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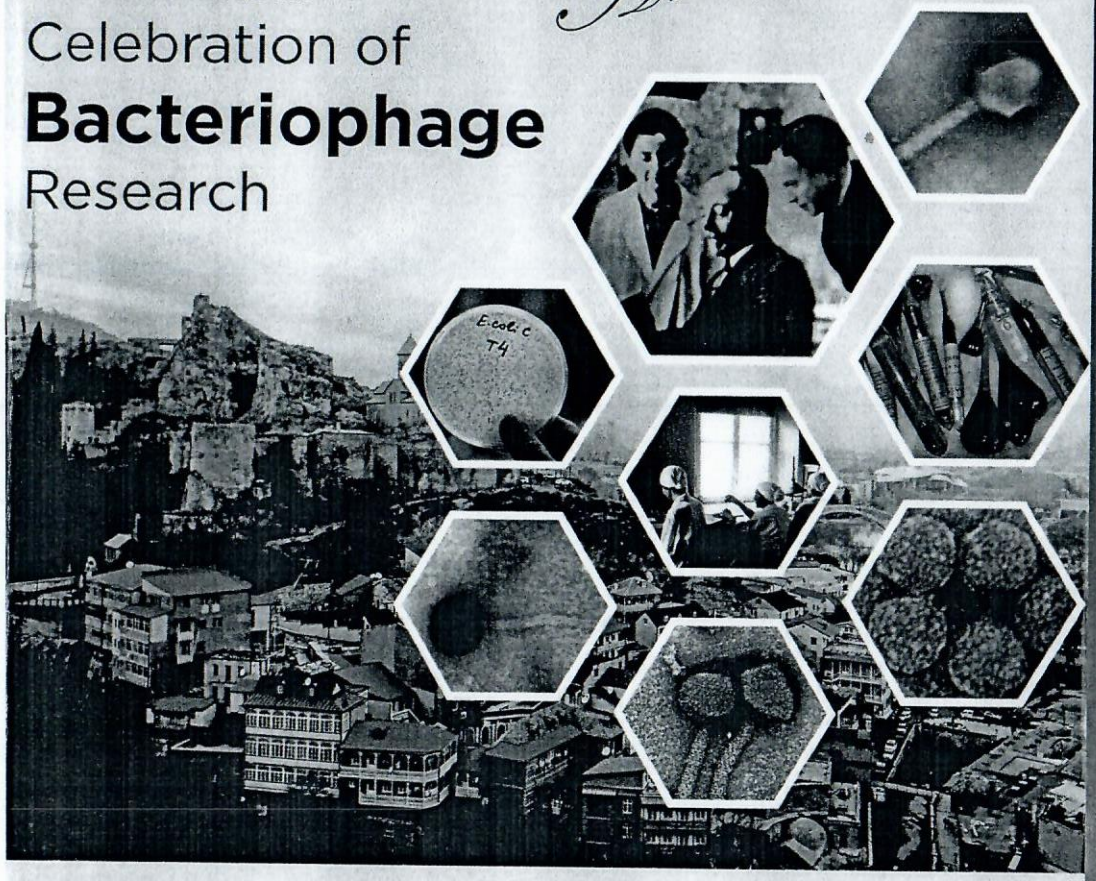
100 Anniversary

Centennial Celebration of **Bacteriophage** Research



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ABSTRACT BOOK

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P4. CHARACTERIZATION OF THE PHAGES ACTIVE AGAINST THE DRUG-RESISTANT STREPTOCOCCUS STRAINS CAUSING OF ORAL INFECTIONS

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Nowadays oral infections are a widespread problem in medicine. About 75% of diseases are caused by bacterial pathogens. Usual treatment includes antibiotics with general action, which is damaging normal oral microbiota as well. Bacteriophages have been considered as alternatives of antibiotics. Phage therapy is advantageous in comparison with antibiotics because of its high specificity and safety, no side effects have ever been registered.

The goal of our work was to isolate, characterize the phages active against *Streptococcus* sp. causing oral infections, with the purpose of their future incorporation into the medical preparation.

The research was carried out using the bacterial strains received from different dental clinics and the Eliava Diagnostic Center. Forty seven strains related to *Streptococcus* spp (*S. pyogenes, S. pneumonia, S. agalacteace, S. mitis*) isolated from the oral samples. The strains were screened against the existing phage collection relevant to *Streptococcus* sp.

Clinical *Streptococcus* strains were studied for antibiotic- and phage- susceptibility. Six strains: *S. pyogenes 33, S.pyogenes 2071, S.pyogenes 134, S.pneumonia6303, S.agalactiae279, S.agalactiae2134* demonstrating resistance to both antibiotics and phages were chosen for further studies. In particular, they were used as host strains for isolation of specific bacteriophages. As a result, six new phage clones vB_GEC_Spy_134, *vB_GEC_Spy_2071, vB_GEC_Ag_279, vB_GEC_Spy_33, vB_GEC_Ag_2134* and vB_GEC_Spn_6303 were isolated from sewage water samples. Transmission electron microscopy analysis showed, that the majority of them belong to the family-Siphoviridae, while *vB_GEC_Ag_2134* attributes to the family - Myoviridae.

The study of single growth cycles of the phages demonstrated that adsorption time for *vB_GEC_Spy_134* and *vB_GEC_Ag_M_2134* is 13-15 min during which 96-99% of phage particles are adsorbed on cell surface, length of latent period is 17-18 min., burst size-68-73 phage particles/cell. These characteristics are typical for temperate phages, therefore the above mentioned phages may not be included into the phage preparation. Adsorption time for other phages is between 6-7 min during which 90-91% of the particles are adsorbed, latent period continues for 20 min, and the burst size is 400-500 particles/cell. These parameters are typical for lytic phages.

Impact of the environmental factors (temperature and UV) on each phage was studied as well. The phages: *vB_GEC_Spy_S_134, vB_GEC_Spy_S_2071* vB_GEC_Spy_S_33 and *vB_GEC_Ag_S_279* rather high resistance to UV irradiation as their titer dropped for one log (from $1*10^9$ to $1*10^8$) after 30 min at 365 nm. Similar results were demonstrated by the phages: *vB_GEC_Ag_M_2134* and *vB_GEC_Spn_S_6303*, but after irradiation size increase of negative colonies was observed. Heat treatment of phages at 65 °C for 30 min caused decrease of phage titers for 3-4 logs. The studied phages appeared genetically diverse as well.

After incorporation of the above listed phages into the experimental series of Streptococcal mixture the host range of this preparation appeared to become broader than an initial cocktail and covered up to 70%. Thus, the studied phages were considered as candidates to be included into the Streptococcal phage cocktail.

P5. ISOLATION, PARTIAL CHARACTERIZATION OF BACTERIOPHAGES AND SCREANING FOR SUSCEPTIBILITY OF A VIRULENT ACINETOBACTER BAUMANNII STRAINS

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Background. Despite the emergence of *Acinetobacter baumannii* as a multidrug resistant worldwide pathogen with increasing prevalence, little is know about its virulence mechanisms. Since phage-encoded virulence factors are found in many bacterial pathogens we identified novel phages in *A. baumannii* strain ATCC 17978 and characterized their potential role in virulence. A. baumannii bacteriophages were also screened for potential endolysins, which are peptidoglycan (PG)-degrading proteins that allow the phage to escape from the bacterial cell during the lytic cycle resulting in a rapid cell lysis. Endolysins and bacteriophage therapy represent a promising and novel class of antibacterial agents.

Methods. Bacteriophages were purified from the sequenced strain *A. baumannii* ATCC 17978 using the QIAprep Spin M13 kit as specified by the manufacturer. Phage DNA sequences were obtained from restriction fragments of the phage genome cloned into pUC19. To identify prophage sequences within bacterial genomes of *A. baumannii* ATCC 17978 the PHAge Search Tool (PHAST) was used. Prophage samples were visualized by transmission electron microscopy (TEM) after samples were negatively stained with phosphotungstic acid (PTA). On the other hand, we are screaning for bacteriophage able to kill *A. baumannii* strain isolate for a patient. We analize susceptibility of this strain againts bacteriophages from ELIAVA Institute.

Results and conclusions. Partial sequencing and blast analysis of the *A. baumannii* ATCC 17978 genome revealed prophage DNA clustered in three chromosomal regions termed φLB1-3 which overlapped with those predicted by PHAST analysis. The φLB1-3 mix was capable of infecting *Vibrio cholerae* 0395 but not a TCP2 pilus-negative strain, suggesting that at least one of the three prophages utilize pili as a receptor. Analysis by TEM showed particles consisting of an icosahedral head and a short tail. Blast analysis of genes encoded within φLB1-3 prophages identified numerous potential virulence determinants involved in iron-acquisition, multidrug efflux, DNA methylation and peptidoglycan degradation as well numerous genes of unknown function. Importantly, we identified two endolysins (lysozymes) located in the φLB2 and φLB3 genomes which have now been recombinantly expressed and are being tested as potential anti-*A. baumannii* agents. Adittionaly, bacteriophages with different level of lisys of *A. baumannii* strain isolate from patient were found.

P15. CHARACTERIZATION AND LYTIC ACTIVITY OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) PHAGES ISOLATED FROM SEWAGE AT HOSPITAL

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Background: Methicillin-resistant Staphylococcus aureus (MRSA) is an important pathogen in humans. Currently MRSA is the most commonly identified antibiotic-resistant pathogen in hospitals that significant and enduring problem to the treatment of infections by MRSA. Therefore there is an urgent need to develop novel antibacterial agents to control this pathogen. Bacteriophages are a potential alternative treatment for MRSA infections.

Aims: Our objectives in this study were to characterize the lytic activity and morphology of methicillin-resistant Staphylococcus aureus (MRSA) phages from a tertiary pediatric hospital.

Methods: MRSA strain was isolated from patient blood. Phages were isolated from the sewage at the tertiary pediatric hospital. Lytic activity was determined with a spot test, while the titers of phage lysates were measured using the DLA technique. The morphology was assessed using electron microscopy, and the latent period time and burst size were determined.

Results: Electron microscopy showed MRSA phages' resemblance to members of the family Siphoviridae, serogroups A and F. They exhibited a latent period of 70 minutes and a relatively burst size of 2,400 plaque-forming units (PFU) / infected cell. The titer of the phages was determined to be 3x109 PFU/mL using the by DLA technique.

Conclusion: In this study, two phages from the family Siphoviridae were isolated and characterized from sewage at a tertiary pediatric hospital; these phages specifically target MRSA.

Keywords: Bacteriophage, Methicillin-Resistant Staphylococcus aureus (MRSA), Hospital Sewage

P16. AN IMPORTANCE OF THE ADAPTATION PROCEDURE FOR IMPROVEMENT OF THE QUALITY OF PHAGE-BASED PREPARATIONS

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The commercial bacteriophages-based preparation Pyo-bacteriophage composed of the phage lines active against purulent infections caused by *Staphylococcus* spp., *Streptococccus* spp., *P. aeruginosa*, *P. mirabilis* and *E.coli* was developed by Felix d'Herelle in the 1930s. Since then it has been manufactured and widely used in the FSU and post Soviet countries. According to the Soviet regulations the phage-based commercial preparations had to be regularly (minimum once per 6 months) checked for their activity and efficiency against the newly emerging strains provided from different geographical regions and clinics of the various profiles. The current Georgian regulations for phage production were developed on the base of the above mentioned Soviet approach.

The aim of the study was to check the efficiency of the existing commercial Pyo-bacteriophage against the urological strains circulating at the Tsulukidze Urology Center (Tbilisi, Georgia) and perform adaptation to these strains with the purpose of their further application in the randomized, placebo-controlled, double-blind clinical trial.

The double blind clinical trial started in January 2016. Since then through June 2017 over three hundred urine samples have been analyzed at the TUC bacteriology laboratory and 172 suitable positive cultures have been sent to Eliava IBMV, among which *E.coli* (20.4%), *Enterococcus* spp. (38.4%), and *Streptococcus* spp. (32.6,5%) predominated, other species represented only 8.6%. In 16.8% of cases the infection was caused by more than one pathogen (29 cultures). Twelve adaptation cycles were performed since the beginning of the project, including about 150 clinical strains received from the TUC during October 2014- December 2015 prior to the start of the clinical trial. The recently obtained 172 cultures showed 83% susceptibility to the commercial Pyo-bacteriophage (including sensitive - 49% and intermediate -34% results), the rest 17% of isolates appeared to be resistant (mainly the mixed cultures). Considering the fact that an initial efficacy of Pyo-bacteriophage prior to adaptations was around 37% we may conclude about an importance of this procedure for improvement of the quality of phage-based preparations The adapted phages are used in the double blind, randomized, placebo supported clinical trials. Presently 71 patients with sensitive bacterial culture are involved into the clinical trial. The final outcome of the trial will be known by the end of 2017 after un-blinding the results. However, due to the positive dynamics of phage activity achieved due to adaptation cycles it is presumed that the adapted phages might be applied as a replacement of antibiotic therapy.

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P33. BACTERIOPHAGE VB_EFM_9 ADVANTAGES AGAINST MULTI-RESISTANT ENTEROCOCCUS FAECALIS

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Background: One of the major problems in modern medicine is the rising prevalence of multidrug resistant bacteria. As resistance continues to increase and new strains of multidrug resistant bacteria are beginning to emerge, and antibiotic development is no longer progressing it seems that the only hope lies in finding new ways of treatment and Bacteriophages as an older alternative to antibiotics is coming to light. Phage therapy has a number of advantages. First of all, the frequency of resistance development is lower than the one with antibiotics. Phages are strictly specific and affect only target bacteria and do not destroy the human microbiome.

Material/methods: In this study 20 Enterococcus faecalis strains were used, isolated from January 2014 to April 2014 period. For antibiotic resistance test were used erythromycin, levofloxacin, tetracycline and vancomycin. For determine antimicrobial resistance antibiotic disk diffusion method were used. Phage vB_EfM_9 was isolated and lytic activity on these strain were studied. Biological properties such are: phage morphology, host range, growth patterns, adsorption rate, thermal and pH stability and nucleic acid composition were studied by using classic methods; Genome analysis were conducted by ARTEMIS, PFAM, GENIUS and HMMER.

Results: Antibiotic sensitivity showed that 18 strains from 20were resistant to erythromycin, 16 strains showed resistance to tetracycline, relatively low resistance were showed to vancomycin 11 strains from 20 and 7 from 20 strains were resistant to levofloxacin. Unlike antibiotics, phage vB_EfM_9 activity was significantly high, only 4 strains were resistant. Electron microscopy study shows that phage vB_EfM_9 belongs to the Myoviridae family. Host range experiment showed that phage vB_EfM_9 is very specific in lysing the respective E. faecalis strains. Phage vB_EfM_9 has high reproductive rate and is stable against thermal and UV stress. Phage vB_EfM_9 was sequenced and analyzed. According to the sequence data phage vB_EfM_9 is lytic, does not contains genes for integration and is similar to lytic phages IME-EFm1 and IME-EFm5 available in NCBI data. However IME-EFm1 and IME-EFm phages genomes contain predicted metalo-beta-lactamase genes, this gene was not found in our phage.

Conclusions: According to above mentioned results the specificity of the phage vB_EfM_9 ensures that it will not affect native bacterial flora while targeting the host of interest. Wide –spectrum antibiotics typically affect a broad range of bacterial species, including those that may be beneficial. Phage vB_EfM_9 exhibits number of properties of potential value for phage therapy.

P34. ISOLATION AND LYTIC ACTIVITIES OF BACTERIOPHAGES TO CMS

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Clavibacter michiganensis subsp. *sepedonicus* is a causative pathogen of highly infectious disease of potato bacterial ring rot. *Cms* poses a constant threat to potato growers worldwide. The pathogen's latency, ability to be present in plants without symptom development and longevity complicate both ring rot detection and control. Currently there are no resistant varieties, crop rotation is not a viable option and no pesticides are currently recommended. Management of ring rot of potato are especially difficult in storage places, where the pathogen, being in a latent form, may infect almost all tubers. Some disinfectants or fumigants are recommended on seed potatoes and during storage. Achieving sustainable agriculture necessitate the search for safer, more specific and environment-friendly control methods. Phage therapy is an attractive option for biocontrol of bacteria pathogens. The search of bacterial viruses to phytopathogens is of great importance in order to find a specific control without any harm to environment and human beings.

In potato tubers samples collected in storage places and in market, Georgia *Cms* were detected by specific molecular method. Several pure *Cms* isolates were recovered from these samples and identified by conventional PCR using specific pair of primers according to EPPO recommendations.

Four bacteriophages have been isolated from potato tubers on *Cms* isolates, # 30, 45, 96 and 97. They displayed different size small transparent plaques with diameter ranging from 1 to 2 mm. Susceptibility of *Cms* isolates to the phages have been studied. Two phage lysed almost all studied *Cms* isolates as well as *Cms* reference strain NCPPB 2137. Two other phages expressed different lytic activities to different isolates. Phages lysed also another subscecies of *C.michiganensis - Clavibacter michiganensis subsp. michiganensis*, reference strain NCPPB 2979. None of the phages lyse other species bacteria, such as isolated by us *Xanthomonas vesicatoria* and *Ralstonia solanacearum* Ref. NCPPB 4256 .

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This is to certify that

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