TRANSDUCTION OF IMIPENEM RESISTANCE BY THE PHAGE F-116 FROM A NOSOCOMIAL STRAIN OF *PSEUDOMONAS AERUGINOSA* ISOLATED IN SLOVAKIA

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Summary. – Generalized transducting *Pseudomonas aeruginosa* phage F-116 was propagated in an Imipenem (IMI)-resistant clinical isolate of *P. aeruginosa* No. 191 and the phage was then used to transduce the IMI-resistance to a susceptible auxotrophic mutant strain of the same species. Transduction seems to be a suitable method for study of resistance determinant(s) and mechanism of resistance in this important species. It was found that the IMI-resistance of the clinical strain No. 191 is caused by production of a betalactamase specifically hydrolyzing this antibiotic.

Key words: F-116 phage; transduction; Imipenem-resistance; *Pseudomonas aeruginosa*

Introduction

In a previous communication (Blahová *et al.,* 1993*a*) we reported our findings that many nosocomial strains of *P. aeruginosa* resistant to IMI originating from the University Clinics in Frankfurt/M., Germany, are lysogenic. From plaques of such phages we prepared bacteriophages which were used to transduce antibiotic resistance from multiple drug-resistant strains of *P. aeruginosa* to susceptible recipient strains.

IMI is the newest broad-spectrum carbapeneme antibiotic which is clinically highly effective also against strains of *P. aeruginosa.* Quite recently, IMI-resistant strains emerged in two hospitals in Bratislava, the Pediatric University Clinic, newly opened in 1991, and the National Cancer Institute (Krčméry Jr. and Trupl, 1994). In contrast to multiple resistance of strains of *P. aeruginosa* from the Frankfurt University Clinic to new anti-pseudomonadal drugs (Blahová *et al.,* 1993*a*), the first IMI-resistant strain, originating from the two hospitals in Bratislava, was resistant to IMI, but susceptible to other specific anti-pseudomonadal drugs like Ceftazidime (CTZ), Aztreonam (AZA) and Ofloxacin (OFL). None of IMI-resistant strains of *P. aeruginosa* transferred Imipenem resistance by bacterial conjugation. As IMI-resistant strains of *P. aeruginosa* were non-lysogenic, we tried to transduce the IMI-resistance by means of a generalized transducing phage, F-116, of *P. aeruginosa.* This phage has been used to map the chromosome of *P. aeruginosa* (Haas and Holloway, 1977) and to transduce genes for resistance to Meropenem, quinolones and Cefsulodin into this species (Masuda and Ohya, 1992).

Materials and Methods

Bacterial strains. The donor (host) strain of *P. aeruginosa* No. 191 was isolated at the Pediatric University Clinic in Bratislava in May 1993 from a patient receiving IMI for 2 weeks. This prototrophic wild-type strain could grown on minimal media with salts and glucose. It was resistant to IMI (minimal inhibitory concentration (MIC) 32 mg/l) and, from drugs effective against *P. aeruginosa* , to Kanamycin (KAN) (MIC 64 mg/l), Carbenicillin (CAR) (MIC 256 mg/l) and moderately resistant to Cefotaxime (CTX) (MIC 8 mg/l). As indicated, the strain was susceptible to CTZ, AZA and quinolones. A single colony obtained by streaking of the strain No. 191 on Nutrient Broth Difco with CTX plus IMI was used for propagation of the transducing phage F-116.

The recipient strain was an auxotrophic mutant of *P. aeruginosa*, designated PAO-1670 ($ade^{-}leu^{-}rif^{+}$) highly susceptible to antibiotics (MIC for IMI was 0.1 mg/l, for CTX 0.4 mg/l, for KAN 1.0 mg/l and for CAR 8 mg/l).

Bacteriophage. The transducing bacteriophage F-116 (Haas and Holloway, 1977; Masuda and Ohya, 1992) was donated to us

by Prof. S. Mitsuhashi, Maebashi, Japan, together with a PAO-1670 strain of *P. aeruginosa* which can be used for its propagation.

Propagation of bacteriophage F-116 in P. aeruginosa No. 191 was performed by double-layer method as described previously (Knothe *et al.*, 1981; Blahová *et al.*, 1993*a*). After propagation the bacteriophage-containing soft layer was washed with L-broth and centrifuged twice for 30 mins. The final titer of the bacteriophage preparation used for transduction experiments was 5×10^9 PFU/ml.

Transduction of IMI-resistance was performed as described previously (Blahová *et al.*, 1993*a*). Recipient strain PAO-1670 was shaken for 6 hrs in Nutrient Broth Difco and adjusted to 1×10^9 CFU/ml by centrifugation. Bacteriophage F-116/191 was added to obtain a multiplicity of infection of 0.5 PFU/cell and the mixture was allowed to stand at 35 °C for 1 hr for phage adsorption.

Experimental (with the phage) and control cultures (without the phage) were plated after centrifugation into the surface of L-Agar Difco plates containing individual antibiotics: 5, 10 and 15 mg/l of IMI, 10 mg/l of CTX, and 50 mg/l of KAN or CAR. The plates were then incubated overnight at 34 °C and the number of transductant colonies on each selection plate was estimated.

Spectra of transduced resistance were obtained by an indirect selection method (Blahová *et al.*, 1993*a*). 25 or 50 transductant colonies (there were no colonies on control plates) were tested for auxotrophy and rifampicin resistance of PAO-1670 and for presence of directly non-selected antibiotic resistance determinants. Individual transductant colonies were picked up into minitubes with 0.5 ml of Nutrient Broth Difco and incubated for 12 hrs. The cultures were then applied by means of a multiloop applicator technique on the surface of agar plates containing an individual antibiotic present in the spectrum of the donor strains. Thus each transductant colony was tested for the resistance to all antibiotics regardless of the drug used for its original isolation.

Betalactamase determination was performed by a micro-iodometric method described by Neu (1985). Relative hydrolytic activity (V_{max}) for individual antibiotics was always compared with that for Penicillin G (PEN-G) ($V_{max} = 100$).

Conjugal transferability of the resistance from the strain *P. aeruginosa* No. 191 was tested in mixed cultures with two rifampicin-resistant strains, PAO-1670 and ML-1008, as described previously (Blahová *et al.*, 1993*b*).

Results

Transduction of antibiotic resistance by bacteriophage F-116/191

Phage F-116 propagated on a multiple drug-resistant clinical strain of *P. aeruginosa* No. 191 transduced the IMI-resistance and also resistance to CTX, KAN and CAR to a susceptible recipient strain PAO-1670. The results are presented in Table 1 and Figs. 1-4. Although IMI-resistant transductants were selected on media with 5 – 15 mg/l of IMI they were all resistant to 15 mg/l of the drug. It seems evident that the core of the spectrum of transduced resistance is the set of three frequently used drugs, i.e. CAR, KAN, CTX. Each IMI-selected transductant was resistant to all four drugs: IMI, CAR, KAN and CTX. However, transductants selected with CAR, KAN or CTX, were resistant to the block of the three drugs mentioned above but not to IMI. This seems to indicate that the IMI resistance determinant joined the set of three-drug resistance determinants more recently in correlation to its most recent introduction into therapy in clinics which from the strain No. 191 has been isolated (Krčméry Jr. and Trupl, 1994).

Table 1. Frequency of transduction to P. aeruginosa PAO-1670 and spectra of transduced determinants of resistance to antibiotics obtained by the phage F-116/191

Antibiotic in selection medium (mg/l)	Frequency of transduction	Spectra of transduced determinants of resistance to antibiotics	
IMI (15)	9.0×10^{-7}	IMI CAR KAN CTX	(100%)
CTX (30)	6.5×10^{-8}	CTX KAN CAR	(100%)
KAN (100)	5.0×10^{-8}	KAN CAR CTX	(100%)
CAR (100)	4.5×10^{-8}	CAR KAN CTX	(100%)

The spectrum of resistance of the host strain *P. aeruginosa* No. 191 was IMI CAR KAN CTX.

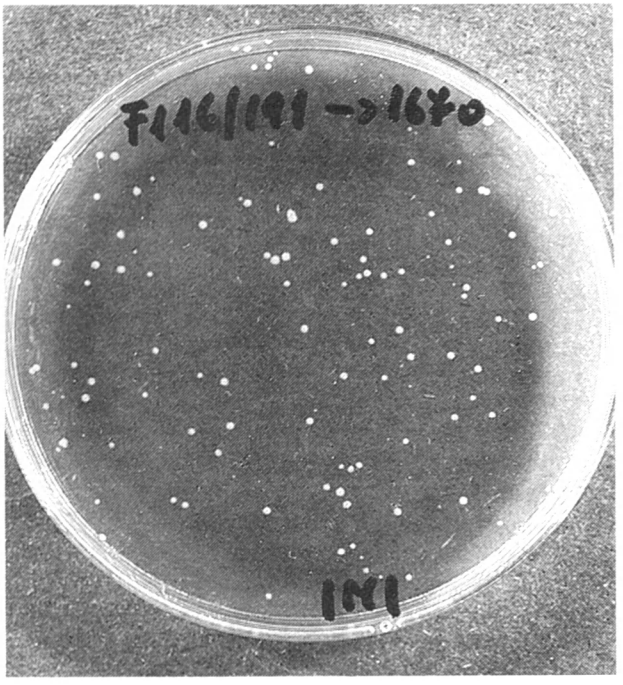

Fig. 1
Transductants produced by F-116/191 phage transduction to P. aeruginosa PAO-1670, selected on a plate with IMI (15 mg/l)

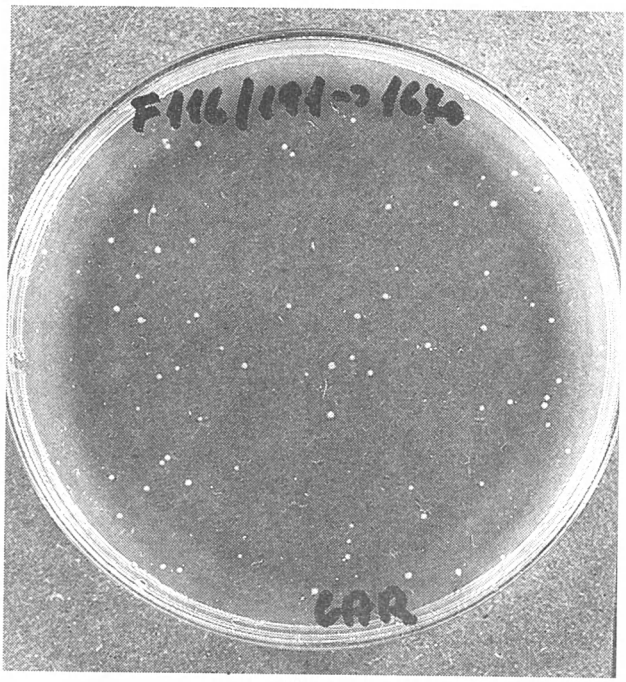

Fig. 2
Transductants produced by F-116/191 transduction to P. aeruginosa PAO-1670, selected on a plate with CAR (100 mg/l)

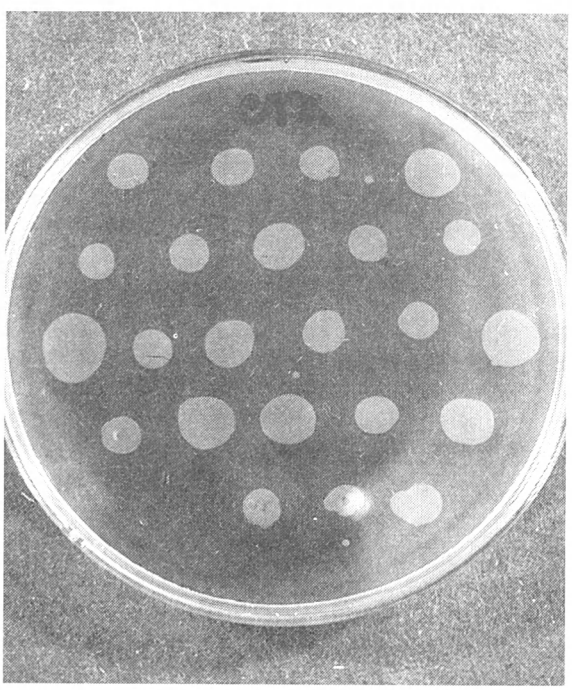

Fig. 3
Analysis of transductants by the indirect selection method on a medium with CTX (30 mg/l)
No. 24 is the control (recipient strain) which is negative due to its susceptibility. Transductants No. 1-14 were picked up from a selection plate containing CTX (30 mg/l). No. 15-23, were picked up from a plate containing IMI (15 mg/l).

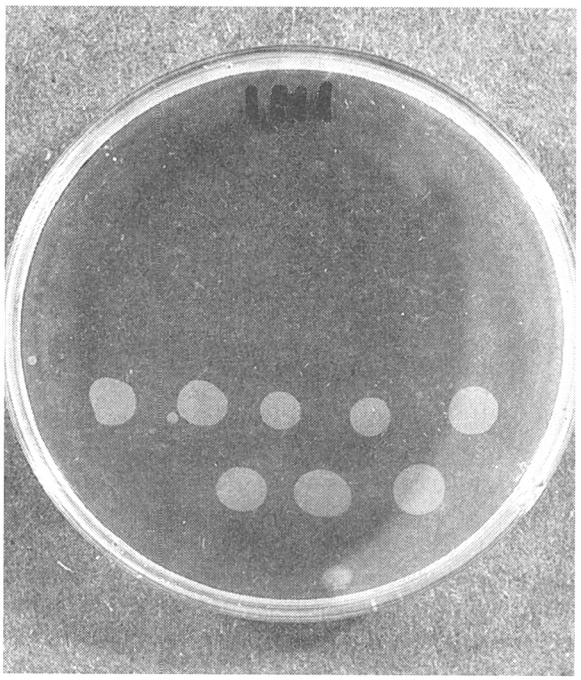

Fig. 4
Analysis of transductants on a medium with IMI (15 mg/l)
It can be seen that transductants isolated with CTX (No. 1-14) are susceptible to IMI. IMI-resistant are transductants No. 15-23 isolated with IMI (see Fig. 3).

Hydrolysis of IMI by transductants of PAO/IMI

IMI-resistant transductants of PAO-1670 were tested for the ability to hydrolyze this antibiotic. The relative rate of hydrolysis (V_{max}) of IMI was compared with that of hydrolysis of PEN-G and of CTX. Results of these experiments are presented in Table 2. The recipient strain PAO-1670 itself cannot hydrolyze IMI as it is highly susceptible to the drug. The original strain *P. aeruginosa* No. 191 and the culture of a transductant colony of PAO/IMI hydrolyzed IMI very actively in comparison with virtually no hydrolysis of IMI by lysates of two transductant colonies not possessing the determinant of resistance to IMI (selected with CTX or CAR) (Table 2). Thus by this separation of the IMI resistance determinant it could be demonstrated that the transduced gene of resistance to IMI codes in the strain PAO-1670 for production of a betalactamase capable to hydrolyze the antibiotic.

Transferability of the resistance by conjugation

In contrast to the strains of *P. aeruginosa* from Frankfurt (Hupková *et al.*, 1993), no resistance determinants were transferred from *P. aeruginosa* No. 191 to recipient strains of *P. aeruginosa* PAO-1670 or ML-1008 by conjugation.

Table 2. Hydrolysis (V_{max}) of IMI by transductants possessing and lacking the determinant of resistance to IMI

Substrate	Original strain *P. aeruginosa* No. 191[a]	Transductants (CAR)[b]	(CTX)[b]	(IMI)[a]
PEN-G	100	100	100	100
CTX	31	25	28	24
IMI	224	4	0	300

[a]IMI-resistant
[b]IMI-susceptible

Discussion

The importance of this report of the use of a generalized transducing phage like F-116 to transduce and analyze the resistance to antibiotics is stressed by the fact that first IMI-resistant strains which began to appear in Slovak hospitals were apparently non-lysogenic. They had spread as serotype O11 from the Pediatric University Clinic to the National Institute of Oncology, both in Bratislava, causing there 40 infections of patients (6 deaths) (Krčméry Jr. and Trupl, 1994).

The experiment with phage F-116 helped us in gaining new information about the resistance of *P. aeruginosa* to IMI. Firstly, it could be demonstrated in strains with much less complicated spectrum of resistance than in strains from Frankfurt University Clinic (Blahová *et al.*, 1993*a*) that the determinant of IMI-resistance was added to the spectrum of anti-pseudomonadal drugs recently and, apparently, it was not selected by the use of other drugs except IMI itself. This helps to rotate drugs like IMI with other antibiotics.

Secondly, by the separation of transductants carrying the IMI-resistance determinant from those not possessing it, it could be demonstrated that IMI-resistance was caused by production of a betalactamase hydrolyzing actively this antibiotic.

Livermore (1993) described in *P. aeruginosa* enzymes of a metallo-betalactamase type hydrolyzing IMI and other carbapenem antibiotics in non-pathogenic bacteria. Activity of this enzyme was completely blocked by EDTA (10 mg/ml). Nevertheless, the hydrolysis of IMI by original strain of *P. aeruginosa* No. 191 and by two IMI-resistant transductants was not inhibited by EDTA (data not shown). Thus, the nature of the IMI hydrolyzing enzyme in *P. aeruginosa* No. 191, studied by us is apparently from that described by Livermore (1993) and remains to be elucidated.

In non-lysogenic *P. aeruginosa* strains, the transduction of antibiotic resistance by generalized transducing phages seems to be a suitable method of separation analysis and transfer of genes determining antibiotic resistance.

References

Blahová, J., Hupková, M., Babálová, M., Krčméry, V., and Schäfer, V. (1993*a*): Transduction of resistance to imipenem, aztreonam and ceftazidime in nosocomial strains of *P. aeruginosa*. *Acta virol.* **6**, 429–436.

Blahová, J., Hupková, M., Krčméry, V., and Kuboňová, K. (1993*b*): Transfer of resistance to new betalactams in a *P. aeruginosa* from surgery by conjugation and transduction. *Biológia* **3**, 274.

Haas, D., and Holloway, B.W. (1977): Genetic organisation of arginine biosynthesis in *P. aeruginosa*. *Mol. gen. Genetic* **154**, 7–22.

Hupková, M., Blahová, J., Babálová, M., Krčméry, V., and Schäfer, V. (1993): Transferable resistance to imipenem in hospital isolates of *P. aeruginosa*. *J. Chemother.* **5**, 14–16.

Knothe, H., Lebek, G., Krčméry, V., Seginková, Z., and Mitsuhashi, S. (1981): Transduction of amikacin, gentamicin and tobramycin resistance in *P. aeruginosa* with phage F-116. *Zentralblatt f. Bakteriologie A* **250**, 506–510.

Krčméry, V.Jr., and Trupl, J. (1994): Nosocomial outbreak of meropenem resistant *P. aeruginosa* infections in a cancer centre. *J. Hosp. Infect.* **26**, 101–103.

Livermore, D. (1993): Carbapenemases, the next generation of beta-lactamases. *Amer. Soc. Microbiol. News* **59**, 129–135.

Masuda, N., and Ohya, S. (1992): Cross-resistance to meropenem, cephems and quinolones in *P. aeruginosa*. *Antimicrob. Agents Chemother.* **36**, 1847–1851.

Neu, H.C., (1985): Current mechanisms of resistance to antimicrobial agents in microorganisms causing infection in the patient at risk for infection. *Amer. J. Med.* **5**, 11–27.