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

R. Egger and G. Lebek
Institute for Hygiene and Medical Microbiology, University of Bern, CH-3010 Bern

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*

S. Abdel-Sayed and F. Schmid
Institut de Microbiologie, CHUV, Université de Lausanne, CH-1011 Lausanne

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

W. Tosch and E. Sigarlakie
Ciba-Geigy Ltd, Pharmaceutical Research Laboratories, CH-4002 Basel

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

M. Michéa-Hamzehpour and J. C. Pechère
Département de Microbiologie, Faculté de Médecine, CH-1211 Genève

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*

R. Labbia, J. S. Pitton and M. Heitz
CNRS-Cercoa, F-94320 Thiais (France), Anamed 2000, CH-1201 Geneva, and Institute of Microbiology, University of Lausanne, CH-1011 Lausanne

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*

C. Sutter and G. Lebek
Institute for Hygiene and Medical Microbiology, University of Bern, CH-3010 Bern

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.