ceftriaxon (MIC 2.5 µg/ml), which was rather surprising. Further treatment of this strain with increasing ceftriaxon concentrations over several passages, resulted in a substrain with a MIC for ceftriaxon of 100 µg/ml. This increase could be correlated with an augmented β-lactamase secretion. These findings do have a clinical importance. A ceftriaxon therapy of an *E. coli* infection may fail if the patient has been treated before with ampicillin or another similar β-lactamase antibiotic.

Outer membrane proteins in a chloramphenicol-resistant strain of *Pseudomonas aeruginosa*

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The outer membrane of a clinical isolate of *P. aeruginosa* resistant to chloramphenicol (CM), and its derivative sensitive to the drug were analyzed by polyacrylamide gel electrophoresis. A major outer membrane polypeptide with an apparent mol. wt of 50,000 daltons found in sensitive cells is almost lacking in the resistant cells, thus supporting the view of altered outer membrane permeability to CM in this strain. In vitro polypeptide synthesis experiments demonstrated that the ribosomes of the resistant strain were sensitive to the action of CM. Cell-free extracts of the sensitive mutant acetylated CM as well as its resistant parent strain. Moreover, intact cells of the resistant strain inactivated CM 9.06 times less than the sensitive mutant. The later accumulated ^{14}C-CM two times more than the resistant strain. These results clearly indicated that the resistance of the clinical isolate of *P. aeruginosa* to CM was due to reduced permeability towards the drug.

Induction and reversion of methicillin-resistant *Staphylococcus aureus* (MRSA) by antibiotics

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Induction and reversion were defined as an increase or decrease in MIC. After incubation with sub-inhibitory or inhibitory concentrations of methicillin and thienamycin, induction occurred which was reversible, depending on duration of growth without antibiotic. Population analysis showed MRSA to consist of several populations of differing sensitivities, but the populations of methicillin-sensitive strains (MSSA) were more homogeneous (similar sensitivity of all bacteria). The greater resistance of induced MRSA seems to be due to selection of more resistant populations, rather than to induction of metabolic processes. Reversion is then only a matter of overgrowth of the faster-growing sensitive population and depends on duration of growth, as demonstrated.

In vivo acquired resistance to beta-lactam compounds and fluoroquinolones: an experimental model

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To reproduce in vivo acquired resistance to new expanded-spectrum cephalosporins and fluoroquinolones, a murine peritonitis model has been developed. 2 h after i.p. challenge with *Enterobacter cloacae* (E.c.) – 10^8 CFU – animals received a single antibiotic shot = Ceftriaxone (CTX): 50 mg kg^{-1} b.wt; Pefloxacine (PFX): 25 mg kg^{-1} b.wt; Amikacine (AMK): 15 mg kg^{-1} b.wt. 24 h later, peritoneal E.c. populations were analyzed on Szibalski gradient agar. With CTX, shift towards resistance multiplied the MICs by a factor of 100–1000 (34/35); hyperproduction of beta-lactamase, and altered OMPs PAGE patterns were observed in all resistant variants (4 E.c. strains tested). With PFX, a shift was also observed, but to a lesser extent, and less frequently. AMK did not shift significantly E.c. populations.

Resistance of *Klebsiellae* to cephalosporins. Particular properties of beta-lactamases isolated from *K. oxytoca*

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K. pneumoniae and *K. oxytoca* are usually resistant to penicillins, and this resistance is normally associated with a beta-lactamase production. *K. pneumoniae* which produce a penicillinase are virtually sensitive to all cephalosporins, whereas *K. oxytoca* are more or less resistant to these antibiotics. We have studied beta-lactamase production of 10 strains of *K. oxytoca* isolated in Switzerland. All strains produce a single beta-lactamase as shown by iso-electric focusing. Four different patterns have been obtained with major bands at pI: 5.2, 5.7, 6.0 and 6.3. The specific activities of the crude extracts were condensed between 15 and 9000 mU/mg. This did not seem to be related to the pI. The kinetic constants were determined for a large set of beta-lactam antibiotics and the four enzymes showed the similar properties:

- hydrolysis of most of the tested beta-lactams, including the methoxy-imino-cephalosporins, such as cefuroxim and the 3rd generation of cephalosporins: cefoperazon, cefotaxim and ceftriaxon,
- cephamycins (cefoxitin and cefotetan), moxalactam and ceftazidim are very resistant to hydrolysis,
- all the enzymes are very sensitive to the action of clavulanic acid.

These properties are very different from those observed with other enterobacteria.

Intrafamiliar long-time epidemiology of drug-resistance factors and other plasmids in fecal *E. coli*

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We investigated fecal coligerms of five small families whether and when they contain R-factors and other plasmids. We paid particular attention to know at which moment after birth the first drug resistance factor appears in the coligerms of babies. To determine the intrafamiliar plasmid movement we used the identification of R-factors, colicinogenic factors and cryptic plasmids. To identify the hot-strains we used the methods of serotyping, colicinotyping and lysotyping and also the properties for lactose fermentation, hemolysis and mobility.

Amazingly we found only rare evidence in the families for a toilet respectively kitchen community of the coliflora with regard to the plasmid and the colitype content. Plasmid and coligerm transfer were observed between newborn and mother, between newborn and sisters and brothers but only once between newborn and father. Without influence of antibiotics the plasmid status of each member of the families was astonishingly stable for long periods.

The susceptibility of *Pseudomonas pseudomallei* to six β-lactams and five other antibiotics in vitro

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We determined the minimal inhibitory concentrations (MIC) and the minimal bactericidal concentrations (MBC) of 11 compounds against 20 clinical isolates of *Pseudomonas pseudomallei* from Thailand. We worked with Mueller-Hinton broth (microdilution technique, NCCLS) and Mueller-Hinton agar. The most active compounds were weight-for-weight (μg/ml) in terms of MIC_{90}, MBC_{90}, Range (MIC): Ro 17-2301 (4, 2, 1–128), Ceftazidime (CAZ) (64, 2, 0.5–128), Ciprofloxacin (CIP) (8, 8, 2–8), Co-trimoxazole (SXT) (2, 4, 1–4). Although most strains were resistant to both sulfamethoxazole (S) and trimethoprim (T), the combination SXT was highly active against all isolates tested. Ro 17-2301 and CAZ were as a rule fourfold more active than ceftriaxone, latamoxef and aztreonam. We conclude that structurally related β-lactams may have different potency in vitro against *P. pseudomallei* and that Ro 17-2301 and ceftazidime were the most active of those tested.

Antibiotic susceptibility of anaerobic bacteria determined by agar dilution

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250 anaerobic bacteria, being probably of clinical significance, were identified by gram stain of culture smears, gas liquid chromatography (Holdeman et al., Anaerobe laboratory manual, 1977) and with API 20 A. Susceptibility testing was performed according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (M 11-T 1982) using Wilkins-Chalgren agar containing 5% sheep blood and Adatab antimicrobial tablets (Mast). The breakpoints were chosen as recommended by the NCCLS M 7-T 1983: Penicillin G 0.125 mg/l, Amoxycillin/Clavulanate 8/4 mg/l, Carbenicillin 32 mg/l, Cefoxitin 8 mg/l, Metronidazole 16 mg/l, Chloramphenicol 8 mg/l, Clindamycin 0.5 mg/l, Tetracyclin 1 mg/l. In agreement with other publications, Amoxycillin/Clavulanate and Chloramphenicol proved to be very efficient antimicrobials against anaerobic germs in vitro. Furthermore, it is noteworthy that 30% *Peptococcus* and 70% *Peptostreptococcus* species were resistant to Metronidazole. Therefore, Metronidazole should not be considered as universal agent against infections caused by anaerobic bacteria.

OXA-2 and TEM-1 beta-lactamase DNA probes for epidemiological studies

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DNA probes seem promising for molecular epidemiology of antibiotic resistance. Two probes have been produced as markers of beta-lactamase (Bla) producing strains. TEM-1 Bla probe consists of λbb DNA carrying Tn 2301 originated from plasmid R 111. OXA-2 Bla probe was constructed with plasmid R 46 as donor and pBR 327 as vector. pBR 327 was digested by *Bam* HI and *Pst* I, leading to isolation of a fragment carrying the origin of replication, without the AMP gene. Plasmid R 46 was digested by *Bam* HI, and the fragment carrying the *Bla* gene was purified and digested by *Pst* I. Vector and donor *Bam* HI–*Pst* I fragments were ligated and introduced into *E. coli* C600 by transformation. One clone producing OXA-2 Bla has been then isolated. These TEM and OXA_2 Bla DNA probes have been tested against a collection of strains producing known Bla, and will be used as epidemiological markers.

Beta-lactamases produced by *Campylobacter jejuni*

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Out of 20 sporadic isolates of *C. jejuni* (6 resistant and 14 susceptible to amicillin (A), 18 had Betalactamase (Bla) activity (0.01 to 1.5 IU per ml of undiluted sonic extract). Bla have been characterized after partial purification by gel filtration: profil of activity by a bio assay, relative rates of hydrolysis, inhibition profile and immunological specificity at the spectro, IEF in polyacrylamide gel and mol. wt in column. Bla type A (18 out of a collection of 23 Bla producing strains) is active against peni (P), oxa (O) and to a lesser extend carbenicillin (C), pI: 8.3, mol. wt: 30 K; neutralized by homologous rabbit antiserum. Bla type B (1/23 strain) is similar to Bla type A, but also hydrolyzes cephaloridine and cefuroxim; IEF: 1 major band at 8.6, accessory band at 8.3 and 8.1. Bla type C (1/23) is active against P, A, C, O, cephalothin, cephaloridine and cefotaxime, but not cefuroxim. pI: 8.3, mol. wt: 45 K, neutralization by anti Bla type A antiserum. Bla type D (1/23) is mainly active P, less against A, O and C, pI: 7.4, mol. wt: 45 K, no neutralization by anti Bla type A. Bla may be involved.

Immunology

Suppressive mechanism of cyclosporin A: blocked IL-2 secretion of Con A activated T-cell hybridomas

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The fungal metabolite cyclosporin A (Cy A) is a powerful immunosuppressant with apparent selective action on lymphocytes (mainly T-cells). The mechanism of suppression is still controversial. The examination of the mechanism, by which Cy A suppresses cellular immune responses, is complicated by the fact that T-cell activation is dependent on the presence of antigen presenting cells, lymphokines, expression of lymphokine receptors and immune-response related cell proliferation. In order to further define the suppressive mechanism of Cy A, we searched for an assay system which is free of these complications. The activation of T-cell hybridomas with Con A, in the absence of accessory cells, provides the desired experimental conditions. We found that pharmacologic levels of Cy A (25 ng/ml) strongly inhibited IL-2 secretion, but had no effect on DNA synthesis of T-cell hybridomas. This observation suggests, that the primary mechanism of Cy A mediated suppression is due to the blocking of the T-lymphocyte activation pathway, leading to IL-2 secretion.