TRANSDUCTION OF ANTIBIOTIC RESISTANCE INCLUDING IMIPENEM RESISTANCE BY WILD TYPE PHAGES FROM NOSOCOMIAL STRAINS OF *PSEUDOMONAS AERUGINOSA*

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Summary. – In this report we describe transduction of antibiotic resistance determinants by three wild type bacteriophages isolated from three *Pseudomonas aeruginos*a strains. The strains showed evident plaques of a lysis caused by a bacteriophage. The strains were identified as lysogenic among 31 imipenem (IMP)-resistant *P. aeruginosa* strains isolated at the National Institute of Oncology in Bratislava. The carbenicillin (CAR) resistance determinant was transduced by all the three phages to four *P. aeruginos*a recipients – PAO-1670, ML-M-88, ML-1292 and ML-1008. The gentamicin (GEN) resistance was transduced to ML-1008 only. The kanamycin (KAN) resistance was transduced in the following systems (combinations): "phage AP-37 to M-88", "phage AP-38 to PAO-1670, ML-1292 and M-88", and "phage AP-40 to M-88". The IMP resistance determinant was transduced by all the three phages to *P. aeruginosa* recipient strains. All transductant colonies were tested for the presence of directly not selected but co-transduced resistance determinants. Whereas transductants selected on media with IMP were resistant to five antibiotics (IMP, CAR, streptomycin (STR), KAN and GEN), transductants selected on CAR, KAN, STR, or GEN were resistant to a block of four of these antibiotics but not to IMP.

Key words: *Pseudomonas aeruginosa*; antibiotic resistance; transduction

Introduction

Resistance to antibiotics can be transferred among *Pseudomonas aeruginos*a strains by conjugation (Hupková *et al.*, 1993; Watanabe *et al.*, 1991), transduction (Blahová *et al.*, 1993; Lebek *et al.*, 1981; Masuda and Ohya, 1992) or transposition (Yobe *et al.*, 1996). Wild type bacteriophages isolated from lysogenic nosocomial polyresistant *P. aeruginosa* strains were found to transduce to susceptible recipient strains of this species determinants of resistance to aminoglycoside antibiotics (Knothe *et al.*, 1981) and to β-lactam antibiotics (Blahová *et al.*, 1993; Seginková *et al.*, 1986).

In this report we describe transduction of antibiotic resistance determinants by three wild type bacteriophages isolated from three multiple drug-resistant *P. aeruginosa* strains isolated at the National Institute of Oncology in Bratislava. The strains showed evident plaques of a lysis caused by a bacteriophage. Two of them (No. 37 and 40) were isolated from drug-resistant *P. aeruginos*a strains infecting the hospitalized oncologic patients. The third bacteriophage (No. 38) was isolated from a *P. aeruginosa* strain from a haemodialysator equipment at the same hospital.

Materials and Methods

Bacterial strains. Donor lysogenic strains of *P. aeruginosa* No. 37, 38 and 40 were resistant to a series of antibiotics including STR, KAN, GEN, CAR and IMP. As recipient strains in transduction experiments were used four antibiotic-susceptible auxo-

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Abbreviations: CAR = carbenicillin; CEP = cephaloridine; CFU = colony forming unit; GEN = gentamicin; IMP = imipenem; KAN = kanamycin; MIC = minimal inhibitory concentration; STR = streptomycin

trophic mutants of *P. aeruginosa* strains PAO-1670 (*ade⁻ leu⁻ rif^I*), ML-1008 (*trp⁻ leu⁻ arg⁻ ile⁻ val⁻ his⁻ rif^I*), ML-1292 (*trp⁻ met⁻ ile⁻ val⁻ his⁻*), and ML-M-88 (*leu⁻ trp⁻ str^I*). The strains were obtained from Prof. S. Mitsuhashi, Maebashi, Japan.

Transduction experiments. Preparation of phage lysates from lysogenic strains of *P. aeruginosa*, propagation of the obtained phages to a titer of 10^{10} PFU/ml, transduction procedure, selection of transductants on monoantibiotic plates and analysis of transduced resistance determinants in individual recipient strains were described previously (Blahová *et al.*, 1994; Seginková *et al.*, 1986; Holloway *et al.*, 1975; Olsen and Thomas, 1973). Multiplicity of infection was 0.1 PFU per cell. One ml of bacterial suspension of recipient strains contained approximately 5 x 10^9 CFU/ml. Transductants were selected on agar (Nutrient agar, Difco) plates containing following concentrations of single antibiotics present in the spectrum of multiple drug resistance of donor strains: 100 mg/l CAR, 30 mg/l GEN, 100 mg/l KAN, and 15 mg/ml and 30 mg/l IMP.

Hydrolytic activity of strains. Relative rate of hydrolysis (V_{max}) of cephaloridine (CEP), CAR and IMP by crude sonicates of original strains of *P. aeruginosa* and of transductants of *P. aeruginosa* was measured by a macroiodometric method (Neu, 1986) and was calculated in relation to CEP (V_{max} = 100%).

Table 1. Relative rates of hydrolysis (V_{max}) of CEP, CAR and IMP by sonic extracts of lysogenic Pseudomonas aeruginosa isolates No. 37, 38 and 40 and of transductant colonies of Pseudomonas aeruginosa PAO 1670 rif^I IMP^R

Sonic extract	Relative rate of hydrolysis (V_{max}) in %[1]		
	CEP	CAR	IMP
No. 37 (original strain)	100	120	35
Transductant IMP^R (obtained by the phage AP-37)	100	140	35
No. 38 (original strain)	100	105	35
Transductant IMP^R (obtained by the phage AP-38)	100	135	40
No. 40 (original strain)	100	120	45
Transductant IMP^R (obtained by the phage AP-40)	100	140	45

[1]Calculations were performed according to the procedure described by Neu (1986). The rate of hydrolysis of CEP was taken for 100%. Transductant colonies were taken from selective plates containing 15 mg/l IMP. CEP = caphaloridine; CAR = carbenicillin; IMP = imipenem.

Results

All the three phages transduced determinants of antibiotic resistance to the four *P. aeruginosa* recipient strains. Transduction of the STR resistance could not be studied in recipient strain ML-M88 due to its mutational resistance to this drug. The CAR resistance determinant was transduced by all the three phages to all the four recipients. The frequency of transduction was 4.6 x 10^{-8} to 9.8 x 10^{-7}. The GEN resistance was also generally transduced (frequency 2 x 10^{-9} to 2.9 x 10^{-7}) (Fig. 1) with exception of the experimental system "phage AP-37 to ML-1008" in which no transductants appeared on media with GEN. The KAN resistance was transduced in the systems "phage AP-37 to M-88" (frequency 1.4 x 10^{-8}), "phage AP-38 to PAO-1670" (frequency 5 x 10^{-8}), "phage AP-38 to ML-1292" (frequency 1.0 x 10^{-8}), "phage AP-38 to M-88" (frequency 2.3 x 10^{-7}) and "phage AP-40 to M-88" (frequency 7 x 10^{-7}) but not in other combinations of bacteriophages with individual recipient strains. The IMP resistance determinant was transduced by all the three phages in frequency of 1.7 x 10^{-7} (system "phage AP-40 to M-88") to 3.0 x 10^{-9} (system "phage AP-37 to PAO-1670") to all the four recipient strains (Fig. 2).

All transductant colonies were tested for the presence of directly not selected but co-transduced resistance determinants (Knothe *et al.*, 1983). The transductants selected on media with IMP were resistant to five antibiotics: IMP, CAR, STR, KAR and GEN, while transductants selected on CAR, KAN, STR or GEN were resistant to a block of four of these antibiotics but not to IMP.

The eventual hydrolytic character of the IMP resistance of transductants was tested by a iodometric method of Neu (1986) (Table 1). From these results it can be concluded that the transductants tested were capable to hydrolyze IMP.

Discussion

Transduction of determinants of antibiotic resistance, including the IMP resistance, by the three wild type bacteriophages isolated from multiple drug-resistant strains of *P. aeruginosa* seems to be an important finding in view of steadily increasing occurrence of IMP-resistant strains causing deaths of patients under the neoplastic chemotherapy at the National Institute of Oncology in Bratislava (Krčméry Jr. and Trupl, 1994).

Although the identity of spectra of multiple drug-resistance and of the serotype and phagotype of the strains (i.e. No. 37, 38 and 40) might point to the possibility of a transmission of a single strain from the haemodialysis equipment to both patients, minor differences in the transduction capability (both in frequencies and in resistance spectra transduced) might indicate that the bacteriophages isolated from these strains cannot be regarded as identical.

The IMP resistance was caused in donor wild type strains and in transductants tested, at least partially, by the ability to hydrolyze this antibiotic. This hydrolytic ability against IMP can be caused in several species of bacteria by the serine type of β-lactamases (Rasmussen *et al.*, 1996). Genes regulating such hydrolysis are located either in the chromosome (Marumo *et al.*, 1996) or in plasmids (Kelly *et al.*, 1995; Senda *et al.*, 1996), however, their transferability was not tested. It can be caused in *P. aeruginosa* by the activity of metallo-β-lactamases (Senda *et al.*, 1996) which have been detected in other non-fermenting species e.g. *Stenotrophomonas maltophilia* (Kelly *et al.*, 1995).

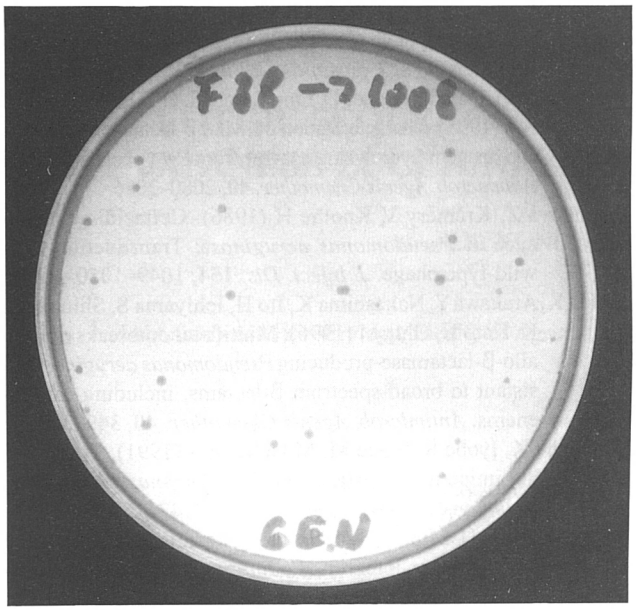

Fig. 1
Transduction of the GEN resistance from *P. aeruginosa* No. 38 to *P. aeruginosa* ML-1008 by phage AP-38
Transductant colonies of *P. aeruginosa* ML-1008 were selected on media with 30 mg/l GEN. This concentration was well above the minimal inhibitory concentration (MIC) for the recipient strain (0.9 mg/l) to avoid eventual appearance of spontaneous mutants.

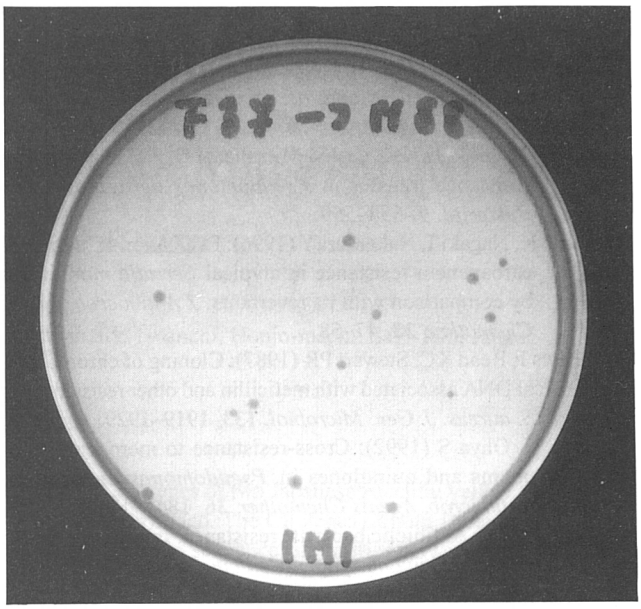

Fig. 2
Transduction of the IMP resistance from *P. aeruginosa* No. 37 to *P. aeruginosa* M-88 by phage AP-37
Transductant colonies of *P. aeruginosa* M-88 were selected on media with 15 mg/l IMP. MIC of IMP for the recipient strain was 0.45 mg/l.

Alternatively, an impairment of transport of carbapenems due to absence or production of some specific porin proteins (Masuda and Ohya, 1992), or its combination with hydrolysis, can cause a decreased susceptibility of *P. aeruginosa* to IMP and other carbapenems (Ballestero *et al.*, 1996). Our results indicate that the IMP resistance of the donor strains and transductants obtained by the three bacteriophages isolated from them was caused by their ability to hydrolyze this antibiotic. Transduction of determinants of antibiotic resistance from resistant lysogenic to susceptible *P. aeruginosa* by bacteriophages might represent an eventual mechanism of dissemination of resistance genes in an epidemical manner as it has been demonstrated for penicillinase in *Staphylococcus aureus* (Matthews *et al.*, 1987).

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