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Effectiveness of Polyvalent Bacterial Lysate and Autovaccines Against Upper Respiratory Tract Bacterial Colonization by Potential Pathogens: A Randomized Study

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 Olaf Zagólski
ABCDEFG 2 Paweł Stręk
BCD 3 Andrzej Kasprowicz
BCD 3 Anna Białecka

1 Department of Otorhinolaryngology, St. John Grande's Hospital, Cracow, Poland
2 Department of Otolaryngology, Jagiellonian University, Cracow, Poland
3 Centre of Microbiological Research and Autovaccines, Cracow, Poland

Corresponding Author: Olaf Zagólski, e-mail: olafzag@gmail.com
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Background: Polyvalent bacterial lysate (PBL) is an oral immunostimulating vaccine consisting of bacterial standardized lysates obtained by lysis of different strains of bacteria. Autovaccines are individually prepared based on the results of smears obtained from the patient. Both types of vaccine can be used to treat an ongoing chronic infection. This study sought to determine which method is more effective against nasal colonization by potential respiratory tract pathogens.

Material/Methods: We enrolled 150 patients with aerobic Gram stain culture and count results indicating bacterial colonization of the nose and/or throat by potential pathogens. The participants were randomly assigned to each of the following groups: 1. administration of PBL, 2. administration of autovaccine, and 3. no intervention (controls).

Results: Reduction of the bacterial count in *Streptococcus pneumoniae*-colonized participants was significant after the autovaccine ($p<0.001$) and PBL ($p<0.01$). Reduction of the bacterial count of other β-hemolytic streptococcal strains after treatment with the autovaccine was significant ($p<0.01$) and was non-significant after PBL. In *Haemophilus influenzae* colonization, significant reduction in the bacterial count was noted in the PBL group ($p<0.01$). Methicillin-resistant *Staphylococcus aureus* colonization did not respond to either treatment.

Conclusions: The autovaccine is more effective than PBL for reducing bacterial count of *Streptococcus pneumoniae* and β-hemolytic streptococci, while PBL was more effective against *Haemophilus influenzae* colonization.

MeSH Keywords: Autovaccines • Bacteria, Aerobic • Bacterial Vaccines • Nose Diseases • Pharyngeal Diseases

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Background

An increasing number of otorhinolaryngological consultations have been performed due to symptoms of chronic infection within the nose and/or throat [1]. In individuals in whom bacterial colonization of the upper respiratory tract is the only cause of the symptoms, treatment should logically consist of improving effectiveness of the mucosa-related immunological system in order to eliminate pathogens [2]. This can be achieved by using commercially available bacterial lysates or by administering an autovaccine.

Polyvalent bacterial lysate (PBL) is an oral immunostimulating vaccine consisting of bacterial standardized lysates obtained by lysis of different strains of Gram-positive and Gram-negative pathogens causing respiratory tract infections [3]. PBL exerts a therapeutic and preventive effect on acute and recurrent infections of the upper respiratory tract. It also reduces their frequency, severity, duration, and indications for antibiotics [4]. Most previous studies were conducted using PBL administered sublingually for 10 days for 3 consecutive months [3,5,6]. They confirmed a stimulating effect of PBL both on humoral and cellular immune responses and activation of the interleukin (IL)-2 receptor (IL-2Ralpha) on different lymphocyte subsets (B, CD4+ T and CD8+ T cells) involved in humoral and cellular immune responses [3]. Also, induction of cytokine synthesis (IL-2, IL-10, IL-12, interferon gamma) in the immune-competent cells that initiate and regulate immune responses proved to be significant after oral administration of PBL [3]. Generation of CD4+ and CD8+ effector T cells as well as activation and enhancement of both immunoglobulin (Ig) M memory B lymphocytes (CD24+/CD27+ cells) and IL2 receptor-expressing lymphocytes (CD25+ cells) involved either in humoral or cellular immunity proved to be markedly stimulated by oral PBL [3,5–7]. Recent studies have provided evidence that oral administration of PBL is capable of attenuating allergic airway inflammation in animal models, which may be associated with the expansion of T regulatory cells [8]. In humans, studies with oral PBL show a decrease in the frequency of upper respiratory tract infectious episodes in the short term and chronic tonsillitis in long-term follow-up [9–11]. The supposed mechanism is potentiation of the antibody-mediated arm of the immune response [11,12].

Bacterial ribosomal lysates have also been found effective as inducers of specific immune responses [13,14].

Autovaccines are individually prepared based on results of smears obtained from the patient. The pathogens are inactivated and then administered orally in increasing concentrations [15]. Autovaccines can be used to treat ongoing chronic infection and can therefore be considered therapeutic vaccines [15]. Autogenous vaccines are strain-specific, which permits treatment of infections caused by bacteria against which no classical preventive vaccine has been available [15].

Oral administration of PBL or the autovaccine initially activates gut-associated lymphoid tissue (GALT). Subsequently, it activates local immunological systems of the upper respiratory system mucosa in bronchial-associated lymphoid tissue (BALT). The active immunostimulating substance, attached to specialized endothelial cells, is presented in a processed form to lymphocytic endothelial cells, migrating to Peyer's patches and stimulating their activity. Stimulated B lymphocytes migrate from Peyer's patches to mesenteric lymphatic nodes, where they are further differentiated and subsequently migrate to the bloodstream. Competent lymphocytes are found on mucous membranes, where they are further differentiated into plasmocytes, producing different classes of antibodies [5,15,16].

The purpose of this study was to compare the results of 2 vaccination procedures for mucosal immunization – PBL and the autovaccine – in eliminating bacterial nasal and pharyngeal colonization by the most frequent potential pathogens: *Streptococcus pneumoniae*, β-hemolytic streptococci, *Haemophilus influenzae* and Methicillin-resistant *Staphylococcus aureus* (MRSA). Among the rich and diverse bacterial assemblages normally present in the adult upper respiratory tract, the pathogens examined in the current study belong to those most frequently isolated [17,18]. These effects of the autovaccine and PBL have never before been compared. In particular, we sought to determine which treatment method is more effective against the colonization of the nose and/or throat by particular potential respiratory tract pathogens.

Material and Methods

Patients

A total of 150 patients aged 15–63 years (mean=32.6, SD=17.8, 74 women and 76 men), with aerobic bacterial culture (Gram stain) results indicating bacterial colonization of the nose and/or throat by potential pathogens, were included in this prospective, randomized study. A parallel trial was conducted from January 2012 to February 2014 with allocation ratio "one": each new patient included in the study was allocated respectively to group 1, then group 2 and then group 3; the next one was allocated to group 1, repeating the cycle. They completed the trial and their results were analyzed. The medical histories of the patients included in the study were unremarkable. In the period of the study we examined 16 individuals with a history of diabetes, 3 with cancer, and 1 with confirmed immunodeficiency, who presented with aerobic bacterial culture results indicating bacterial colonization of the nose and/or throat by potential pathogens, but they were not invited to participate

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in the study. Four other patients aged 72–76, fulfilling the remaining criteria of participation in the study, were not included in the analysis since advanced age is a factor favoring bacterial colonization and infection [19]. The other 21 did not appear at the follow-up visits and were excluded. Bacterial cultures were obtained using sterile cotton-tipped wire swabs, from the region of the middle nasal meatus and/or from the surface of the palatine tonsils. The bacterial counts graded by the laboratory from 1 to 5 were analyzed. The 2 criteria for inclusion were: confirmed nasal and/or pharyngeal bacterial colonization by potential pathogens, and no history of antibiotic treatment both during the study and at least 3 weeks preceding the initial examination. The criterion for exclusion of patients with pathological results of nasal smears was abnormality in sinus CT scans suggestive of chronic rhinosinusitis. The criterion for exclusion of patients with pathological results of pharyngeal smears was evidence of chronic tonsillitis or recurrent tonsillitis with indications for tonsillectomy.

Randomization for interventions

The participants were randomized to 1 of the following interventions: 1. administration of PBL (n=52; mean age=30.3 years; 24 females, 28 males), 2. administration of autovaccine (n=50; mean age=32.5 years; 26 females, 24 males), or 3. no intervention (controls; n=48; mean age=32.2 years; 24 females, 24 males). The study sample size was estimated with the use of a minimum expected difference of 1, an estimated standard deviation of variables of 1.0, with a resulting desired test power of 0.8 and a value of $p<0.05$ considered significant. The first author generated the random allocation sequence as well as enrolling and assigning participants to the interventions. The patients were not blinded as to which treatment was administered, as only objective measures (bacterial counts and results of blood tests) were the subject of the study. The bacterial smears were repeated after 16 weeks.

Interventions

Each patient in the PBL group received one 3-mg tablet of the lysate containing 1×10^9 of each of: *Staphylococcus aureus*, *Streptococcus mitis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Moraxella catarrhalis,* and *Haemophilus influenzae* (Luivac, Sankyo Pharma, Japan), daily for 28 days, followed by a treatment-free period of 28 days. This was followed by another 28 days of treatment with PBL, after which there was a 28-day treatment-free period. The frequency of all adverse incidents was 9.3%: 3 participants complained of mild abdominal pain and another 2 of loose stools.

Oral personalized autovaccines were produced by the Centre of Microbiological Research and Autovaccines in Kraków. The microbes were isolated from smears and subsequently processed according to a standardized procedure, and underwent 3 cycles of heat-inactivation at 60°C for 4 hours. There was 1 level containing 1×10^8–1×10^9 units germs/ml. The production complied with the principles of Good Manufacturing Production rules. The autovaccine capsules were administered orally daily for 30 days, followed by a treatment-free period of 14 days. This was followed by another 30 days of treatment. The incidence of adverse effects was 7.8%: 2 participants complained of mild abdominal pains and another 2 of loose stools.

The period of administration of the oral PBL used in the study was determined by its manufacturer. The different periods of administration of the autovaccine were based on our clinical experience with this preparation.

The examination lasted 4 months with 3 scheduled patient visits. Laboratory studies were performed at baseline and at 4 and 16 weeks after treatment termination, in order to assess the condition of the immunological system. The purpose of the complete blood count and serum IgA, IgG, and IgM levels was to rule out immunodeficiency as a contraindication to treatment with PBL or the autovaccine. Levels of immunoglobulins were determined using the SERION ELISA classic method (Institut Virion/Serion GmbH, Würzburg, Germany).

Statistical analysis

To determine if there were significant differences between levels of immunoglobulins and lymphocyte count scores obtained in patients and controls, an unpaired t test was performed with Statistica software (Statsoft, Inc. Tulsa, Oklahoma, USA), version 5. A paired t test was used to establish if there were significant differences between the immunoglobulins' blood concentrations and lymphocyte levels obtained in the participants before the administration of PBL or the autovaccine, 4 and 16 weeks after the treatment termination; as well as the bacterial counts obtained in the 1st and 2nd bacteriological examinations. ANOVA was used to determine age differences of participants within groups. Prior to the examination, informed consent was obtained from all individual participants included in the study. The research plan was approved by the local medical ethics committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of the medical ethics committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Results

The mean age and sex of the patients did not significantly differ between the groups. Colonization by MRSA was confirmed in 60 participants (40 in the throat and 20 in the nose);

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Table 1. Effectiveness of treatment with autovaccine and polyvalent bacterial lysate in reducing bacterial count of pathogens colonizing the upper respiratory tract mucosa.

Bacterial species	Intervention	1st examination – mean bacterial count grade 1–5 (SD)	2nd examination – mean bacterial count grade 1–5 (SD)	Statistics
Staphylococcus aureus (MRSA*)	PBL**	4.2 (1.6)	4.1 (2.1)	NS***
	Autovaccine	4.3 (1.1)	4.2 (1.5)	NS***
	Controls	4.2 (2.0)	4.5 (2.1)	NS***
Haemophilus influenzae	PBL**	3.8 (1.2)	1.9 (0.8)	p<0.01
	Autovaccine	4.1 (2.5)	4.2 (1.9)	NS***
	Controls	3.6 (2.1)	3.8 (2.1)	NS***
Streptococcus pneumoniae	PBL**	4.3 (1.8)	2.2 (1.4)	p<0.01
	Autovaccine	4.2 (3.6)	1.1 (0.3)	p<0.001
	Controls	4.8 (3.1)	4.8 (4.2)	NS***
β-haemolytic streptococci	PBL**	4.6 (3.3)	4.5 (1.8)	NS***
	Autovaccine	4.6 (1.1)	1.7 (1.0)	p<0.01
	Controls	4.2 (2.7)	4.3 (1.7)	NS***

* MRSA – methicillin-resistant *Staphylococcus aureus*; ** PBL – polyvalent bacterial lysate; *** NS – no statistical significance.

with *Haemophilus influenza*, 31 (throat – 16 and nose – 15); with *Streptococcus pneumonia*, 23 (throat – 12, nose – 11); and with other β-hemolytic streptococci, 56 patients (throat – 48 and nose – 8). In the majority of the examined individuals, colonization by multiple pathogens was noted, but the bacterial strains were analyzed separately. Physiological flora was detected in 130 participants. Pharyngeal smears disclosed *Streptococcus viridians* in 101 participants (mean bacterial count 3.2, SD=1.1) and *Haemophilus parainfluenzae* in 18 individuals (mean bacterial count 4.3, SD=2.3). Nasal smears disclosed *Staphylococcus epidermidis* in 39 participants (mean bacterial count 2.1, SD=2.7). These results were not influenced by either PBL or the autovaccine.

Colonization by other bacteria was not analyzed. IgA, IgG, and IgM levels did not differ between patients and controls prior to the treatment initiation. Reduction of the bacterial count in *Streptococcus pneumoniae*-colonized participants was significant after the autovaccine and PBL. Reduction of the bacterial count of β-hemolytic streptococcal strains was significant after treatment with the autovaccine and non-significant after administration of PBL (Table 1). In *Haemophilus influenzae* colonization, significant reduction in the bacterial count in the colonized patients was noted exclusively in the PBL group. MRSA colonization did not respond to either treatment method. Statistically significant reduction in IgA blood level was noted in patients treated with the autovaccine 16 weeks after the treatment termination (Table 2). The same treatment method resulted in significant increase in IgG levels after the same period. Differences in levels of IgA, IgM, and IgG in the participants treated with PBL were insignificant. Total lymphocyte count prior to and after the therapy did not differ significantly after either treatment method.

Discussion

The data from this study demonstrate that there might be marked differences in the effects of treatment with the autovaccine and PBL aimed at eliminating nasal and/or pharyngeal colonization by potential pathogens. To date, the use of PBL and autovaccines has received limited research attention.

Our study is based on the clinical results obtained in groups of volunteers and its aim was not to investigate immunological mechanisms. Therefore, other tests, such as flow cytometry and the specific increase in lymphocyte counts as a parameter directly related to B cell proliferation, were not performed.

Previous reports on PBL confirmed a white blood cell count drop in the treated group as opposed to an increase in the untreated group, but there were no statistically significant differences in the intergroup or intragroup analysis [6]. There were also no clinically relevant changes in laboratory parameters after the treatment with PBL [4]. These observations were confirmed in the current study, contrary to another study reporting a statistically significant increase of serum immunoglobulins after PBL therapy [20]. The observed increase in IgG concentration after autovaccine administration suggests that it exerts a stronger influence on lymphocytes B than PBL. IgG

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Table 2. Results of serum levels of immunoglobulins (Ig) and total lymphocyte count before the treatment initiation, 4 and 16 weeks after treatment termination.

		Before treatment I	4 weeks after treatment termination II	16 weeks after treatment termination III	Statistics
Autovaccine	Ig A (mg/dl)	172	170	154	I–III p<0.05
	Ig G (mg/dl)	992	1020	1140	I–III p<0.05
	Ig M (mg/dl)	91	95	77	NS*
	Lymphocytes (%)	36.9	35.8	33.1	NS*
PBL**	Ig A (mg/dl)	193	188	197	NS*
	Ig G (mg/dl)	1044	1042	1499	NS*
	Ig M (mg/dl)	139	136	122	NS*
	Lymphocytes (%)	36.5	37.1	33.1	NS*

* NS – no statistical significance; ** PBL – polyvalent bacterial lysate; mg/dl to g/l conversion factor: 0.01.

constitutes about 2/3 of blood immunoglobulins and they can be detected in the blood after IgM class antibodies disappear (hence the significant increase in their concentration after a longer period following treatment termination) [5].

Staphylococcus aureus has been found to be hard to eliminate from the upper respiratory tract [21], which is consistent with results from the current study. It remains unclear why in some patients with the same bacteria colonizing the same anatomical region, autovaccine or PBL treatment is effective and in some it is not. Possible explanations include the fact that the presence of specific bacteria does not imply homogeneity of the flora, which was confirmed by high phenotypic diversity in *Staphylococcus aureus* strains infecting respiratory tract mucosa [22]. Nasal carriage of *Staphylococcus aureus* is a risk factor for infection, yet the interactions between *Staphylococcus aureus* and other members of the bacterial flora may determine colonization by the former [23], which was not accounted for in our study. Healthy carriers of different *Staphylococcus aureus* strains constitute the majority of individuals with nose and throat colonization by this pathogen [24].

Nasal and pharyngeal colonization by *Streptococcus pneumoniae* is a prerequisite to its spreading to the lungs or bloodstream. This organism is capable of colonizing the mucosa of the upper respiratory tract, where it can reside, multiply, and eventually overcome host defences, to invade other tissues of the host [25]. Vaccination with a specific *Streptococcus pneumoniae* vaccine has been found to be a superior protection against pneumococcal invasive disease, compared with the commercially available vaccines [26], which could confirm our observations of the superior effectiveness of autovaccines over PBL against the upper respiratory tract colonization by this pathogen.

Preparing personalized autovaccines is a complex task to perform in a large population of patients with recurrent respiratory tract infections. A commercially available PBL can be used for the population at large. Our results suggest that the autovaccine is specifically more effective in eliminating microbial colonization by different potentially pathogenic bacteria. However, its cost is approximately 4–5 times higher that that of a PBL. Before the treatment is initiated, it is necessary to consider whether the potentially better results obtained using the autovaccine compensate for the work and costs needed for its preparation.

The strengths of this study include the direct comparison of statistically significant groups of participants treated with relatively rarely-used methods. Weaknesses include the fact that there could be other factors influencing results of the bacterial cultures detecting bacterial colonization, particularly in the individuals in whom differences in the culture results were noted over time, without any intervention. A probable explanation is that the immunological system eliminated the bacteria temporarily colonizing the mucosa of the upper respiratory system. Determining the mechanism of this phenomenon could be an interesting topic of future research. Also, analysis of immunoglobulin levels and lymphocyte count is sufficient for excluding immunodeficiency. However, PBL and the autovaccine could influence the counts of different lymphocyte subsets and blood levels of humoral immune-response components, which were not examined in the current study.

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Previous reports confirmed that in bacteriological studies of nasal colonization, recovery rates might vary significantly between different types of swabs. Even the choice of the swab or sponge used for the examination could have a great impact on the laboratory result [27,28].

Several important conclusions can be drawn from this study. The results obtained will assist in evaluating patients with upper respiratory tract colonization by potential pathogens. Based on the obtained results, we suggest treatment with the autovaccine for patients with upper respiratory tract colonization by *Streptococcus pneumoniae* and β-hemolytic streptococci, and with PBL for those with *Haemophilus influenzae* colonization. Bacterial colonization by MRSA requires further studies, with an emphasis on bacteriophage therapy.

Conclusions

The autovaccine is more effective than PBL for reducing the bacterial count in patients with *Streptococcus pneumoniae* and β-hemolytic streptococci colonization, while PBL is more effective in those with colonization by *Haemophilus influenzae*. Neither method proved effective against MRSA colonization.

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