of 20 lyophilized substrates are read with a dichromatic spectrophotometer and compared with a data base. Identifications with probabilities (PR) are printed after 4–5 h at 37 °C.
6 E strains (str.) were each tested three times in two different lots of cuvettes with sterile distilled water (SDW, pH 6.3) and SDW ad infundibilia (SDWI, pH 6.0). Different biocodes but identical sp. diagnoses at 97.6% PR were obtained. 594 Str. (479 E, 80 NF, 35 V), identified by CDC methods (E) or API 20 NE (NF, V) were tested for indentification. SDW (262 E+ (NF+V) str.) and SDWI (332 E+ (NF+V) str.) gave no significantly different results. At > 80–99% PR, 77% each of E and NF+V were diagnosed correctly; from < 50% upward, figures were 92.5% and 79%. 24 str. were misidentified, 16 only to species. 62.5% of E were diagnosed at 4 h; the rest and all NF+V at 5 h. Problems occurred with *P. mirabilis, S. liquefaciens, S. paratyphi A,* and *Yersinia* spp. and *E. agglomerans.*

Evaluation of the Cobas-Bact® automated antimicrobial susceptibility testing system

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The Cobas-Bact® is an analyzer for the microbiological laboratory capable of producing 5 h susceptibility tests and kinetic growth curves. The Cobas-Bact® system was evaluated for its efficacy by determining the susceptibilities of 2000 clinical, nonfastidious, falcultative and aerobic isolates compared with standard Kirby-Bauer disk diffusion. For gram-positive isolates, overall full and essential agreements between Cobas-Bact® and reference antibiograms were 90% and 95.5% respectively. For Enterobacteriaceae the overall full and essential agreements were 91% and 95.5% respectively. With *Pseudomonas* spp. the full and essential agreements were 90% and 96% respectively. Reproducibility studies gave essential agreement in 98% of cases. On the basis of this preliminary evaluation, it seems that the Cobas-Bact® can yield rapid, reasonably accurate and reproducible results when testing gram-positive cocci, Enterobacteriaceae and *Pseudomonas aeruginosa.*

A simple rapid technique to measure minimal bactericidal concentrations and combined antimicrobial action

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The methods used most frequently in vitro to measure the interaction of antibiotic combinations are 1) chessboard method (MIC and/or MBC), 2) killing curves and 3) diffusion tests, e.g. the cellophane transfer method of Chabbert. None of these is satisfactory. I have developed a new technique which combines many advantages of the known methods without their major disadvantages – especially the 'carry over' of antibiotics.
Chessboard titrations are done in tissue culture plates (Costar 3424, Mark II, 24 holes). After overnight incubation the plates are centrifuged in an oblique position of 45°. The supernatant is removed and the sediment may be washed several times to remove the antibiotics. After resuspension of the bacteria a double strength agar medium is added. After reincubation for another 24 h 99% or 99.9% bactericidal activity is calculated for each combination of antibiotics.

Evaluation of the Api 20 Strep system for species identification of viridans streptococci from blood cultures

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87 (100%) strains of Aerococci and viridans streptococci isolated from blood cultures in the years of 1983 and 1984 were classified according to a modification of Facklman's methods: 3 Aerococci, 10 *S. bovis* I, 3 *S. bovis* II, 15 *S. sanguis* I, 21 *S. sanguis* II, 12 *S. mitis*, 14 *S. milleri*, 4 *S. salivarius*, 2 *S. mutans*, 3 strains unclassified. Api system identifications: 28 (32%) strains after 4 h of incubation by the profile index. 68 (78%) strains after 24 h. 73 (84%) strains by additional testing: Optochin reaction and polysaccharid production. 74 (85%) strains by computer identification. 76 (87%) strains by computer identification and additional testing. 3 strains were misidentified. 8 (9%) strains remained unidentified, including the 3 unclassified strains by the reference method.
The Api system proves to be an accurate method for species identification of viridans streptococci. With additional testing and identification by the profile index the rate of identification was 84%.

Plasmids and Transposons

Replication of the tetracycline resistance plasmid pSC101

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We have a series of mutations in the replication genes of the *E. coli* plasmid pSC101 caused either by insertion in vivo of the transposon Tn*1000*, or in vitro of a segment of DNA carrying an antibiotic resistance marker. These mutations have enabled us to map the origin of replication, a gene, *repA*, whose product is essential for replication and regions implicated in the regulation of replication. The RepA protein represses transcription of its own gene. We are currently isolating and analyzing mutations which affect replication control and using gene fusion techniques to map transcriptional units within the replication origin. We want to elucidate the molecular mechanisms which determine plasmid copy number.

Ω mutagenesis in gram-negative bacteria: a selectable DNA fragment which terminates transcription in a wide range of bacterial species

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A novel method for in vitro insertional mutagenesis of genes cloned in *E. coli* has been previously described (Prentki and Krisch, Gene *29* (1984) 303). It employed the Ω fragment, a 2.0 kb DNA segment consisting of the antibiotic resistance gene *aadA*$^+$ (Sm^r/Spc^r) flanked by small inverted repeats carrying transcription and translation termination signals and synthetic polylinkers. In *E. coli* the Ω fragment has been shown to terminate RNA and protein synthesis prematurately at the site of insertion. In this communication we demonstrate that Ω mutagenesis is equally effective in a wide range of gram-negative bacteria other than *E. coli.* To facilitate this analysis, we constructed in a broad host range vector, a hybrid plasmid which contains the entire *meta*-cleavage pathway operon of the *Pseudomonas putida* TOL plasmid pWWO. Since one of the downstream genes in the polycistronic mRNA encodes an easily assayable enzyme, the Ω fragment was inserted between this gene and the promoter. Comparison of the enzyme levels produced by the plasmid with and without the Ω insertion indicates that in all the strains examined Ω reduced transcription beyond the point of insertion at least 50-fold. We conclude that Ω mutagenesis is equally applicable as a method to study gene structure and function on these organisms.