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COMPARISON OF *IN VITRO* LYTIC ACTIVITIES OF THREE BACTERIOPHAGE PREPARATIONS STAFAL®, STAPHYLON® AND PYOBACTERIOPHAGUM LIQUIDUM AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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In this work we summarize the results of in vitro susceptibility testing of methicillin-resistant S. aureus strains to bacteriophage preparation STAFAL® compared with strain susceptibilities to Pyo Bacteriophagum liquidum, Intesti Bacteriophagum liquidum and STAPHYLON® produced by Eliava Biopreparetions and Eliava Phages in Georgia.

Introduction. *Staphylococcus aureus* is one of the most common gram-positive opportunistic pathogens in humans causing from minor skin to severe systemic infections. The newly acquired genes responsible for virulence and resistance to antibiotics are rapidly disseminated in the staphylococcal population and multiple-resistant *S. aureus* strains represent a significant medical problem. Bacteriophages with a broad host range are suitable for fighting these pathogenic bacteria as an alternative to antibiotic therapy.

STAFAL® is an antistaphylococcal phage lysate for topical application, containing highly effective virulent phage particles of Twortlikevirus genus of family *Myoviridae* [1] with a strong and rapid lytic and polyvalent effect produced under GMP by IMUNA s.r.o. in the Czech Republic. The preparation is standardized in its efficacy according to the concentration not less than 1 x 10^7 of specific phage particles per 1.0 ml. STAFAL® is designed exclusively for topical application in infections caused by staphylococcal strains. It can be used both in human as well as in veterinary medicine in all forms of staphylococcal infections. It is used for the destruction of staphylococcal cells in the site of progressing infection. The preparation is administered mainly for the elimination of causative agents of staphylococcal infection in the foci of infections (e.g. purulent processes of the skin, subcutis and in skin adnex) as well as in potential reservoirs (particularly in nasopharinx, intestinal and urinary tract). STAFAL® presents a significant therapeutical agent in the complex treatment of chronic form of staphylococcal infections (purulent affections, abcesses, fistulae, infections affecting deeply located soft tissues). STAFAL® is also an important part of preventive measures in pre-operation preparation with the aim of preventing the occurrence of superponed pyogenic complications after operation interventions.

Materials and Methods. Set of 120 MRSA strains was collected from hospital microbiology departments of the Czech Republic in 1999 – 2011. The MRSA strains were classified by MLST [2], spa typing [3] and SCC*mec* typing [4] to 50 different genotypes. In addition to the commercial bacteriophage preparations, following polyvalent bacteriophages were used for susceptibility testing (their propagating strains are bracketed): K (*S. aureus* RN4220) [5], 812 (*S. aureus* CCM 4028) [6], 131 (*S. aureus* SA 6409) [7], U16 (*S. epidermidis* V505) [8] and SK311 (*S. carnosus* TM 300) [9].

Individual phage lysates were adjusted to the titer 2-3 x 10^7 PFU ml^{-1}. For estimating the susceptibility of staphylococcal strains to the phages under study, the spot test on nutrient agar plates containing calcium chloride was used. The strain tested was considered to be susceptible to a given phage if confluent or semiconfluent lysis and/or plaques were observed in the spot area, and resistant if no lysis in the spot area (no zone) was detected. If a phage tested formed a turbid spot area (turbid zone), the test was repeated three times. If at least one of these repeated tests gave confluent or semiconfluent lysis in the spot area, the tested strain was considered to be susceptible to the given phage or phage mixture.

Results and Discussion. The estimated susceptibilities of the 120 MRSA strains to bacteriophage medications and individual phages are given in Table 1. Twelve strains were completely resistant to both the preparation and all the phages tested. These resistant strains fell to sequence types ST 45, ST 80 and ST 239 whereas the susceptible strains belonged to ST 1, ST 5, ST 8, ST 20, ST 22, ST 30, ST 36, ST 111, ST 225, ST 228 and ST 247. STAFAL® exhibited *in vitro* about 10% broader host-range than Pyo-Bacteriophagum liquidum or Intesti- Bacteriophagum liquidum. Interestingly STAPHYLON® had very limited host-range and only MRSA isolates belonging to Brazilian MRSA clone (ST239/spa-type t030/SCC*mec* III) were susceptible to this medication. On the other hand this MRSA clone was resistant to STAFAL® and Pyo-Bacteriophagum liquidum.

Table 1. - Per cent of MRSA strains (n=120) which are susceptible to phage preparations and reference phages.

Phage	STAFAL®	PYO BACTERIOPHAGUM	INTESTI BACTERIOPHAGUM	K	812	131	U16	SK311
Per cent of susceptible strains	83	73	72	60	63	69	65	52

Different restriction-modification systems in the strains of distinct ST types indicate that the insensitivity of particular strains could be caused by restriction of phage DNA. Nevertheless, in the set of strains belonging to ST 5 and ST 8 that are generally susceptible to polyvalent phages, some isolates exhibited resistance to all polyvalent phages tested. In these cases, the insensitivity may be caused by a prophage which interferes with reproduction of polyvalent phages. This phenomenon was observed after laboratory lysogenization of some strains with temperate phages of the serogroup B.

Conclusion. Ninety per cent of MRSA strains tested were susceptible to at least one preparation or polyvalent phages. Broad host-range of the preparation STAFAL® (83%) indicates that its use for treatment of MRSA infections seems promising however, the clinical efficacy must be further proved. MRSA strains resistant to all *Myoviridae* phages tested belonged to ST 45, ST 80 and ST 239.

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СПЕЦИАЛИЗИРОВАННЫЙ ПРОДУКТ ПИТАНИЯ «ФУДФАГ» В ПРОФИЛАКТИКЕ ПИЩЕВЫХ ИНФЕКЦИЙ

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