INFLUENCE OF BACTERIOTHERAPY ON NASAL AND TONSILLAR MICROBIOTA IN INFLUENZA PATIENTS

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Abstract: This paper deals with study of species composition and population level of nasal and tonsillar microbiota in adults. Contamination of mucous membranes with S.aureus, S.pyogenes, S.viridans, H. influenzae, K.pneumoniae, E. coli, S. pneumoniae, fungi of Candida genus in adults infected with influenza viruses had determined. It had been established in vitro the influence alike antagonistic activity of Bacillus subtilis and Bacillus licheniformis strains towards inhibition of bacterial growth of Staphylococcus aureus, Streptococcus pyogenes strains: after 24 hours for 24,15±0,17%, after 48 hours – to 44,65±0,22% and after 72 hours – to 58,94±0,23%. Permanent antagonistic activity had appeared onto S. pyogenes also in 24 hours, gradually increased and reached up to 73,33±0,19% in 72 hours. It was observed the decreasing of population level of pathogenic and opportunistic organisms of tonsillar and nasal mucosae against the background of complete elimination of S. aureus, K. pneumonia, S. pyogenes, S.viridans, S. epidermidis, H. influenzae, S. pneumoniae, E. coli, fungi of Candida genus ($p<0.05$).
Keywords: species composition, population level, microbiota, antagonistic activity, influenza, complication.

Introduction

Influenza is ubiquitous, but complications are more common in the intertropical zone because of local climatic and socioeconomic conditions. According to the WHO, worldwide, the annual epidemics of the disease result in about 3 to 5 million cases of severe illness, and about 250.000 to 500.000 deaths.

Viral influenza is a seasonal infection associated with significant morbidity and mortality. In the USA, more than 35,000 deaths and 200,000 hospitalizations due to influenza occur annually, and the number is increasing. Annual vaccination is the cornerstone of prevention, but some older patients may derive less benefit from immunization than otherwise fit individuals. If started promptly, antiviral medications may reduce complications of acute influenza, but increasing resistance to amantadine and perhaps neuraminidase inhibitors underscores the need for novel prevention and treatment strategies (Glezen, 2008). Pulmonary complications of influenza are most common and include primary influenza and secondary bacterial infection. Staphylococcus aureus, including methicillin-resistant strains, is an important cause of secondary bacterial pneumonia with high mortality (Rothberg, 2008).

In spite of a great quantity of modern medicines for a treatment of patients with influenza, there is still often development of bacterial complications. From one side it determined by dysfunction of systemic immunity, especially cellular section, and from another it realized due to activation of opportunistic microorganisms, which are present on the mucous membranes of nasal and tonsillar surfaces. By the Cochrane databases, staphylococci and streptococci are the most distributed pathogenic and conventionally pathogenic microbes persisted on tonsillar and nasal mucosae. Thus primary their role in development of purulent bacterial complications on the background of acute respiratory infections posted as a great (Joseph, Togawa and Shindo, 2013).

Recent scientific interest increased in the field of microbiology as well as the place and role of probiotics in pharmacotherapy of infectious diseases. There are few experimental research dedicated to positive influence of bacillus contained bacterial preparations due to their antagonistic activity towards pathogenic strains (Shobharani, Padmaja and Halami, 2014). However, analysis of available literature data proved that current clinical study with experimental microbiologic part had conducted firstly in adults with seasonal influenza A and B in Ukraine and Eastern Europe.

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It is predicted that Bifidobacterium and Bacillus strains may express antagonism versus staphylococcus and streptococcus, hence in fact it may use for prevention of secondary bacterial influenza complications.

Method

Current prospective study enrolled 107 patients aged 18-25 (average 21.7 years old) had conducted in Infectious Disease Department of Chernivtsi Municipal Clinical Hospital. All enrolled was infected with seasonal influenza viruses A and B. Gender allocation included 62 (56.9 %) females and 45 (43.1 %) – males. Investigated persons were belonging to Caucasian race. All enrolled persons having the same high risk to get influenza virus because of student activity based on the epidemiologic data being upon same exposure during communication and overcrowding. One hundred and nine young patients with clinical features and laboratory findings (acute onset with hyperthermia more 38.5^0C, headache, intoxication syndrome, etc., positive epidemiologic data) were investigated during October-December 2015 at the Dept. of Droplet Respiratory Infections in the Municipal Clinical Hospital, Chernivtsi (South Western region of Ukraine, Eastern Europe).

Research material (smears from nasopharynx, tonsils) of case (influenza virus infected persons) and control groups – 30 healthy volunteers of same age and gender allocation, had delivered to Microbiological Clinical Laboratory of Municipal Clinical Hospital (Chernivtsi, Ukraine) with purpose to evaluate a species composition and population level of nasal and tonsillar microflora. Cultures of facultative anaerobic and aerobic bacteria had cultured in an incubator (37^0C) for 24-48 hours. Obligate anaerobic bacteria had grown in the stationary anaerostat «CO2-Incubator» T-125" (Sweden, EU) during 5-7 days, rarely up to 14 days. Then received single-type colonies had studied for each genus of the microbes, from the colonies there had obtained pure cultures of obligate and facultative anaerobic and aerobic microorganisms. Pure culture identified by genus (species) by morphological, tinctorial, cultural and biochemical properties.

Identification of isolated microorganisms was done by Bergey's Manual of Systematic Bacteriology. Antagonistic activity studied by classical microbiologic experiment in vitro as simultaneous cultivation in selective nutrient media (yolk-salt agar, serum agar) of bacillus-contained probiotic and clinical strain of microbes from patients mucous membrane (S. aureus, S.pyogenes mostly).

The 67 persons infected with influenza A & B had agreed to participate with further clinical part of the research. After detailed explanation of the methodic of bacillus-contained probiotic application, they signed up the "Patient consent form" and continued the multimodality treatment with drops of bacillus-contained probiotic (content of ampule with dry powder was diluted in 2 ml of isotonic solution, then applied 2 drops into nose, 2 drops into each tonsil surface, within 7 days) and orally intake capsules of Bifidobacterium-contained probiotic by scheme (1 capsule three times per day, 7 days).

Mathematic, statistical analysis of the results was performed by the method of variation statistics with the definition of average value, average error, and probability of possible error by statistical Student's t-test by means of Biostat® PC program (USA).

Results

All enrolled patients had admitted to Infectious Department of Municipal Clinical Hospital (Chernivtsi, Bukovina) with purpose of stationary treatment. Influenza caused by A/H3N2 and A/H2N2/ in investigated young patients characterized mostly by a moderate severity course. Influenza caused by B type virus had mild severity course.

Examined patients complained on moderate headache, dry persistent cough, general weakness, louse of appetite, pain in chest (98,17±1,28), pain an eyeballs (96,33±1,80), myalgia (88,99±3,00) and arthralgia – in 76,15±4,08 cases.

The diagnosis of influenza had proved by clinical data, epidemiologic anamnesis. The result of serological authentication proved: influenza A/N2N2 – in 46,78±4,78% cases, influenza A/N3N2 – in 40,36±4,70% and B – at 6,42±2,34% patients at Bukovina (Western Ukraine) during the epidemic season 2014-2015 years.

Authors defined several microorganisms persisted on nasal and tonsillar mucous membranes of infected patients with seasonal influenza: Staphylococcus aureus, Streptococcus pyogenes, Streptococcus epidermidis, Streptococcus viridans, Streptococcus pneumoniae, Enterobacter (Escherichia, Klebsiella pneumoniae), Haemophilusinfluenzae, Candida. The foregoing bacteria were detected (in 60,55±4,68 % patients) more often as monoculture, and also as associations which include two (in 35,78±4,59% cases) and three (in 2,75±1,46% patients) species of opportunistic microorganisms. Their population level as quantitative figure reached high critical level – more than

four lgCFU/ml that proved potential possibility of development of purulent secondary complications (bacterial lobar pneumonia, acute sinusitis, lacunar tonsillitis, etc.).

Initially, the simultaneous 72 hours cultivation of Staphylococcus aureus and Streptococcus pyogenes clinical strains with the bacillus–contained probiotic had performed on selective medium (in vitro). Resulted this, Bacillus subtilis & Bacillus licheniformis as antagonistic active components of mentioned above bacterial preparation depressed the growth and reproduction of S. aureus clinical strain: after 24 hours for 24,15±0,17%, after 48 hours – to 44,65±0,22% and after 72 hours – to 58,94±0,23% comparatively with cultivation without bacillus-contained probiotic. Permanent antagonistic activity had appeared onto S. pyogenes also in 24 hours, gradually increased and reached up to 73,33±0,19% growth inhibition in 72 hours.

It was observed the decreasing of population level of pathogenic and opportunistic organisms of tonsillar and nasal mucosae against the background of complete elimination of S. aureus, K. pneumonia, S. pyogenes, S.viridans, S. epidermidis, H. influenzae, S. pneumoniae, E. coli, fungi of Candida genus ($p<0.05$).

Discussion

By current study, the quantitative and qualitative parameters of the microflora of tonsils and nose mucosa in patients infected with influenza viruses A/N2N2 and A/N3N2, B subtypes during the season from October to March, which is typically for eastern European countries and Ukraine particularly had defined; generally, the data matched with other researches. As Raid Yaqoub Yousef demonstrated (2014), staphylococcus aureus is the most commonly grown organism in the core of the tonsil and/or surface culture (65 patients out of 73) (89.05%); group A beta-hemolytic streptococci was isolated in 53.43%; Haemophilus influenza was isolated in 46.58% patients who need tonsillectomy because of recurrent chronic tonsillitis. It is well-known fact that viral respiratory infections including influenza viruses pathophysiological lead to different disorders of immune system; logically, that “did a favor” for persisting pathogenic or either conventionally pathogenic bacteria strains present over nasal and pharyngeal mucous membranes of human.

Many studies investigating the effect of probiotics conclude that they confer their benefits on health by boosting the immune system (Ashraf and Shah, 2014). Kobayashi M. et al. (2011) introduced heat-killed Lactobacillus pentosus b240 into a mouse model infected with influenza virus A/H1N1. Although their study did not show that the probiotic affected the replication of the virus and cytokine production, it showed that the probiotic regulated gene expression in the influenza viruses found in the lung of the mouse. This result is important because it demonstrates that probiotics may disarm cold and flu viruses without necessarily killing them off. The task of destroying the viruses rests with immune cells such as leucocytes and NK cells. In addition, the results demonstrated that this inhibition of influenza virus occurred in the lungs. Moreover, antiviral effect of probiotics occurs in the respiratory tract, the very organ-system most affected by respiratory viruses. By T. Kawahara, T. Takahashi (2015), Bifidobacterium, one of the major components of intestinal microflora, shows anti-influenza virus potential as a probiotic, partly through enhancement of innate immunity by modulation of the intestinal immune system. Bifidobacterium longum MM-2, a very safe bacterium in humans, was isolated from healthy humans and its protective effect against infection in a murine model shown.

The results show that species composition of tonsillar and nasal microflora in patients infected with seasonal influenza viruses A/H3N2, A/H2N2 and B type defined as different compared with healthy volunteers. Authors revealed the contamination of mucous membranes with S.aureus, S.pyogenes, S.viridans, H. influenzae, K.pneumoniae, E. coli, S. pneumoniae, fungi of Candida genus. Moreovermentioned above pathogens in 60,55±4,68 % patients detected as monoculture, and bi-associations in 35,78±4,59% cases. Obviously, high population levelof coccihardly multiplies the risk for development of secondary bacterial complications. This research provides powerful evidence that antagonistic activity of Bacillus- and Bifidobacterium-contained probiotics towards S. aureus, S. pyogenescould inhibit them up to total elimination from nasal and tonsillar microbiota in vitro and in vivo. Multimodality treatment with drops of bacillus-contained probiotic (content of ampule with dry powder was diluted in 2 ml of isotonic solution, then applied 2 drops into nose, 2 drops into each tonsil surface, within 7 days) and orally intake capsules of Bifidobacterium-contained probiotic by scheme (1 capsule three times per day, 7 days). Second microbiological investigation of tonsillar and nasal microflora after probiotics application proved the elimination of S.aureus, S.pyogenes in mostly cases ($p<0.05$). The resultsdemonstratethe effectiveness of combination probiotic therapy confirmed firstly in vitro, thus further in clinics enrolled patients within 7 days in holiatry of influenza A & B in adults.

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