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Isolation and characterization of virulent phages of *Staphylococcus saprophyticus*.

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Abstract
Staphylococcus saprophyticus is a Gram-positive, coagulase-negative microorganism belongs to *Micrococcaceae* family. It is a unique uropathogen associated with uncomplicated urinary tract infections (UTIs), the second most common pathogen after *Escherichia coli*. During our investigation 11 strains of *S. saprophyticus* isolated from patients with urinary tract infections were characterized with multiple antibiotic resistances - 73% of strains revealed resistance against β-lactam antibiotics, aminoglycosides and 91% of strains were resistance towards macrolides. From environmental source two new active phages - vB_S.s.1and vB_S.s.2 - against target pathogen *S. saprophyticus* have been isolated. Phages were belonged to *Syphoviridae* family, were characterized with high percentage of adsorption and big burst size. Formation of phage resistant mutant towards both phages was low.

Keywords: Bacteriophages, bacterial strain, antibiotic resistance

Introduction

Staphylococcus saprophyticus is a Gram-positive, coagulase-negative microorganism belongs to *Micrococcaceae* family. *S. saprophyticus* is a frequent colonizer of the human gastrointestinal tract, cervix, urethra, vagina, perineum, and rectum. *S. saprophyticus* also is part of the gut and rectal flora of livestock, including pigs and cattle, and a frequent contaminant of meat and fermented food products. *S. saprophyticus* also has been recovered from polluted aquatic environments. [1, 2]

The role of *S. saprophyticus* in human disease dates from 1961 when it was recognized as the cause of urinary tract infection (UTI) in young women. [3] In 1962, Torres Pereira first isolated coagulase-negative Staphylococci with antigen 51 from women with acute UTI. This antigen was later classified as *S. saprophyticus*. [4]

S. saprophyticus is second only to *E. coli* as the most frequent causative organism of uncomplicated UTI in women. The vast majority of infections occur in young sexually active women. In females ages 16 to 25, it causes up to 42% of all infections**.** Over 40% of all young, sexually active women contain *S. saprophyticus* as part of their normal genitourinary flora. *S. saprophyticus* can also cause UTI in males of all ages. [5, 6]

Bacteria contain the urease enzymes that hydrolyze the urea to produce ammonia. The urease activity is the main factor for UTIs infection. Apart from urease activity it has numerous transporter systems to adjust against change in pH, osmolarity, and concentration of urea in human urine. [7]

After severe infections, *S. saprophyticus* causes various complications. Complications include acute pyelonephritis and nephrolithiasis and, in the case of male patients, urethritis, epididymitis, and prostatitis. BSIs with *S. saprophyticus* that are not related to urinary tract infections, such as septicemia, endocarditis. [8] Patients with nosocomial UTIs, the elderly, pregnant patients, and those with urinary catheterization have an increased incidence *S. saprophyticus* colonization. *S. saprophyticus* is also a common culprit involved in polymicrobial UTIs. [6]

Antibiotic resistance is increasing among *S. saprophyticus* strains isolated from urinary tract infection. [9] *S. saprophyticus* strains possess ability to form biofilms. Biofilms

constitute effective barriers against host-immune evasion and low urine pH. *S. saprophyticus* biofilms were reported to be resistant to antibiotics used in the empirical treatment of UTI and to biocides used for decontamination of these agents. [1] These necessitate alternative therapies. As alternatives to antibiotics bacteriophages have been attracting considerable attention. Phage products are shelf-stable, safe, easy to apply and can be regularly modified in response to changes in the susceptibility of target pathogens. [10]

Thus, it is very important the selection, study of biological properties and creation of collection of new phages against pathogenic and conditionally pathogenic multiple-resistant microorganisms causing various infectious diseases.

The aim of our investigation was isolation and study of virulent bacteriophages towards coagulase negative *S. saprophyticus.*

Material and method

Bacterial strains

In these study were used 11 strains of *S. saprophyticus*, 31 strains of *S. aureus*, 26 and 12 strains of *S. epidermidis* and *S. haemolyticus* respectively and 3 strains of *S. hominis* from G.Eliava institute collection. Cultivation of strains was performed on Brain heart infusion (BHI) medium and selective Mannitol salt agar.

To study antibiotic sensitivity of *S. saprophyticus* strains were used 20 antibiotic standard disks – amoxicillin, ampicillin, ampicillin sulbactam, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, gentamicin, amikacin, erythromycin, azithromycin, clarithromycin, doxycycline, ciprofloxacin, moxifloxacine, levofloxacin, ofloxacin, rifampicin, clindamycin, cotrimoxazol.

Antibiotic sensitivity was determined by Kirby Bayer`s method using Mueller-Hinton agar. [11]

Bacteriophages

To determine activity against *S. saprophyticus* strains by spot test were used 2 freshly isolated phages of *S. saprophyticus* (vB_S.s.1and vB_S.s.2), 5 pages of *S. aureus* and 4 phages of *S. epidermidis* from Department of Phage and Strain collection of G.Eliava institute.

For isolation of new active phages from environmental source 20 different samples were collected in from rivers in Georgia (rivers Mtkvari and Digmula).

Isolation and plating of phages from environmental samples, characterization of bacteriophages specific to S. saprophyticus - phage nucleocapside morphology, lytic spectrum, stability, parameters of the intracellular phage growth cycle (IPGC), and frequency of formation of phage resistant mutants - were done by standard methodology. [12, 13, 14]

Results and Discussion

During our investigation we studied antibiotic sensitivity of 11 strains of *S. saprophyticus* isolated from patients with urinary tract infections towards 20 antibiotics of different groups. It was found out that 73% of strains revealed resistance against β-lactam antibiotics, aminoglycosides and 91% strains were resistance towards macrolides. 64% of bacteria have showed sensitivity separately against rifampicin and doxycycline and 73% of strains towards - quinolones. Among the investigated microorganisms 5 strains were resistant against 17 antibiotics from 20. Host strain *S. saprophyticus* 129 of new virulent phages also was resistant towards 17 antibiotics and sensitive only against 3 antibiotics – ciprofloxacin, moxifloxacine and levofloxacin.

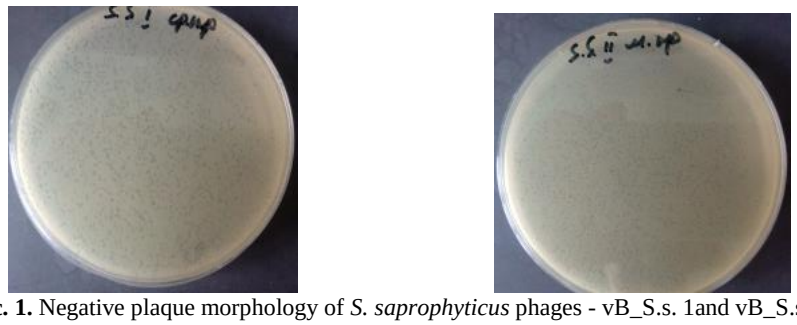
Screening of 5 phages of *S. aureus* and 4 phages of *S. epidermidis* showed that all strains of *S. saprophyticus* were resistant to S.aureus phages, while 18% of *S. saprophyticus* strains were revealed sensitivity towards 2 phages of *S. epidermidis*.

All strains of S. saprophyticus were investigated on phage content by Fisk method and were found out that none of strains contain phage.

In order to isolate new active phages against target pathogen *S. saprophyticus* the 20 series of enrichment procedures have been done and 2 primary phage isolates have been obtained.

Based on series of cloning and purification were obtained 2 phages - vB_S.s.1and vB_S.s.2 - revealing activity against one strain of *S. saprophyticus* 129.

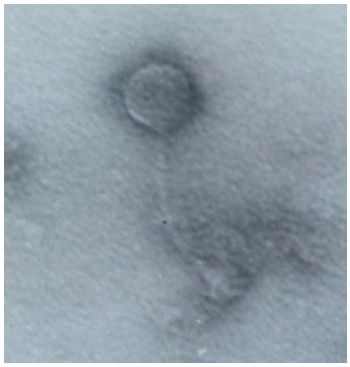
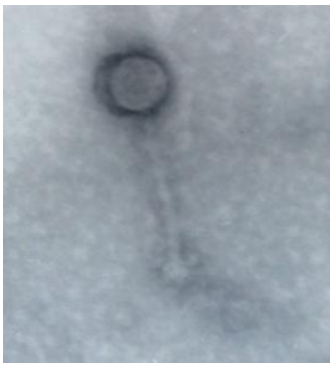
Depending on negative plaques morphology *S. saprophyticus* phages were formed similar polymorphic colonies (turbid, clear, and dotted) characterized with 1-2mm size plaques. (Picture 1)



Pic. 1. Negative plaque morphology of *S. saprophyticus* phages - vB_S.s. 1and vB_S.s. 2

The titer of phages on solid media comprised $4x10^9$pfu/ml for vB_S.s.1and $7x10^9$pfu/ml for vB_S.s.2. In liquid media titer of both phages was 10^{-4} pfu/ml. Appelman's method meintened stability of both phages during 24 hr. The phage titer remained 10^{-4} pfu/ml.

Phage clones expressing lytic activity towards *S. saprophyticus* 129 strain revealed virion morphology consistent with *Siphoviridae* family of bacteriophages. Size of head of both phages were 63,6 x 63,6 nm and the size of tail of vB_S.s.1 was 154,5x13,6nm and for vB_S.s.2 - 122,7x9nm. (Picture 2)

1.  2. 

Pic. 2. Virion morphology of *S. saprophyticus* phages – 1) vB_S.s. 1; 2) vB_S.s. 2

For investigation of range of action of new phages screening experiments were performed on 83 strains of *Stapylococcus* genera: 52 strains of coagulase negative *S. saprophyticus, S. epidermidis, S. haemolyticus, S. hominis* and 31 strains of coagulase positive *S. aureus*. Based on screening experiments new phages reviled activity only against host strain *S. saprophyticus* 129.

During investigation period was determined formation of phage resistant mutant of *S.saprophyticus* 129 strain. It was found out that frequency of formation of mutants against phage vB_S.s.1 was $0.15X10^{-7}$and towards phage vB_S.s.2 – $0,4\ X10^{-7}$.

The initial steps of host-phage interactions, namely some parameters of vB_S.s.1and vB_S.s.2 phages one step growth cycle have been determined. (Table 1)

Table 1. Virion morphology of Parameters of one step growth cycle *S. saprophyticus phages* – 1.vB_S.s.1; 2. vB_S.s.2

Phage	Strain	Adsorbtion time in min	Adsorbtion %	Latent period in min	Burst size	Lysis time in hr
vB_S.s.1	*S.saprophyticus 129*	17	82,5	120	161,3	7
vB_S.s.2		20	90	120	128	6, 5

The obtained results showed that phages reviled different adsorption time and percentage: for phage vB_S.s.1adsorbtion time was 17min and for vB_S.s.2 phage – 20min, while adsorption percentage was– 82,5% and 90% respectively. Investigated phages were characterized with non-identical burst size and lysis time - for phage vB_S.s.1 burst size was 161,3 (CFU/infected cell) and lysis time - 7hr; for vB_S.s.2 phage burst size was 128 (CFU/infected cell) and lysis time – 6,5hr. Similar for both phages was latent period 120min.

Conclusion

As a result of our work it was found that *S. saprophyticus*, which is one of the most common pathogen among Gram-positive cocci causing urinary tract infections, were characterized with multiple antibiotic resistances.

Based on efforts done during investigation period for isolation phages from environmental source two active phages vB_S.s.1and vB_S.s.2 against target pathogen have been isolated. According to phage virion morphology phages belonged to *Syphoviridae* family. Both phages revealed similar lytic spectrum towards 83 represents of related species – they were lysis only host strain *S. saprophyticus*129, but in future to determine application potential (therapeutic or diagnostic) of new phages, they will be tested on more strains of *S. saprophyticus*. The low incidence of formation of phage-resistant mutants of new *S. saprophyticus* phages is one of the important properties for their therapeutic potential. A study of one step growth cycle, have determined that investigated phages have big burst size which is main characteristic for phages potential for practical usage.

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