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BACTERIOPHAGE TRANSDUCTION OF MOBILE GENETIC ELEMENTS IN METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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This research was aimed at characterizing transduction of mobile genetic elements in methicillin-resistant strains of Staphylococcus aureus. In this work it has been proved that bacteriophages efficiently transferred antibiotic resistance and virulence genes between clinical S. aureus strains. Furthermore, successful transduction was performed using prophages of lysogenic laboratory and clinical strains.

Introduction. *Staphylococcus aureus* is an important bacterial pathogen constituting a serious problem for human health. One of the most noticeable features of this species is the rapid evolution leading to substantial strain variability and appearance of novel and dangerous antibiotic-resistant clones [1]. This evolution is pushed forward by horizontal transfer of mobile genetic elements (MGE), such as plasmids, transposons, cassette chromosomes, pathogenicity islands and genomic islands, carrying antibiotic resistance genes and virulence factors, which provide the host bacterium with a selective advantage. The most common mechanism of horizontal gene transfer in *S. aureus* is apparently transduction, because there is a little evidence that transformation occurs and conjugative plasmids or transposons are not widespread in *S. aureus* [2]. Many transduction experiments have been conducted intending to prove the mobility of variable genetic elements with genes coding for antibiotic resistance or toxins [3, 4, 5]. Ability of bacteriophages to transduce plasmid-borne and chromosomal genes has been well documented in most staphylococcal bacteriophages of serological group B, such as φ11, φ80 and φ80α [6, 7]. An important role in transferring MGE play also prophages. As the majority of clinical *S. aureus* strains harbour one or more prophages [8], efficient transduction

can follow after prophage induction from the host strain, which was recently demonstrated in *S. aureus* USA300 clone [9].

Materials and Methods. Five clinical *S. aureus* strains (Jevons B, 07/759, 08/019, 08/629 and 08/986) were chosen as donors of plasmids (4.4kb pT181 tetracycline resistance plasmid and 28kb penicillinase plasmid of Jevons B strain, 27kb pUSA300-HOUMR-like penicillinase plasmids of 08/019, 08/629, and 08/986 strains and 31kb pUSA300-HOUMR-like penicillinase plasmid of 07/759 strain). For transduction purposes with induced phage lysate, the lysogen 07/759 (φJB$^+$) was constructed by inserting φJB into its chromosome as previously reported [10]. Three laboratory strains SA113, NCTC 8325-4, and RN4220 and two clinical strains 07/235 and 07/759 were used as recipients. Clinical genome-sequenced strain COL was used for propagation of transducing bacteriophages followed by detection and quantification of MGE in phage particles. Induction of prophages by UV light and transduction experiments were performed as described previously [9].

The plasmid DNA from donors and transductants was isolated using the High Pure Plasmid Isolation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol with following modifications of cell lysis. Eight ml of overnight culture was washed twice in phosphate buffered saline and resuspended in 235 µl of Suspension Buffer with RNase A + 15 µl of lysostaphin (Dr. Petry Genmedics, Reutlingen, Germany) (0.5 mg/ml). Then,

it was left incubating at 37 °C for 15 min. After the treatment, 250 µl of lysis buffer was added.

Bacteriophage integrase types and morphogenesis gene types corresponding to serological groups of prophages in the chromosomes of the strains were identified by multiplex PCR assay as previously reported [11].

Detection and quantification of different MGE types directly inside phage particles was performed by quantitative real-time PCR (qPCR) as described previously [12].

Results and Discussion. For the transduction experiments, well-characterized methicillin-resistant *S. aureus* strains containing different types of plasmids were used as donors for transferring these plasmids to recipient strains.

Preliminary experiments resulted in successful transfer of 4.4kb pT181 tetracycline resistance plasmid and 28kb penicillinase plasmid from methicillin-resistant donor Jevons B to laboratory recipient strains SA113, NCTC 8325-4 and RN4220. The transfer was mediated by prophage φJB induced by UV light from the chromosome of Jevons B strain to titre of 10^9 PFU/ml without the need of further propagation.

Afterwards, we focused on transferring the penicillinase and tetracycline resistance plasmids by bacteriophages φ80α and φJB between clinical isolates belonging to the USA300 clone. Phages were propagated on four donor strains 07/759, 08/019, 08/629, 08/986 possessing 27kb (31kb in case of 07/759) pUSA300-HOUMR-like penicillinase plasmids, which were successfully transferred to clinical recipient strain 07/235. As none of the USA300 donors naturally contain any tetracycline resistance plasmid, the pT181 plasmid was transduced from the Jevons B strain by means of φ80α to the strain 08/019. Subsequently, transductions of pT181 from such prepared strain were performed using φ80α and φJB into other strains of the USA300 clone.

In further experiments, 31kb penicillinase plasmid of lysogen 07/759 (φJB$^+$) was transferred into the strain 07/235 by prophage φJB. Another donor strain used was the lysogenic transductant 07/235, pUSA300-HOUMR-like (φ80α$^+$) containing the φ80α prophage and 27 kb penicillinase plasmid of the 08/986 strain. The UV-induced φ80α successfully transduced the plasmid into RN4220 strain. This shows that if the transductant is lysogenized, the plasmid can be very effectively mobilized.

The transductants obtained were tested for the presence of transferred plasmid and their genetic background was characterized in detail. In all experiments, high transduction frequencies

(10^{-5}–10^{-6} CFU/PFU) were observed (Table 1) using phages propagated on donor strains as well as prophages induced from donors by UV light.

QPCR was employed to detect penicillinase plasmids in transducing phage particles and determine the ratio of transducing particles in phage lysates to infectious phage particles (determined as approximately 1 : 1700).

Further, phages φ11, φ80, φ80α and φ81 were propagated on *S. aureus* COL strain and afterwards different types of MGE were detected inside their capsids using qPCR. These were parts of SCC*mec* (*mecA* and *ccrA1* genes), parts of SaPIs (Sa1*int* and *seb* genes), parts of genomic islands (*set5* and *lukD-lukE* genes) and genes *clfB*, *sspA* and *nuc* localized on bacterial chromosome. Total amount of bacterial DNA in phage capsids quantified by qPCR was described as $\log_{10}$ of mean gene copies per 1 ng phage DNA and frequencies of phage transducing particles carrying the targeted genes were finally calculated.

Table 1. - Transduction frequencies obtained with phages propagated on donor strains (f80a) as well as prophages induced from donors by UV light (fJB).

Donor strain	Transducing bacteriophage	Transduced plasmid (size; genes)	Recipient strain	Transduction frequency (CFU/PFU)
07/759	f80a	31kb; *blaZ*, *cadD*	07/235	1.5×10^{-5}
08/019	f80a	27kb; *blaZ*, *cadD*	07/235	9.2×10^{-6}
08/629	f80a	27kb; *blaZ*, *cadD*	07/235	1.1×10^{-5}
08/986	f80a	27kb; *blaZ*, *cadD*	07/235	7.9×10^{-6}
08/019, pT181	f80a	4.4kb; *tetK*	07/759	4.6×10^{-6}
07/759	fJB	31kb; *blaZ*, *cadD*	07/235	5.0×10^{-6}
08/019	fJB	27kb; *blaZ*, *cadD*	07/235	1.1×10^{-6}
08/629	fJB	27kb; *blaZ*, *cadD*	07/235	2.7×10^{-6}
08/986	fJB	27kb; *blaZ*, *cadD*	07/235	9.0×10^{-7}
08/019, pT181	fJB	4.4kb; *tetK*	07/759	2.8×10^{-6}
07/759 (φJB^{+})	φJB	31kb; *blaZ*, *cadD*	07/235	2.3×10^{-6}
07/235 (φ80α^{+})	φ80α	27kb; *blaZ*, *cadD*	RN4220	3.1×10^{-6}

Conclusion. The outstanding transduction abilities of serological group B phages φ80α and φJB have been proved by aforementioned experiments. Moreover, the efficient transfer of antibiotic resistance plasmids within methicillin-resistant *S. aureus* USA300 clone shows that such plasmids can be easily disseminated in bacterial populations by transducing bacteriophages. In addition to plasmids, also other types of MGE were detected inside transducing phage particles using qPCR, such as SCC*mec*, SaPIs and genomic islands. These findings indicate that bacteriophages play an important role in spreading the virulence and resistance determinants among bacterial strains and contribute to their evolution.

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