Application of microbiological quantitative methods for evaluation of changes in the amount of bacteria in patients with wounds and purulent fistulas subjected to phage therapy and for assessment of phage preparation effectiveness (in vitro studies)

Lecion D1,*, Fortuna W1,2, Dąbrowska K1, Międzybrodziek R1,2, Weber-Dąbrowska B1,2, Górski A1,2

1 Bacteriophage Laboratory, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
2 Phage Therapy Unit, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

* CORRESPONDING AUTHOR:
Bacteriophage Laboratory,
Ludwik Hirszfeld Institute of Immunology and Experimental Therapy,
Polish Academy of Sciences,
Rudolfa Weigla 12,
53-114 Wrocław, Poland
Tel. +48 71 337 1172, Fax: +4871 337 2171
email: lecion@iitd.pan.wroc.pl (Dorota Lecion)

ABSTRACT

Purpose: Quantitative microbiological studies may provide important information required for successful phage therapy (PT), however methods for PT monitoring of purulent wounds and fistulas has never been reported before. Therefore our goal was to determine and apply microbiological quantitative methods (MQMs) for monitoring experimental PT.

Methods: Samples from agar plates with growing bacteria were collected using dry and wet sterile compresses, or swabs. After shaking the sample in saline the amount of bacteria in suspension was determined. The method was standardized. The MQM using compress was applied for comparison of in vitro activity of phage preparations with other agents for wound rinsing. The usefulness of this swabbing method was tested in the Phage Therapy Unit for monitoring of experimental PT of patients with chronic wounds or purulent fistulas.

Results: Minimum, maximum and standard deviation values used for standardization of the studied method showed that data repeatability was good; thus the method was used for quantitation of bacteria taken both from plates in vitro and patients samples. Effectiveness of phage preparations was compared to gentamicin in vitro. Phages were as effective as antibiotics in reducing the amount of bacteria on agar plates, and this effect was not only due to simple mechanical removal of bacteria, but dependent on their antibacterial activity. We have also observed that the results of bacteria quantitation may correlate with the local status of a wound/fistula in a particular stage of PT.

Conclusion: The standardized swabbing method of bacteria quantitation can be used for PT monitoring. Presented MQMs are simple and may help to monitor the therapy process and to decide on its duration, frequency and a kind of the phage applied. They can also be applied in other antibacterial treatment strategies.

Key words: Bacteriophages, Phage preparation, Phage therapy, Wound infection, Purulent fistula

INTRODUCTION

Bacteriophages (phages) are viruses of bacteria that can kill and lyse the bacteria they infect. After their discovery in the early 20th century, phages were used to treat bacterial diseases in people and animals. At present, increasing levels of antibiotic resistance constitute one of the main problems in medicine, so phage therapy (PT) became an alternative way to eliminate bacterial infections [1,2]. Only a few institutions in Eastern Europe continue to study and use phages as
therapeutic agents for human infections (including the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, IIET) [2]. One of the best known centers of bacteriophage study and production of phage preparations for the treatment and prophylaxis of human infections has been the George Eliava Institute of Bacteriophages, Microbiology and Virology in Tbilisi, Georgia. Both centers published many papers on successful clinical application of PT [3-5]. Recently the results of standardized controlled clinical trials on PT safety as well as effectiveness done by other groups were also published [6-9].

The Phage Therapy Unit (PTU) at the IIET has extensive experience in the bacteriophage treatment of patients suffering from chronic bacterial infections in which standard antibiotic treatment was ineffective [5]. Our recent studies have confirmed that phages can be highly effective in treating many different types of bacterial diseases. Specificity of phages and the safety of applying phages make them efficacious antibacterial agents [5,10,11] that may also be less expensive than antibiotic treatment [12].

In the Bacteriophage Laboratory of the IIET that cooperates with PTU, monitoring of the PT is carried out such as phage typing of pathogenic strains isolated from patients. The quantitative monitoring of bacteria during PT has been introduced by the Laboratory since 2009. The justification for these studies comes from our previous observations revealing that a number of patients, although treated with active preparations, did not improve as expected. PTU is an ambulatory care unit, so the best possible and safe way of collecting patients' material was the use of sterile cotton swabs or compresses (as 'stamps').

Typical quantitative methods for determination of the amount of bacteria in wounds are: wet compresses, wet swabs, microscopy (smear of swabs or homogenized tissue obtained from biopsies), Gram staining of wound smears, and others [13-15]. Cotton swabs are usually considered as possible sampling devices, but considered to provide semi-quantitative quantification [13-16]. There is no general agreement as to the results of comparison between wound tissue samples and bacterial swabs. [15,17,18]. Nevertheless, most of these comparative studies were conducted in animal models, which allow for a large number of probes to be performed. These methods, although well accepted and reliable, are difficult to transfer to human patients. Currently, quantitative biopsy is considered as the most reliable method for assessing of infection dynamics, because it allows investigators to determine the number of bacterial cells per gram of infected tissue [19]. Usually, biopsy of infected tissues alone, or pairing of biopsy and swabs, is considered the most reliable. Nevertheless, when taken from various areas of a wound, neither swabs nor biopsy are very likely to give the same results. This probability was estimated at approx. 50% (for both swabs and biopsy) [15]. Additionally, swabs offer a larger, i.e. more complete, spectrum of aerobic bacterial species than in biopsy. However, in a large wound the biodiversity of microorganisms on its surface and underneath may be impossible to detect, whether with swabs or with biopsy [13]. Some bacterial strains are less likely to be detected by biopsy than by swabs [16]. Interestingly, in the case of Staphylococcus aureus its detection by cotton swabs on the surface is connected with the presence of these bacteria in the deeper tissue [17]. Of course, technical details of swabbing are important for the result, which also correlates with the tendency for analyzing all samples in the same laboratory [16,17].

Histological methods, although probably the most reliable for monitoring of the infected wound, can be used only in high-specialized health care units and they are neither universal nor common [13]. Biopsy seems to be highly respected, but as an invasive method it requires special conditions, qualified staff and constant accessibility of a patient as probes have to be collected every 3-4 days. Therefore it is often impossible to apply [13].

The methods applied in our laboratory resemble the quantitative methods by Gardner or Levine [18,20]. Some investigators use only the swab method, mostly because of its lower costs, lower risk of additional infection resulting from the procedure and probable negative results of biopsy [21]. In pneumonia, these kinds of microbiological diagnostics were performed with cut histological brushes [22]. An ophthalmic clinic has developed an individual protocol of eye swabbing [23]. Recently, a quantitative method of bacterial count in the purulent discharge taken from the ear using a cotton swab was used as one of the parameters to test the efficacy of phage application for treatment of chronic Pseudomonas aeruginosa otitis in man during controlled clinical trial [6].

The aim of this study is to propose a simple standardized method using swabs and compresses for estimation of the number of viable bacterial cells present in an infected site, and for monitoring the effectiveness of PT during treatment of the purulent wounds and fistulas with the perspective of other microbiological applications.

### MATERIALS AND METHODS

**Patients**

Patients with different chronic bacterial infections were qualified for phage treatment in PTU under the protocol of therapeutic experiment “Experimental phage therapy of drug-resistant bacterial infections, including MRSA infections”. The detailed inclusion and exclusion criteria as well as PT protocol was described by Międzybrodzki et al. [5].

**Materials**

The method was standardized for Staphylococcus aureus, from a clinical sample, since S. aureus was the most frequent bacterial species infecting patients’ wounds. Bacterial isolates...
were stored in culture medium supplemented with 25% glycerol at −70°C. Bacterial cultures were carried out in COS medium (BioMérieux). Plate cultures were used up to 48 h (maximum 5 passages). Quantitation of bacterial number was carried out with Mueller-Hinton Agar 2 (MH2; BioMérieux) and with two different size: thick (the diameter 5 mm) or thin (the diameter 2.5 mm) sterile cotton swabs (CULTIPLAST, LP Italiana SPA) and sterile cotton compresses 25×25 mm² or 30×30 mm² (Toruth, Poland). Other materials used included sterile 0.9% NaCl for wetting swabs or compresses and sample dilutions, sterile 30 ml PP flasks. All the materials used had been purchased from qualified companies.

Staphylococcus aureus bacteriophages P4/6409, liz/80 and 676/F were derived from the IIET phage collection. These therapeutic phage lysates (produced by the BIOMED Kraków, Poland) were routinely used in the treatment in PTU. They were prepared as according to modified Ślopek's method [5].

**Standardization of the method of collecting samples for quantification of bacteria**

Staphylococcus aureus 27821 (clinical strain) culture was prepared at 2.0 MF (McFarland scale) that is 9.0×10⁶ CFU/ml. This was calculated based on the dilution method. Three independent samples of 2.0 MF were diluted in sequence from 10⁻¹ to 10⁻⁸. Each sample dilution was applied (0.1 ml) on an MH2 culture plate, and spread. After 18 h incubation bacterial colonies were counted (when there were 30 to 300 colonies per plate). Calculation was done with regard to the dilution and the final value was an average for three samples.

For standardization of the method for quantification of bacteria using compresses twenty MH2 culture plates were covered with S. aureus 27821 (2.0 MF), dried at 37ºC (30 min). Ten compresses were soaked with sterile 0.9% NaCl in sterile PP flasks, while ten other were used dry. Both wet and dry compresses were put on the surface of the culture plates previously covered with S. aureus for 10 s (the compresses were evened using a sterile plastic inoculation loop). The same person did the sampling in all cases, to ensure the same pressure on the swab or compress was applied (for repeatability of results). Next, compresses were transferred to sterile flasks with 10 ml of 0.9% NaCl (in the case of wet compresses the rest of the saline left after pre-soaking was applied). After 5 min each sample was vortexed twice for 30 s at 1600 rpm, each time. The number of viable bacteria in these saline samples was counted using the dilution method (from 10⁻¹ to 10⁻⁸). Each sample dilution was applied on an MH2 culture plate in volume of 0.1 ml, and spread. After 18 h incubation bacterial colonies were counted (when there were 30 to 300 colonies per plate). Calculation of bacterial number in sample was done with regard to its dilution and the final value was calculated per square centimeter.

For standardization of the swabbing method, S. aureus cultures were prepared as described above. Forty plates covered with bacteria were marked with 1 cm markers. Each of 20 thick swabs or 20 thin ones were rolled left and then right at a 1 cm distance (the contact time was 10 s), immersed into 2 ml of sterile 0.9% NaCl, and then processed identically to compresses. Calculation of bacterial number in sample was done with regard to its dilution and the final value was calculated per milliliter.

**Comparing antibacterial activity of phage lysates with other typical means for rinsing bacteria off the surface.**

Five Staphylococcus aureus clinical strains were selected from the IIET Microbial Collection. Tab. 1 presents MIC (minimal inhibitory concentration) values, for gentamicin, established with E-tests (BioMérieux) by the Microbiological Department of the 4th Military Clinical Hospital in Wroclaw. All these strains were susceptible (had the same pattern of sensitivity) to phages P4/6409, liz/80, 676/F used in the experimental therapy prepared by IBSS Biomed S.A. in Cracow, Poland. These three phages were selected for in vitro study due to the best activity against strains isolated from wounds in PTU patients. Water and saline were used as controls to rinse the plate surface to determine the effect of mechanical removal of bacteria from the plate.

Five milliliters of suspensions of a selected staphylococcal strain (2.0 MF) were poured on each culture plate, and excess liquid was removed from the plate. Then plates were dried at 37ºC for 30 minutes. Each sample was tested in triplicate. Control plates were not washed with any agent. Other groups were washed with 5 ml of: sterile saline, sterile deionized water, sage infusion (Salvia officinalis), gentamicin (3 mg/ml, 10–15 µg/ml = 10×MIC, 2–3 µg/ml = 2×MIC) or phages in different titers as shown in Fig. 1. Washing was done by five round moves during 3 s, then the agent was poured out, and the plate’s brim was dried on sterile blotting paper. The plates were incubated at 37ºC for 6 h. The number of bacteria was counted on the plates using the standardized compress method (as described earlier). However in non-diluted samples of gentamicin (3 mg/ml), 10⁴ PFU/ml of the phage liz/80, 10⁶ PFU/ml of the phage 676/F, 1 ml of sample was put on plate instead of 0.1 ml because the number of bacteria on plates was less than 20 colonies. Each test series was conducted for all five S. aureus strains shown in Tab. 1. Results were presented as an average from three independent experiments.

<table>
<thead>
<tr>
<th>Clinical bacterial strains</th>
<th>MIC values for gentamicin in selected Staphylococcus aureus strains.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus 27929</td>
<td>1.0 µg/ml</td>
</tr>
<tr>
<td>Staphylococcus aureus 27935</td>
<td>1.0 µg/ml</td>
</tr>
<tr>
<td>Staphylococcus aureus 27941</td>
<td>1.0 µg/ml</td>
</tr>
<tr>
<td>Staphylococcus aureus 27947</td>
<td>1.5 µg/ml</td>
</tr>
<tr>
<td>Staphylococcus aureus 27956</td>
<td>1.5 µg/ml</td>
</tr>
</tbody>
</table>
Collection of samples from patients for bacteriological quantification tests
The thick or thin swab (depending on the diameter of fistula orifice) was put inside the fistula. If the fistula was dry (with no exudate), the swab was soaked with saline before use. Then it was kept for 10 s, moved round slowly to the right 180º and back to its initial position, and immediately processed in the Laboratory. If the swab contact was not complete, which might be caused by the special characteristics of the fistula (e.g. a stricture just behind the orifice of fistula), this information was recorded in the patient’s card to enable all the tests for a particular patient to be done in the same way. Before the study started, a physician was trained at performing the method. All staphylococcal strains isolated from the fistulas were typed with phages.

RESULTS

Standardization of the method of collecting samples for quantification of bacteria in wounds and for in vitro studies
Standardization was done with clinical Staphylococcus aureus 27821 strain (2.0 MF: $9\times10^7$ CFU/ml – an average from 3 samples). We compared different variants: dry versus wet compresses; thin versus thick swabs; or dry versus wet. Ten samples were collected for each group. The results are presented in Fig. 2.

The method with sterile compresses was selected and devised both for in vitro experiments and for use in patients with extensive wounds. The swabbing method was applied to patients with purulent fistulas and small ulcerations.

In vitro studies: comparison of activity of phage preparation with other agents for rinsing bacteria off the surface
The phage activity in this test was compared with an antibiotic (i.e. gentamicin) and other rinsing agents (Fig. 1). The main criterion for choosing gentamicin was its wide use in the topical wound treatment. Comparative use of water and saline for rinsing the surface of plates was used to demonstrate the specific activity of antibacterial agents not only resulting from simple mechanical removal of bacteria.

Our data showed that rinsing the plates with sterile water, sterile saline, or salvia infusion did not result in a substantial decrease in the amount of bacteria compared to the phage suspensions or antibiotic. Washing with the phage preparation ($10^8$ PFU/ml) or gentamicin (0.3%, as in the pharmaceutical preparation) resulted in a large decrease of bacteria: up to 7 orders of magnitude. Interestingly we have observed that rinsing the plate with three different phages at the same titer of $10^8$ PFU/ml resulted in different efficacy properties towards the five staphylococcal strains tested, although they presented the same sensitivity to the phages in the phage typing procedure, which is routinely applied in our Laboratory. This illustrates the necessity of proper selection of the most active phages. In the same study, we observed the best phage activity with titers of $10^8$ PFU/ml and $10^7$ PFU/ml (in comparison to $10^4$–$10^6$ PFU/ml). Both observations contribute to important aspects that must be regarded in the PT.

Quantitative microbiological tests during PT
This preliminary study was conducted in the PTU between 2008 and 2010. The results of bacteriological quantification of swabs taken at least once before PT and once during or after PT were available for 10 patients with monoinfection (caused by S. aureus, P. mirabilis, or P. aeruginosa). Patients were monitored as out-patients, under the regimen of therapeutic experiment which is not a controlled clinical trial. They came to the PTU form remote parts of the country at their own expenses which made sampling not always possible in some cases (e.g. after the treatment). From this group we selected 6 cases to present in detail (Tab. 2). All patients were infected only by Staphylococcus aureus – a single bacterial strain was detected. Bacteriophage preparations were applied orally (3 x 10 ml per day) or locally (2-6 ml per dose) as wet compresses or for irrigation of fistulas. Microbiological samples were
Table 2. Characteristics of six patients subjected to phage therapy (PT) with staphylococcal phages.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Pathogen causing infection</th>
<th>Microbiological sampling site</th>
<th>Microbiological sampling method</th>
<th>Bacteriophage applied</th>
<th>Phage titer(^a)</th>
<th>Phage application route</th>
<th>Phage application frequency</th>
<th>Concomitant antibacterial treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right femur infection with a purulent fistula in the postoperative cicatrix: patient 1</td>
<td><em>Staphylococcus aureus</em> (n=6): patients 1-6</td>
<td>Fistula (n=5): patients 1-5</td>
<td>Swab (n=6): patients 1-6</td>
<td><em>Staphylococcus aureus</em> phages, different phages for individual patients, monotherapy (n=6): patients 1-6</td>
<td>3.65×10^7 – 6.3×10^8 PFU/ml</td>
<td>Irrigation of the fistula and wet compresses applied on the fistula orifice (n=2): patients 1, and 3</td>
<td>Irrigation of the fistula, 1-2 times daily, (n=1): patients 1-4</td>
<td>No other antibacterials applied (n=6): patients 1-6</td>
</tr>
<tr>
<td>Suppurative infection of the hip joint: patient 2</td>
<td></td>
<td>Fistula (n=5): patients 1-5</td>
<td></td>
<td></td>
<td></td>
<td>Wet compresses applied on the fistula orifice and oral administration (n=1): patient 4</td>
<td>Wet compresses, 3-5 times daily, (n=2): patients 5, and 6</td>
<td></td>
</tr>
<tr>
<td>Tibia bone infection with a purulent fistula in the postoperative cicatrix: patient 3</td>
<td></td>
<td>Ulceration (n=1): patient 6</td>
<td></td>
<td></td>
<td></td>
<td>Wet compresses applied on the ulcerations (n=1): patient 6</td>
<td>Wet compresses applied on the ulcerations (n=1): patients 5, and 6</td>
<td></td>
</tr>
<tr>
<td>Wound infection after cardiac surgery: patient 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibia bone infection with a purulent fistula in the ankle joint area: patient 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facial ulcerations: patient 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of bacteria in samples

<table>
<thead>
<tr>
<th>Patient number evaluated PT scheme</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to PT:</td>
<td>1.9×10^4 CFU/ml</td>
<td>3.5×10^4 CFU/ml</td>
<td>2.3×10^4 CFU/ml</td>
<td>2.6×10^4 CFU/ml</td>
<td>5.3×10^4 CFU/ml</td>
<td>6.0×10^3 CFU/ml</td>
</tr>
<tr>
<td>During/after PT:</td>
<td>Day 32: 2.6×10^4 CFU/ml</td>
<td>Day 7: 5.3×10^4 CFU/ml</td>
<td>Day 102: 1.0×10^5 CFU/ml</td>
<td>Day 42: 6.0×10^4 CFU/ml</td>
<td>Day 115: 3.0×10^3 CFU/ml</td>
<td>Day 14: 6.3×10^4 CFU/ml</td>
</tr>
<tr>
<td>Patient number evaluated PT scheme</td>
<td>42 days of PT</td>
<td>33 days of PT</td>
<td>32 days of PT</td>
<td>22 days of PT</td>
<td>33 days of PT</td>
<td>55 days of PT</td>
</tr>
<tr>
<td>Prior to PT:</td>
<td>2.0×10^4 CFU/ml</td>
<td>5.0×10^4 CFU/ml</td>
<td>1.5×10^5 CFU/ml</td>
<td>1.3×10^5 CFU/ml</td>
<td>1.0×10^4 CFU/ml</td>
<td>2.0×10^5 CFU/ml</td>
</tr>
<tr>
<td>During/after PT:</td>
<td>Day 7: 10 CFU/ml</td>
<td>Day 77: &lt;10 CFU/ml</td>
<td>Day 14: 6.3×10^4 CFU/ml</td>
<td>Day 20: 1.3×10^4 CFU/ml</td>
<td>Day 28: 1.4×10^4 CFU/ml</td>
<td>Day 81: 2.0×10^5 CFU/ml</td>
</tr>
</tbody>
</table>

Response to phage treatment\(^b\)

- Wound healing (n=1): patient 4
- No changes in local status of the fistula (n=2): patients 1, and 3
- Clinical improvement (n=3): patients 2, 5, and 6

Microbiological data for *Staphylococcus aureus* strains isolated form the patients:

- Sensitivity to phages\(^c\)
  - Sensitivity to all phages from the test panel (n=4): patients 1, 2, 4, and 6
  - Sensitivity only to several phages from the test panel (n=2): patients 3, and 5

- Presence of different colony types\(^d\)
  - 2 types with the same phagogram (n=1): patient 1
  - 2 types with different phagogram (n=1): patient 5
  - 3 types with different phagogram (n=1): patient 2
  - No differences (n=3): patients 3, 4, and 6

- Appearance of phage-resistant strain
  - Resistance of isolated staphylococcal strain to all phages from the test panel appeared at the end of the second cycle of PT (n=1): patient 3

\(^a\) PFU – plaque forming unit

\(^b\) In patient 2 the discharge of fistula changed from purulent to serous, and it was associated with improvement of leg mobility and general improvement of well-being, physical activity and reduction of pain observed during PT. In patient 5 the amount of discharge of fistulas gradually decreased, tissue edema and was decreased, and it was associated with reduction of pain. In patient 6 a significant reduction of ulceration dimension had been observed and it was followed by signs of epithelialization.

\(^c\) IIET Bacteriophage laboratory test panel is composed with nine *Staphylococcus aureus* phages.

\(^d\) Presence of different colony types prior to the therapy was not associated with appearance of a resistant strain. In samples from patient 3 *Staphylococcus aureus* strain was without morphological differences.
DISCUSSION

Monitoring of efficacy of treatment against an infection requires an analysis of multiple clinical factors in which microbiological methods are not sufficient. Quantitative methods are only one of many diagnostic aspects. Nevertheless, they are important for antibacterial therapy monitoring [16]. The importance of preparing the detailed method has been noted in numerous articles [15-18,23].

Our studies confirmed that a standardization of simple microbiological quantitative methods using swabs or compresses was possible: minimum, maximum, and SD values (Fig. 2) showed that data repeatability was good. Therefore we decided to conduct an in vitro study and test the robustness of our methods to determine: the efficacy of phage preparation, gentamicin and other agents used for wound rinsing. Similar studies have been conducted in other scientific units on animal models (e.g. goats or rats with model wounds or fistulas) or technical surfaces covered with bacteria (plastic, cement). In these studies, the sterile rinsing methods were saline, water, Ringer buffer, liquid soap, and various antibiotics (compared). Rinsing was carried out with press-ups, syringes, and pulse-pumps [24-27].

The comparison of phage lysozyme to other agents for wound rinsing was only possible with the in vitro study, which used culture plates covered with bacteria. The mechanical rinsing could not substantially decrease the number of bacteria on a plate lawn, in contrast to phage suspensions or antibiotic, which reduced the number of bacteria by 7 orders of magnitude. We therefore conclude that the activity of the phages tested and a typical antibiotic were comparable. We observed that the microbiological data might correlate with clinical status at a particular moment of the therapy: decrease in the amount of purulent discharge in fistulas or small wounds. In two cases (patients No. 2, No. 4, and No. 5) we could observe decrease in bacterial count in the site of infection (reduction by 1-2 log). In one of them (patient No. 4) wound healing was accompanied by almost complete eradication of bacteria (bacterial titer in the sample dropped from 2.0x10^8 CFU/ml before PT to <10 CFU/ml at the end of treatment).

CONCLUSIONS

Our results showed that the method of swabbing with microbiological quantitation can be used to study the amount of bacteria in wounds and microbiological data may correlate with the clinical status of a wound in a particular stage of therapy.
antibacterial therapy. However other bacterial strains should be verified for this correlation in further studies on larger groups of patients. The results of microbiological studies, including quantitative methods, offer important practical information for quantifying PT results. They help to evaluate the efficacy of therapy and may have an effect on therapy duration, frequency and kind of phage applied. Presented microbiological quantitative methods are simple and can be used in most microbiological laboratories.

ACKNOWLEDGMENTS

This work was financially supported by the statutory funds of IIET and the European Regional Development Fund within the Operational Program Innovative Economy, 2007-2013, Priority axis 1. Research and Development of Modern Technologies, Measure 1.3 Support for R&D projects for entrepreneurs carried out by scientific entities, Submeasure 1.3.1 Development projects as project No. POIG 01.03.01-02-003/08 entitled “Optimization of the production and characterization of bacteriophage preparations for therapeutic use.”

REFERENCES


