them, the latter diagnosis being confirmed at autopsy by the presence of hyaline membranes in the lungs. These two cases, as well as other cases previously reported, draw attention to the fact that both septic shock and ARDS can occur, albeit rarely, in patients with disseminated cryptococcosis [2, 9]. The serum cryptococcal antigen test is a rapid method which enables a prompt diagnosis. Nevertheless, empirical treatment with amphotericin B should be administered early to AIDS patients who present with severe pneumonia of unknown etiology, especially if shock and/or ARDS develop.

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High-Frequency Transduction of Antibiotic Resistance in *Pseudomonas aeruginosa* by a Wild-Type Bacteriophage with Restricted Specificity for Recipient Strains

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Antibiotic resistance can be transferred among strains of *Pseudomonas aeruginosa* by conjugation [1], transduction by wild-type phages as well as by generalised transducing phages, such as F-116 or G-101 [2–5], or by transposition of integrons [6]. While determinants of resistance can be transferred by conjugation from *Pseudomonas aeruginosa* to, for example, *Escherichia coli* or *Proteus mirabilis* recipient strains, transduction by bacteriophages is a species-specific event. Nevertheless, it could be also an important factor contributing to the dissemination and spread of antibiotic resistance among *Pseudomonas aeruginosa* in clinical settings [5].

In this report we describe several properties of a new wild-type bacteriophage (no. AP-103) from a ceftazidime-resistant strain of *Pseudomonas aeruginosa* (no. 103) isolated from the urine of a patient hospitalised in the intensive care unit of a large teaching hospital (Bata's Hospital) in Zlín, Czech Republic. This phage, in contrast to bacteriophages described previously, exhibited two rather peculiar properties: the frequency of transduction was unusually high (10^{-5} to 10^{-6} in comparison with, for example, 10^{-7} to 10^{-9} for phages 37, 38, and 40 [5]), and the phage AP-103 was lytic to a single recipient strain, i.e., the PAO strain, and showed no evidence of a lytic reaction to ML recipient strains, i.e., to *Pseudomonas aeruginosa* ML-1008, ML-1292, or ML-M-88. Nevertheless, it transduced genes of antibiotic resistance to both PAO and ML series of strains.

Plaques of phage lysis were detected on the surface of antibiotic-containing media during testing for multiple drug resistance in a donor strain of *Pseudomonas aeruginosa* 103. Isolation of the bacteriophage (AP-103) and preparation of a wild-type phage lysate were performed as described previously [1, 4, 5].

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Transduction procedures were performed as described in detail previously [3, 7, 8]. Four auxotrophic recipient strains of *Pseudomonas aeruginosa* were used in transduction systems: *Pseudomonas aeruginosa* PAO-1670 (*ade– leu– rif+*), *Pseudomonas aeruginosa* ML-1008 (*trp– leu– arg– ile– val– his– rif+*), *Pseudomonas aeruginosa* ML-1292 (*trp– met– ile– val– his–*), and *Pseudomonas aeruginosa* ML-M-88 (*leu– trp– str+*). Auxotrophic recipient strains were obtained courtesy of Prof. S. Mitsuhashi, Maebashi, Japan.

All four recipient strains were highly susceptible to all antibiotics used for selection of transductants (e.g., MIC of cefotaxime, 3.15 μg/ml; MIC of ceftazidime, 0.8 to 1.6 μg/ml), and they were used as 8 h shake cultures in nutrient broth (Difco, USA) (eventually adjusted by centrifugation to a density of 1×10^9 cfu/ml). The sterile phage lysate AP-103 was added to each recipient strain in an amount sufficient to obtain a multiplicity of infection (MOI) of 0.5 pfu/cell. The time allowed for phenotypic expression of transduced determinants was 60 min. The surface of antibiotic-containing plates (with 100 μg/ml of streptomycin, kanamycin, or carbenicillin, or 30 μg/ml of cefotaxime, ceftazidime, or aztreonam) was then inoculated with a mixture of bacteria plus the phage. Identical plates were inoculated in parallel with the control (recipient bacteria only, without phage added). Antibiotic-containing plates were then incubated at 35 °C for 36 h and 48 h, and number of transductants was recorded. Transductants were then picked from each experimental plate and examined by an indirect selection procedure for their complete spectra of transduced resistance.

In contrast to other wild-type phages isolated from multiple-drug-resistant nosocomial strains of *Pseudomonas aeruginosa* [3, 5, 9], the phage lysate of *Pseudomonas aeruginosa* 103 showed a lytic reaction when added, in dilution of up to 10^{-12}, to the recipient strain *Pseudomonas aeruginosa* PAO 1670 (Figure 1) but not to the other strains. This phage was not lytic for ML strains, but we thought it might be a good transducing phage for this series of strains.

Transduction experiments confirmed this hypothesis. A high frequency of transduction of resistance to all β-lactam antibiotics tested and to kanamycin was observed for all four recipient auxotrophic mutants of *Pseudomonas aeruginosa* PAO and ML examined. Genes coding for resistance to ceftazidime, aztreonam, and cefotaxime were transduced to *Pseudomonas aeruginosa* PAO-1670, ML-1008, and ML-M-88 at frequencies of 10^{-5} to 10^{-6} (Figure 2), which was two logarithms higher than the highest frequency obtained with the phages AP-37, AP-38, and AP-40 (10^{-7} for transduction of carbenicillin or imipenem resistance to *Pseudomonas aeruginosa* M-88) and four logarithms higher than the frequency of transduction of imipenem resistance to *Pseudomonas aeruginosa* PAO-1670 [5]. Transfer of resistance to kanamycin was found to occur at a frequency of 10^{-8} to 10^{-9}.

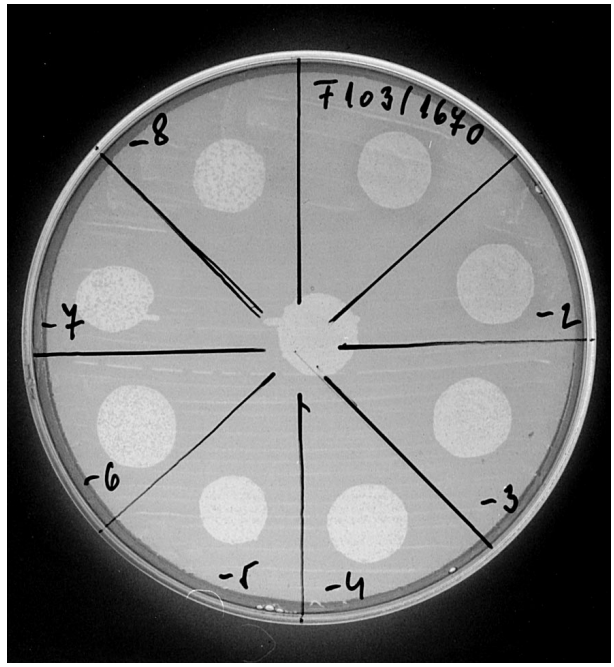

Figure 1 The titre of bacteriophage AP-103 in the recipient strain *Pseudomonas aeruginosa* PAO-1670

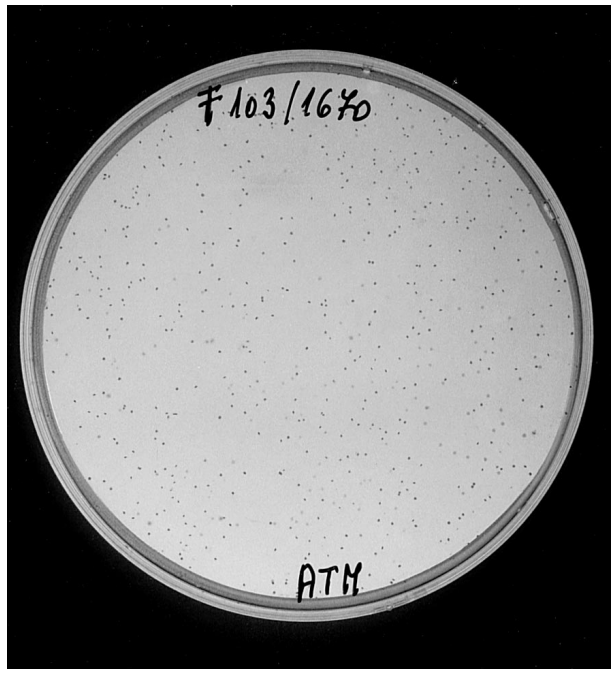

Figure 2 Transduction of aztreonam resistance determinant by phage AP-103 from *Pseudomonas aeruginosa* 103 to recipient strain *Pseudomonas aeruginosa* PAO-1670

Analysis of transductants, selected on any of the single antibiotic media used, showed that all transductants were co-resistant to all five antibiotics, i.e., ceftazidime, aztreonam, cefotaxime, carbenicillin, and kanamycin. The presence of various *bla* genes on a single integron with genes coding the resistance to kanamycin or other antibacterial agents was demonstrated in nosocomial strains of *Pseudomonas aeruginosa* and of other gram-negative bacteria [6, 10, 11].

In conclusion, the results obtained in transduction experiments with the wild-type bacteriophage AP-103 indicate a relation between the lytic and the transducing capacities of a phage preparation. If such a relation is confirmed, the possibility exists that some phages might actually remain undetected in nosocomial strains of *Pseudomonas aeruginosa*. Furthermore, it could be demonstrated that some phages, including phage AP-103 described here, transduce genes of antibiotic resistance, mainly in a block of resistance determinants, to several recipient strains at an unexpectedly high frequency, which might play an important role in the dissemination of antibiotic resistance among nosocomial strains of *Pseudomonas aeruginosa*.

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Prevalence of Hepatitis G Virus Infection among Patients with Chronic Hepatitis

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Despite the use of sensitive and specific serological and molecular diagnostic methods for identifying hepatitis A, B, C, D, and E viruses, 5–20% of acute and chronic hepatitis cases are of unknown etiology. Recently, two novel hepatitis viruses, the hepatitis G virus (HGV) and the GB virus C (GBV-C), were independently isolated by two different research groups [1]. In fact, HGV and GBV-C are separate isolates of the same virus. In this report we use the term hepatitis G virus (HGV) to denote GBV-C.

HGV is a single-stranded, positive-sense RNA virus that belongs to the Flaviviridae family [1]. Like HBV and HCV, HGV is usually transmitted parenterally. HGV has been found in 9% of patients with acute non-A-E hepatitis, in 4–39% of those with chronic non-A-E hepatitis, in 0–50% of those with fulminant hepatitis, and in 14–36% of those with cryptogenic cirrhosis [2–5]. Coinfection with HBV of HCV is common, occurring in 5–15% and 10–20% of HGV infections, respectively [1]. However, the etiological role of HGV in hepatitis is unclear, and the pathogenesis remains to be elucidated. This study was performed to determine the prevalence of HGV infection among patients with chronic hepatitis in our area using the nested reverse transcriptase polymerase chain reaction (RT-PCR).

Eighty-seven patients with chronic hepatitis were included in the study. The diagnosis of chronic hepatitis

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