prevalence group not only for HBV but even more so for HCV.

Conclusion. On the basis of the high prevalence of circulating HCV found with both HCV PCRs, one might speculate that chances for transmission of HCV among IVDU occur more frequently than for transmission of HBV via similar routes. Conversely, instead of transmission through needle sharing or sexual contact, community acquisition of HCV infections might be of particular importance in IVDU.

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Identification of mycobacteria species by 23S ribosomal RNA targeted gene probes

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Conserved molecules like the rRNAs are suitable markers for identification of microorganisms. The most common molecule among the rRNAs is the 16S rRNA, which is used for rapid diagnosis of mycobacteria by direct DNA sequencing techniques[2]. The 23S rRNA is double the size of the 16S rRNA and consists of more high variable regions. The 23S rRNA of the genus *Mycobacterium*, members of the phylogenetic group of Gram-positive bacteria with a high DNA G + C content, contains a characteristic insert of about 100 bases in length. Its primary structure is highly variable. We developed specific oligonucleotide probes, which can rapidly identify and differentiate mycobacteria.

Methods and results. Isolation and purification of genomic mycobacterial DNA was done according to a modified standard procedure[1]. PCR reactions were performed on a Cetus 9600 thermocycler (Perkin-Elmer Cetus Corp., Norwalk, Conn.). For direct sequencing amplicons were purified by spin columns (Pharmacia Biotech, Germany). Sequencing reactions were loaded on a direct blotting electrophoresis (DBE) system GATC 1500 (MWG-Biotech GmbH, Germany) and automatically blotted on a noncharged nylon membrane (direct blotting membrane, GATC GmbH, Germany)[4]. Hybridization with the oligonucleotide probes was performed using a dot format and a modified protocol with tetramethylammonium chloride for washing at a specific temperature[5].

For *M. avium*, *M. nonchromogenicum*, *M. phlei*, *M. bovis*, *M. scrofulaceum*, *M. celatum*, *M. terrae*, *M. flavescens*, *M. xenopi*, *M. fortuitum*, *M. tuberculosis*, *M. gastri*, *M. gordonae*, *M. kansasii*, and *M. malmoense*, the 23S rRNA insert of helix 54 was amplified and the primary structure determined by direct sequencing. The primary structures were aligned and completed using published results[3]. Probes were designed for specific detection of *M gastri*, *M. fortuitum*, *M. nonchromogenicum*, *M. phlei*, *M. celatum*, *M. malmoense*, *M. scrofulaceum*, *M. kansasii*, *M. terrae* and *M. xenopi*. Only *M. gastri* and *M. kansasii* could not be distinguished on the level of the helix 54 insert.

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Use of 16S rDNA targeted oligonucleotide probe to detect phenotypic heterogeneity of *Bacillus mycoides*

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Many *Bacillus* species exhibit a high degree of similarity both in phenotye and 16S rDNA sequence, making their identification somewhat difficult. Examples of such species are those of the *B. cereus* group composed of *B. cereus*, *B. mycoides*, *B. thuringiensis* and *B. anthracis* which demonstrate only small differences in phenotype and high 16S rDNA sequence similarity (>99.4%)[1].

Thirty-four *Bacillus* species isolated from a peat bog sample were identified on the basis of fatty acid analysis as strains of the species *Bacillus mycoides*. The degree of similarity to the fatty acid profile of the *B. mycoides* strain in the fatty acid profile database was in the range 20–80% and *B. mycoides* was considered the best match. In some cases these results were in conflict with the phenotypic data, which showed many of these strains to be motile and all to have a non-rhizoid colony morphology, characteristics different to those described for the species *B. mycoides*[2].

Full 16S rDNA sequence was determined for five repre-

sentatives of these isolates and the five *B. mycoides* strains listed in the DSM Catalogue of Strains 1993. Sequence analysis showed the five isolates, and three of the strains, to have between 99.6 and 100% similarity to the type strain of *B. mycoides* (DSM 2048^T). Two strains considered to be *B. mycoides* on the basis of phenotype, namely DSM 303 and DSM 307, showed 99.4 and 98.8% sequence similarity respectively to the type strain of *B. mycoides*.

Comparison of 16S rDNA sequences of *B. mycoides*, *B. cereus*, *B. thuringiensis* and *B. anthracis* between positions 180 and 199 (*E. coli* numbering) indicated that the region could be useful as an oligonucleotide probe target for the differentiation of *B. mycoides* from the other *Bacillus* species. An oligonucleotide probe of 20 nucleotides was designed, 3'-end labelled with the DIG System (Boehringer Mannheim) and tested against a dot blot of PCR products of all isolates, available *B. mycoides* strains and controls of other closely related *Bacillus* species.

Positive signals were obtained with 33 of the 34 isolates, and with all tested *B. mycoides* strains with the exception of strains DSM 303 and DSM 307. No signals were obtained for the other *Bacillus* species tested. The *B. mycoides* strains DSM 303 and DSM 307 had been assigned to *B. mycoides* on the basis of phenotypic characteristics. Comparison of the sequence of the probe region for these two strains showed strain DSM 303 to differ at one position, while strain DSM 307 differed at four positions.

In order to determine the specificity of the probe in distinguishing *B. mycoides* strains from other closely related species, DNA-DNA hybridization experiments were carried out using the thermal reassociation method. Two of the isolates that gave a positive signal with the oligonucleotide probe had ~70% DNA-DNA similarity to the type strain of *B. mycoides*, but 50–60% to the type strains of *B. cereus* and *B. thuringiensis*, indicating their membership of the species *B. mycoides*. *B. mycoides* strains DSM 303 and DSM 307 which did not give a signal with the probe had ~65% and ~44% DNA-DNA homology to the type strain of *B. mycoides*; such values could speak in favour of their exclusion from the species *B. mycoides*.

This study demonstrates the application of 16S rDNA oligonucleotide probes to the differentiation of species which have highly similar 16S rDNA sequences and are phenotypically difficult to differentiate. A 16S rDNA-based oligonucleotide probe has been designed which differentiates *B. mycoides* strains from the closely related *B. cereus*, *B. thuringiensis* and *B. anthracis*. The specificity of the probe is in accord with results of DNA-DNA hybridization studies. Based on the findings of this study, the species description of *B. mycoides* should be amended to include both rhizoid and non-rhizoid colony morphology and motile and non-motile cells.

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Length polymorphisms detected by PCR for rRNA gene spacers and ERIC2 sequence flanked regions indicate a clonal relatedness among groups of *Staphylococcus aureus* strains

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A clonal relatedness among definite major groups of *S. aureus* strains is already indicated by multilocus enzyme analysis and *Sma*I-macrorestriction patterns[1,2,4]. The aim of our study was to check whether length polymorphisms of rRNA gene spacers and ERIC sequence[3] flanked regions are concordant with this grouping and applicable to epidemiological questions.

Forty-six *S. aureus* strains consisting of 40 strains defined by definite phage- and *Sma*I-macrorestriction patterns and 6 representatives of epidemic MRSA clones[5] have been checked for rRNA gene spacer length polymorphism. DNA extraction and amplification of DNA sequences by PCR have been described previously[5]. The spacer between the 16S rRNA gene and the 23S rRNA gene was amplified by use of primer sequences located in the conserved areas of both flanking genes; I: 5'-TTGTACACACCGCCCGTCA-3'; II: 5'-GGTACCTTAGATCTTTCAGTTC-3'; cycling scheme: 30 cycles 94 °C, 1 min; 55 °C, 1 min; 72 °C, 1 min; last cycle with 4 min at 72 °C. The primer for the ERIC2 sequence[3] was 5'-AAGTAAGTGACTGGGTGAGCG-3'; cycling scheme: 29 cycles: 94 °C, 30 s; 25 °C, 30 s; 72 °C, 30 s; last cycle: 94 °C, 30 s; 25 °C, 30 s; 72 °C, 4 min. *Sma*I-macrorestriction patterns and analysis of fragment similarities (according to molecular masses) were performed as described[4]. rRNA gene spacer size patterns (rRGS) were less discriminative than *Sma*I-macrorestriction patterns. Strains grouped according to macrorestriction patterns and phage-patterns as probably clonally related exhibit common rRGS patterns (fig. 1). This is of special interest for 6 different epidemic MRSA, where 4 strains exhibit similar *Sma*I-macrorestriction and identical rRGS patterns.