**Introduction**

Staphylococcus aureus is one of the most common gram-positive 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. The recent emergence, successful spread, survival and prevalence of virulent and antibiotic resistant clones of hospital- and community-acquired methicillin-resistant S. aureus is an immensely important problem for human health. Bacteriophages with a broad host range are suitable for fighting these pathogenic bacteria as an alternative to antibiotic therapy.

STAFAL® (Fig. 1) is an antistaphylococcal phage lysate for topical application, containing highly effective virulent phage particles (Fig. 3) of Twortlikeus 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 10^10 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 therapeutic agent in the complex treatment of chronic form of staphylococcal infections (purulent affections, abscesses, 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.

Figure 1. STAFAL® and STAFAL® LYO available in liquid or lyophilized form.

**Objectives**

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 Pypo-Bacteriophagum liquidum, Intesti-Bacteriophagum liquidum and STAPHYLON® produced by Eliava Biopreparations and Eliava Phages in Georgia.

**Materials and Methods**

**Origin of bacterial strains**

One hundred and twenty MRSA strains of human origin and veterinary origin of different genotypes were collected by hospital microbiology departments of the Czech Republic in 1999 – 2011.

**Genotyping of strains**

The MRSA strains were characterized by MLST (9), spa typing (3) and SCCmec typing (4). The strains were classified to 50 different genotypes defined by sequence types (ST) and SCCmec types. Twenty-four strains harbored genes for Panton-Valentine leukocidin.

**Sensitivity tests**

Individual phage medications and reference phage lysates were adjusted to the titer 2-3 x 10^7 PFU/ml. In addition to the commercial bacteriophage preparations, following polyvalent bacteriophages were used for susceptibility testing (their propagating strains are bracketed): K (S. aureus RN4220), 812 (S. aureus CCM 4028), 131 (S. aureus SA 6409) [7], U6 (S. epidermidis V605) [8] and SK311 (S. carnosus TM 300) [9]. For estimating the susceptibility of staphylococcal strains to the phages under study, the spot test was used. The strain tested was considered to be susceptible to a given phage if confluent or semi confluent 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 semi confluent lysis in the spot area, the tested strain was considered to be susceptible to the given phage or phage mixture (Fig. 2).

**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 the preparations and all the phages tested. These resistant strains fell to sequence types ST 45, ST 60 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 Pypo-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/SCCmec III) were susceptible to this medication. On the other hand this MRSA clone was resistant to STAFAL® and Pypo-Bacteriophagum liquidum. We found that 99% of 170 recent MSSA isolates tested were susceptible to STAFAL® (Fig. 4).

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 STAFAL®.

**Conclusions**

- Ninety five per cent of MRSA strains tested were susceptible to at least one preparation or polyvalent phages.
- Bacteriophages used in STAFAL® and the preparations from Eliava Institute are related but slightly different in the host-range.
- 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.

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

<table>
<thead>
<tr>
<th>Phage</th>
<th>STAFAL®</th>
<th>PYO</th>
<th>INTESTI</th>
<th>K 812</th>
<th>131</th>
<th>U6</th>
<th>SK311</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per cent of susceptible strains</td>
<td>83</td>
<td>73</td>
<td>72</td>
<td>60</td>
<td>63</td>
<td>69</td>
<td>65</td>
</tr>
</tbody>
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**Fig. 4. Susceptibility of S. aureus to STAFAL®**

2011 MSSA isolates...