Intravesical bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomised, placebo-controlled, double-blind clinical trial

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Summary

Background Urinary tract infections (UTIs) are among the most prevalent microbial diseases and their financial burden on society is substantial. In the context of increasing antibiotic resistance, finding alternative treatments for UTIs is a top priority. We aimed to determine whether intravesical bacteriophage therapy with a commercial bacteriophage cocktail is effective in treating UTI.

Methods We did a randomised, placebo-controlled, clinical trial, at the Alexander Tsulukidze National Centre of Urology, Tbilisi, Georgia. Men older than 18 years of age, who were scheduled for transurethral resection of the prostate (TURP), with complicated UTI or recurrent uncomplicated UTI but no signs of systemic infection, were allocated by block randomisation in a 1:1:1 ratio to receive intravesical Pyo bacteriophage (Pyophage; 20 mL) or intravesical placebo solution (20 mL) in a double-blind manner twice daily for 7 days, or systemically applied antibiotics (according to sensitivities) as an open-label standard-of-care comparator. Urine culture was taken via urinary catheter at the end of treatment (ie, day 7) or at withdrawal from the trial. The primary outcome was microbiological treatment response after 7 days of treatment, measured by urine culture; secondary outcomes included clinical and safety parameters during the treatment period. Analyses were done in a modified intention-to-treat population of patients having received at least one dose of the allocated treatment regimen. This trial is registered with ClinicalTrials.gov, NCT03140085.

Findings Between June 2, 2017, and Dec 14, 2018, 474 patients were screened for eligibility and 113 (24%) patients were randomly assigned to treatment (37 to Pyophage, 38 to placebo, and 38 to antibiotic treatment). 97 patients (28 Pyophage, 32 placebo, 37 antibiotics) received at least one dose of their allocated treatment and were included in the primary analysis. Treatment success rates did not differ between groups. Normalisation of urine culture was achieved in five (18%) of 28 patients in the Pyophage group compared with nine (28%) of 32 patients in the placebo group (odds ratio [OR] 1·60 [95% CI 0·45–5·71]; p=0·47) and 13 (35%) of 37 patients in the antibiotic group (2·66 [0·79–8·82]; p=0·11). Adverse events occurred in six (21%) of 28 patients in the Pyophage group compared with 13 (41%) of 32 patients in the placebo group (OR 0·36 [95% CI 0·11–1·17]; p=0·089) and 11 (30%) of 37 patients in the antibiotic group (0·66 [0·21–2·07]; p=0·47).

Interpretation Intravesical bacteriophage therapy was non-inferior to standard-of-care antibiotic treatment, but was not superior to placebo bladder irrigation, in terms of efficacy or safety in treating UTIs in patients undergoing TURP. Moreover, the bacteriophage safety profile seems to be favourable. Although bacteriophages are not yet a recognised or approved treatment option for UTIs, this trial provides new insight to optimise the design of further large-scale clinical studies to define the role of bacteriophages in UTI treatment.

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For the Georgian translation of the abstract see **Online** for appendix 1

For the German translation of the abstract see **Online** for appendix 2

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Introduction

Urinary tract infections (UTIs) are the most common bacterial infections in the ambulatory care setting and are among the most prevalent health care-associated infections in the USA.[1,2] More than 8 million ambulatory visits and 100 000 hospital admissions associated with UTIs each year result in estimated direct and indirect costs of US$1·6 billion.[1,3] The increasing threat of antimicrobial resistance, mainly due to the overuse of antibiotics,[4,5] and the subsequent absence of access to effective treatments, constitute a challenge for the future.[6] UTIs have an important role in direct antibiotic

Research in context

Evidence before this study

We searched PubMed on March 1, 2019, using the search terms "bacteriophage therapy" AND "phage therapy". We searched for clinical trials for efficacy published between Jan 1, 1990, and March 1, 2019, with no language restrictions. We found two completed phase 1 or phase 2 studies on the use of bacteriophage therapy for the treatment of bacterial infections (in one case for paediatric diarrhoea and in the other study for burn wound infections). A number of case studies and case reports, as well as phase 1 studies, were also found for a number of different clinical indications and target pathogens.

The first successful experiments with bacteriophages for treating bacterial infections in children were conducted by the French-Canadian scientist Félix d'Hérelle in 1919. Since then, bacteriophage therapy has been applied in different fields of medicine for treatment of various bacterial infections. However, after the discovery of penicillin in the 1920s and its introduction in the 1940s, preference was given to antibiotic therapy, although many physicians and researchers in the former Soviet Union republics remained dedicated to bacteriophage therapy and continued to use it as a standalone treatment or in combination with antibiotics. Despite promising results in many case reports, sound evidence in agreement with recent evidence-based standards is lacking, and in the past decade, only three randomised controlled trials for efficacy have been conducted. A trial using bacteriophage therapy in children with acute diarrhoea in Bangladesh was published in 2016 and the first randomised controlled trial using a bacteriophage cocktail for topical treatment of burn wounds was published in 2019. Although both studies demonstrated good tolerability, no claim to efficacy was substantiated. Plausible reasons for such failures could be attributed to methodological limitations (ie, application of low bacteriophage titres, insufficient pathogen coverage, and static bacteriophage products) or poor product stability and specificity.

Added value of this study

To our knowledge, this study is the first randomised, placebo-controlled, double-blind trial investigating bacteriophages for treating urinary tract infections (UTIs). Bacteriophages were actively instilled into the bladder and compared with either bladder instillation of sterile bacteriophage buffer (placebo) in a double-blind manner or to systemically applied antibiotics as an open-label standard-of-care comparator. We observed non-inferiority of bacteriophages in terms of efficacy compared with antibiotics, and high tolerability and safety. However, superiority of bacteriophages over placebo was not observed, most likely because of a therapeutically relevant effect of the placebo treatment caused by unanticipated mechanical reduction of the bacterial load following repeated bladder irrigation over 7 days.

Implications of all the available evidence

Considering the alarming antibiotic resistance worldwide, alternative treatment strategies for bacterial infections are crucial. Despite promising results of bacteriophage therapy in numerous case studies, both recently and historically, the efficacy of this therapy has still not been sufficiently examined and documented in modern times. Favourable safety and tolerability results in the present and aforementioned randomised controlled trials should encourage more high-quality clinical research in humans to answer the questions raised about how to best use bacteriophages and to potentially provide a solution to the global problem of multidrug-resistant bacteria.

selection pressure as they remain among the most common indications for prescribing antibiotics.[7] Thus, well tolerated therapeutic alternatives to treat UTIs and to reduce antimicrobial resistance are highly warranted.

In 1919, Félix d'Hérelle did initial experiments with bacteriophages to treat bacterial infections in human beings and successfully treated several children at the Hôpital des Enfants Malades in Paris, France. However, the discovery of penicillin by Fleming nearly a decade later (in 1928) diminished the interest in bacteriophages in high-income countries.[8] At present, bacteriophage therapy is well accepted and registered in eastern European and post-Soviet countries such as Georgia, Ukraine, and Russia. Lately, the use of bacteriophages as a target therapy against bacterial pathogens has gained a renewed interest. Reviews involving reports of successfully applied bacteriophage therapies for different medical specialisations have been published,[8,9] and the role of bacteriophages as a possible treatment for several types of bacterial infection has been recognised.[8–11] However, there has been criticism of the related studies for not meeting current standards of evidence-based medicine.[11–15]

The role of bacteriophages in infections in the lower urinary tract is becoming increasingly interesting for various fields of research.[16] Our previous in-vitro study showed a high lytic activity of commercially available bacteriophages for the most common bacterial strains found in UTIs,[17] and Khawaldeh and colleagues[11] previously reported on the success of adjunctive bacteriophage therapy after repeated failure of antibiotics alone for the treatment of chronic UTIs. In 2019, the first randomised controlled trial using a bacteriophage cocktail produced with good manufacturing practice for the topical treatment of burn wounds was published, although the trial suffered several technical and methodological setbacks.[18] We aimed to evaluate the efficacy and safety of intravesical bacteriophage therapy in the treatment of UTI compared with placebo and standard-of-care antibiotic treatment.[19]

Methods

Study design

We did a randomised, placebo-controlled, double-blind trial in patients undergoing transurethral resection of the prostate (TURP) in a multidisciplinary setting. All patient-related affairs were conducted at the Alexander Tsulukidze National Centre of Urology, Tbilisi, Georgia. Bacteriophage preparation, the production of the bacteriophage cocktail Pyo bacteriophage (Pyophage; commercially available from Eliava BioPreparations, [Tbilisi, Georgia]) and bacteriophage sensitivity testing was done at the Eliava Institute of Bacteriophages, Microbiology, and Virology, Tbilisi, Georgia.

All eligible patients were thoroughly informed about the study and the procedures and provided written informed consent in the case of study inclusion. The study protocol was approved by the institutional review board and ethics committee at the Alexander Tsulukidze National Centre of Urology, complied with the International Conference on Harmonization Guideline for Good Clinical Practice and the Declaration of Helsinki, and was published previously elsewhere.[19]

Participants

Inclusion criteria for study participation were men (aged >18 years) scheduled for TURP, presenting with non-systemic and non-febrile UTI (including catheter-associated UTIs), defined as positive urine culture harbouring at least 10^4 colony-forming units per mL, and acute intensification of symptoms such as urgency, frequency, or dysuria. All patient urine cultures must have been positive for pathogens covered by the Pyophage cocktail (ie, *Enterococcus* spp, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus* spp, and *Streptococcus* spp) and been sensitive to bacteriophage in vitro. Patients harbouring other typical UTI pathogens, such as *Klebsiella* spp, were not eligible.[17] Local application via intravesical instillation of Pyophage, rather than systemic application, was preferred to avoid potentially more frequent and relevant side effects. The common practice of placing a suprapubic catheter for low-pressure irrigation in patients undergoing TURP was a decisive factor in choosing this patient population.

Patients with asymptomatic bacteriuria, systemic signs and symptoms of infection defined as fever (temperature >38°C), chills, malaise, or elevated inflammation parameter (C-reactive protein >100 mg/L); acute prostatitis; or any rapidly progressing disease or immediately life-threatening illness, were excluded. Further exclusion criteria were concomitant fungal UTIs, or antibiotic treatment within the 7 days before screening.

Randomisation and masking

Patients were randomly assigned to the study treatment groups in a 1:1:1 ratio in a block randomisation setting of nine patients per block, to receive either intravesical Pyophage cocktail, intravesical placebo of bacteriophage buffer, or systemically applied standard antibiotic treatment. Pyophage and placebo were given in a double-blind manner, while antibiotics were open label due to the inability to mask the administration route. The random sequence was generated by the "randomize" tool of the Stata software package. Patients were assigned to the trial groups as defined by the randomisation algorithm by two pre-designated employees of the Eliava Institute of Bacteriophages, Microbiology, and Virology. Pyophage and placebo were prepared in identical vials and delivered to the Alexander Tsulukidze National Centre of Urology. For patients allocated to antibiotic treatment, this information was passed on to the qualified employees at the trial centre. The investigator, site personnel, and monitoring staff were masked for the Pyophage and placebo treatments. The sponsor, statistician, and the microbiologists who did the microbiological evaluations at the beginning and end of the study were masked to all treatment allocations.

Procedures

Urine cultures and subsequent antibiotic sensitivity testing were done. For quantification and qualification of uropathogenic bacteria, the chromogenic UriSelect 4 media (BioRad Laboratories, Marnes-la-Coquette, France) was used. Positive urine cultures were microscopically assessed for Gram stain and morphology. If predefined pathogens potentially targeted by Pyophage were detected, urine cultures were sent for in-vitro sensitivity testing against the Pyophage cocktail with a bacterial cell lysis screening assay.[17] In case of a positive in-vitro sensitivity test concluded within 24 h after receipt of the urine culture, patients were randomly assigned to a treatment group.

The Pyophage cocktail (Georgian pharmaceutical product registration number R-022600), commercially available from Eliava BioPreparations (Tbilisi, Georgia) was used as the bacteriophage treatment intervention in order to cover a broad spectrum of uropathogenic bacteria that cause UTIs. The study product was not produced under good manufacturing practice conditions but was subject to thorough monitoring and produced in accordance with the legislative guidelines of the Georgian Pharmaceutical Agencies. Pyophage is composed of multiple individual bacteriophages active against a range of bacteria. To enhance the spectrum, streptococcus type D (ie, *Enterococcus* spp) bacteriophages were added to the cocktail. The minimum titre was 10^4 plaque-forming units per mL for streptococcus bacteriophages and 10^5 units per mL for all other bacteriophages. The maximum level of endotoxin was in line with the Georgian regulations for Pyophage and did not exceed 0·5 endotoxin units per mL. Pyophage underwent regular stability and activity checks according to local regulatory requirements. Pyophage was regularly

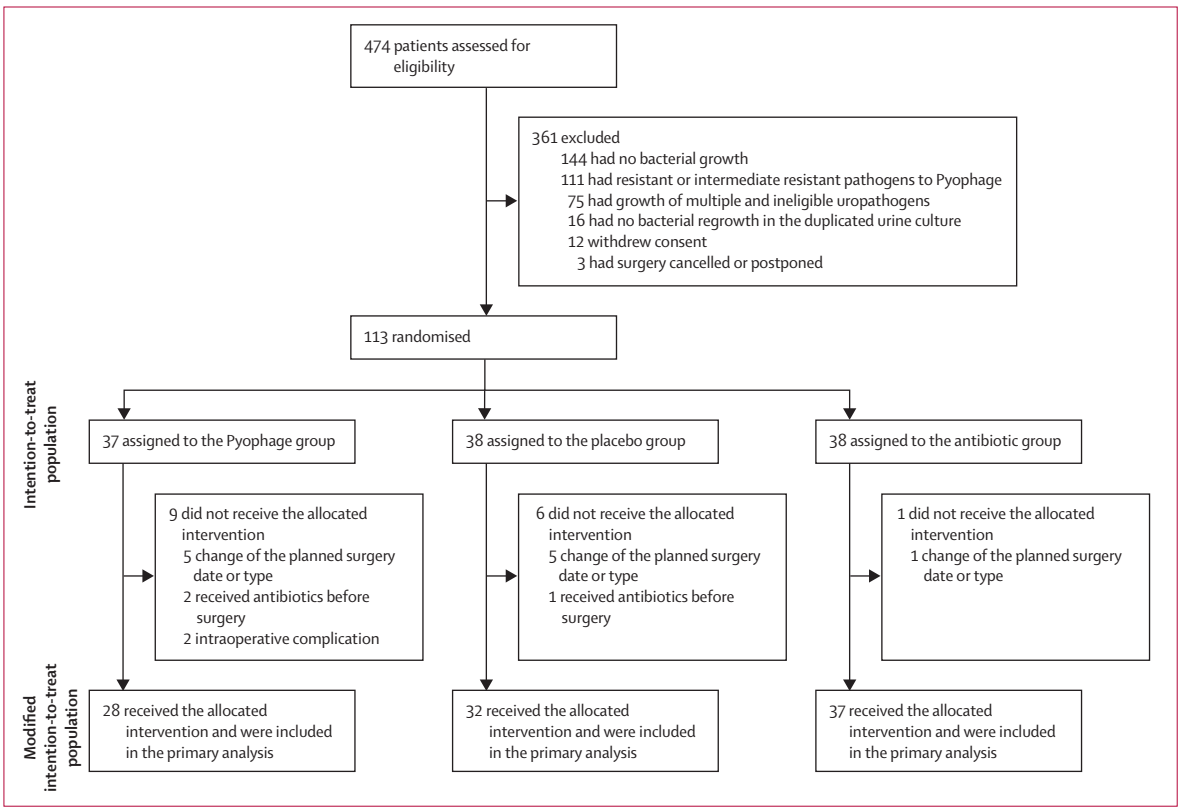

Figure 1: **Trial profile**

adapted according to an Appelmans' base liquid titration protocol for bacteriophages to enhance efficacy and coverage of newly emerging uropathogenic bacterial strains, as described previously.[15,17]

The decision to use a placebo control was justified by the exclusion of patients at high risk of developing severe infection and those with signs of systemic infection, as well as the close monitoring of study participants to allow for immediate treatment modification if required. The bacteriophage buffer used in both the bacteriophage and placebo groups consisted of standard bacteriology media and 0·9% saline, as well as quinazoline as a conservator. The placebo solution was sterile, free from bacterial debris or endotoxins, and prepared to appear visually identical to the Pyophage product in colour, odour, and container.

Antibiotic therapy for the standard-of-care comparator group was administered according to the antibiotic sensitivity test of patient isolates, with either ceftriaxone (1 g once daily intravenously), amoxicillin and clavulanic acid (1 g twice daily orally), or ciprofloxacin (500 mg twice daily orally), each starting approximately 60 min before TURP surgery.

For all patients, prostate size, serum prostate specific antigen level, International Prostate Symptom Score questionnaire[21] values, maximum urinary flow rate, post-void residual volume, bladder diary entries, and urinalysis values were collected before the TURP procedure. Resected prostate volume was documented and histological results were determined. Urine culture sampling was repeated at the end of the treatment period (ie, at the end of treatment on day 7 or at withdrawal from the trial). Preoperative administration of intravesical Pyophage or placebo solution was withheld to avoid a potential systemic bacteriophage dissemination during TURP because, even under low-pressure irrigation, fluid is absorbed through venous sinuses.

Patients in all study groups underwent monopolar TURP.[22] For low-pressure irrigation, a suprapubic catheter was placed, if not already present. Patients received twice daily administration of 20 mL of Pyophage or 20 mL of placebo, depending on treatment group, and no intravesical therapy if they were in the antibiotics group. A maximum period of 6 days was allowed between urine collection and the start of treatment. A specially trained investigator (not involved in the assessment of the clinical outcomes) delivered the solution and taught the health-care provider (for the time during the hospital stay) and the patient (for the time after discharge from hospital) how to instil the solution into the bladder. The solution was instilled using the suprapubic catheter twice per 24 h (at 0800 h and 2000 h) for 7 days, starting the first day after surgery. The health-care providers and patients were instructed to ensure the solution remained in the bladder for 30–60 min. No antibiotic prophylaxis was given to participants in the Pyophage and placebo groups.

At the end of treatment, urinalysis, urine culture, and an International Prostate Symptom Score questionnaire

were taken again for all patients. All patients underwent treatment according to Good Clinical Practice.

Outcomes

The primary outcome was microbiological treatment response at the end of therapy on day 7 or at withdrawal from the trial. A successful treatment was defined by normalisation of urine culture, measured by quantitative microbiological urine assessment (ie, log colony-forming units per mL). Post-hoc analyses were done to investigate clinical symptoms with microbiological normalisation, and the magnitude of change in recorded urine cultures. Secondary outcomes included clinical parameters; clinical parameters were based on the investigator's evaluation of patient's clinical signs and symptoms reported in the bladder and pain diary (eg, an improvement or deterioration of UTI symptoms compared with the status presented at study entry).

Safety assessment included frequency and severity of adverse events during the treatment period; adverse events were categorised according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4 as grade 1 to 5.

Statistical analysis

The protocol was designed both as a superiority trial of bacteriophage versus placebo treatment and for non-inferiority of bacteriophage versus antibiotic treatment. Considering the limited evidence from previous studies, we assumed a treatment success rate of 60% for the Pyophage group and 20% for the placebo group. Regarding the non-inferiority hypothesis, we accepted a non-inferiority margin of 35% between bacteriophage and antibiotic treatment. With a power of 80% and a type I error probability for each null hypothesis of 5%, we determined a minimum sample size of 27 patients per group. Patients withdrawn after randomisation and whom did not receive the allocated intervention were replaced to fulfil the power calculations as needed.

Interval scaled variates are summarised with means and SDs. Categorial variates are described as ratios and percentages. Efficacy and safety analyses were done in a modified intention-to-treat population, which included all randomised patients who received at least one dose of bacteriophage, placebo, or antibiotic treatment. Patients who were randomised but did not receive any dose of the allocated treatment (eg, if their surgery was postponed) were excluded from analysis. If the treatment regimen was deviated from after receiving at least one dose of the allocated treatment, or if a patient did not attend the follow-up at the 7th day after surgery, this was considered to be a failure to treat. To evaluate the clinical plausibility of the modified intention-to-treat population, a full intention-to-treat analysis was done using two different estimations for the post-randomisation excluded patients (either all excluded patients counted as successful treatment, or all excluded patients counted as failed treatment).

	Pyophage (n=28)	Placebo (n=32)	Antibiotics (n=37)
Age, years (SD)	69 (62–76)	68 (59–77)	68 (60–76)
International Prostate Symptom Score*			
Patients with symptoms	19 (13–25)	17 (8–26)	17 (9–25)
Patients with quality of life affected	5 (4–6)	4 (2–6)	4 (2–6)
Free uroflowmetry			
Maximum flow rate, mL/s	9 (6–12)	9 (5–13)	9 (6–12)
Post void residual, mL	165 (25–305)	200 (30–370)	125 (0–270)
Bladder diary			
Daytime frequency	6 (4–8)	6 (5–7)	7 (5–9)
Nighttime frequency	4 (3–5)	4 (3–5)	4 (3–5)
Mean voided volume, mL	180 (95–265)	165 (110–220)	180 (100–260)
Prostate volume, mL	72 (36–108)	76 (38–114)	79 (46–112)
PSA value, µg/L	6 (0–16)	4 (0–8)	6 (0–13)
Indwelling catheter before surgery	9 (32%)	14 (44%)	14 (38%)
Duration of catheterisation before surgery, days	31 (8–54)	37 (8–66)	24 (9–39)
Urinalysis			
Leucocytes	16 (57%)	20 (63%)	17 (46%)
Erythrocytes	12 (43%)	24 (75%)	22 (59%)
Nitrites	10 (36%)	9 (28%)	8 (22%)
Bacterial sampling			
Enterococcus spp	15 (54%)	14 (44%)	15 (41%)
Escherichia coli	5 (18%)	10 (31%)	10 (27%)
Proteus mirabilis	0	1 (3%)	2 (5%)
Pseudomonas aeruginosa	0	0	1 (3%)
Streptococcus spp	8 (29%)	7 (22%)	8 (22%)
Staphylococcus spp	0	0	1 (3%)
Colony-forming units per mL of preoperative urine culture			
10^4 to $<10^5$	0	1 (3%)	0
10^5 to $<10^6$	8 (29%)	9 (28%)	12 (32%)
10^6 to $<10^7$	15 (54%)	19 (59%)	14 (38%)
$\geq 10^7$	5 (18%)	3 (9%)	11 (30%)
Surgical parameters			
Duration of surgery, min	50 (36–64)	54 (39–69)	56 (42–70)
Resected tissue, 10^{-3} kg	38 (23–53)	36 (27–45)	33 (16–50)
Histology			
Malignancy	1 (4%)	4 (13%)	0
Chronic prostatitis	10 (36%)	8 (25%)	11 (30%)
Benign prostate hyperplasia	17 (61%)	20 (63%)	26 (70%)

The modified intention-to-treat population included all patients who received at least one dose of the allocated treatment. Data are mean (95%CIs) or n (%), unless otherwise stated. *Used to determine prostate-related bladder storage and voiding symptoms.

Table 1: **Baseline clinical characteristics of the modified intention-to-treat population**

We compared the primary and safety outcomes between groups using a logistic regression, including the baseline quality-of-life assessment, urinalysis parameters, and the way of bladder emptying (ie, spontaneous *vs* indwelling catheter) as covariates in the analysis. Unadjusted odds ratios (ORs) and adjusted ORs are presented with corresponding 95% CIs. Assessment using the Hosmer–Lemeshow goodness-of-fit test indicated a good model fit. Between-group analyses were assessed using a Kruskal-Wallis test or analysis of variance test where appropriate.

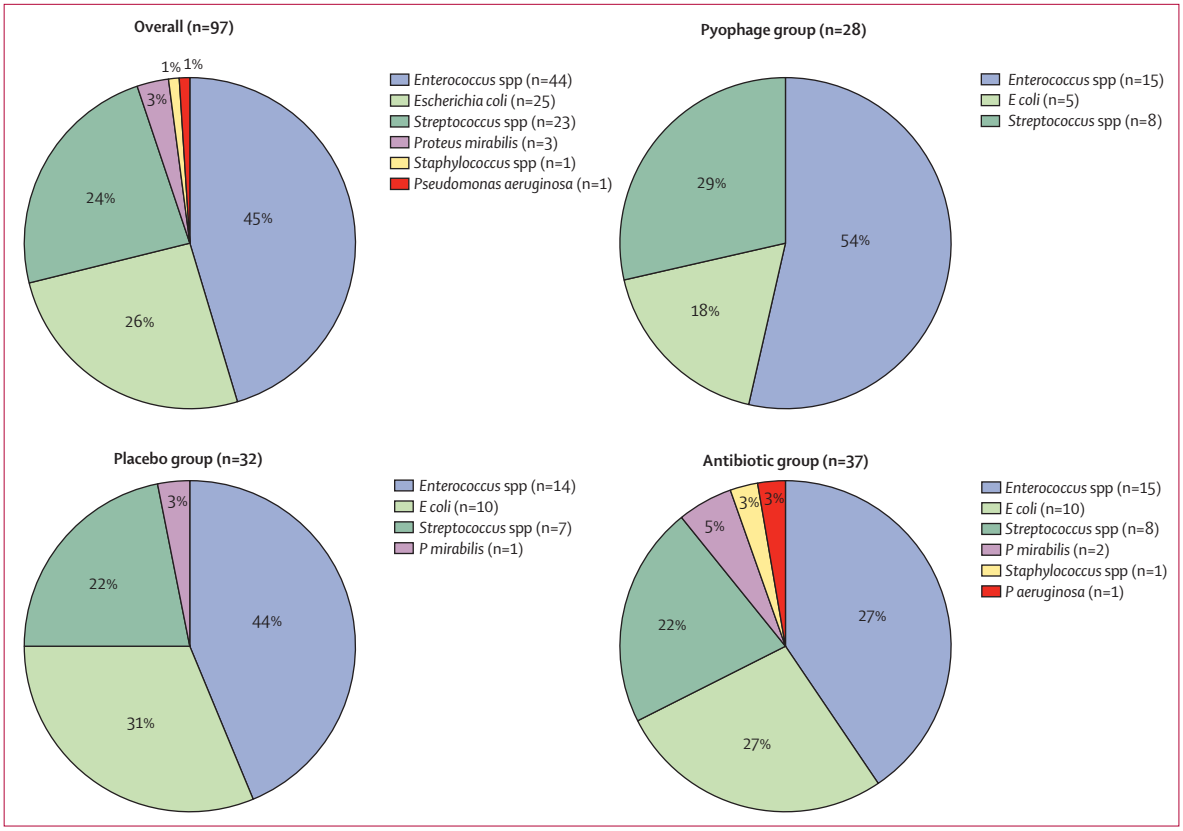

Figure 2: **Distribution of bacterial species by treatment group**

	n/N (%)	Unadjusted analysis			Adjusted analysis		
		OR	95% CIs	p value	OR	95% CIs	p value
Primary outcome: treatment success rate							
Pyophage	5/28 (18%)	1	Ref	..	1	Ref	..
Placebo	9/32 (28%)	1·80	0·52–6·19	0·35	1·60	0·45–5·71	0·47
Antibiotics	13/37 (35%)	2·49	0·77–8·10	0·13	2·66	0·79–8·82	0·11
Post-hoc analysis: reduction in colony-forming unit count per mL of urine culture post-treatment versus pre-treatment							
Pyophage	7/28 (25%)	1	Ref	..	1	Ref	..
Placebo	12/32 (38%)	1·80	0·59–5·49	0·30	1·62	0·51–5·12	0·41
Antibiotics	18/37 (49%)	2·84	0·97–8·29	0·056	2·98	1·00–8·84	0·049
Safety outcome: adverse events							
Pyophage	6/28 (21%)	1	Ref	..	1	Ref	..
Placebo	13/32 (41%)	0·39	0·13–1·25	0·12	0·36	0·11–1·17	0·089
Antibiotics	11/37 (30%)	0·65	0·21–2·03	0·45	0·66	0·21–2·07	0·47

Treatment success was defined as normalisation of urine culture (<10^4 colony-forming units per mL) after treatment. Adjusted analysis included the baseline quality of life assessment, urinalysis parameters, and method of bladder emptying (ie, spontaneous *vs* indwelling catheter) as covariates. OR=odds ratio.

Table 2: **Logistic regression of the primary and safety outcomes, as well as post-hoc analysis of quantitative urine culture parameters, in the modified intention-to-treat population**

Study data were collected and managed using REDCap electronic data capture tools hosted at the Balgrist University Hospital, Zürich, Switzerland. Statistical analyses were done using Stata version 14.2, with a p value of less than 0·05 considered significant.

This study is registered with ClinicalTrials.gov, NCT03140085.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between June 2, 2017, and Dec 14, 2018, 474 patients were screened for eligibility (figure 1, microbiological details provided in appendix 3, p 1). 113 patients were randomly allocated to receive Pyophage (n=37), placebo (n=38), or antibiotic treatment (n=38). 97 (86%) of these patients received at least one dose of their allocated intervention and were included in the modified intention-to-treat population (table 1). Distribution of bacterial strains are shown in figure 2.

Rates of treatment success, defined as urine culture normalisation, did not differ significantly between treatment groups (table 2). After 7 days of treatment,

	Post-treatment count							Improved	Consistent	Deteriorated
	No bacterial growth	10^4 to $<10^5$	10^5 to $<10^6$	10^6 to $<10^7$	$\geq 10^7$	Adverse events	Lost to follow-up			
Pyophage (n=28)										
10^5 to $<10^6$	1	0	1	2	2	2	0	..	..	..
10^6 to $<10^7$	1	0	1	5	4	3	1	..	..	..
$\geq 10^7$	3	0	0	1	1	0	0	..	..	..
Total	5	0	2	8	7	5	1	7 (25%)	7 (25%)	8 (29%)
Placebo (n=32)										
10^4 to $<10^5$	1	0	0	0	0	0	0	..	..	..
10^5 to $<10^6$	5	1	1	0	0	1	1	..	..	..
10^6 to $<10^7$	3	0	1	4	1	7	3	..	..	..
$\geq 10^7$	0	0	0	1	1	1	0	..	..	..
Total	9	1	2	5	2	9	4	12 (38%)	6 (19%)	1 (3%)
Antibiotics (n=37)										
10^5 to $<10^6$	3	2	1	1	0	3	2	..	..	..
10^6 to $<10^7$	8	0	0	3	1	2	0	..	..	..
$\geq 10^7$	2	0	0	3	2	3	1	..	..	..
Total	13	2	1	7	3	8	3	18 (49%)	6 (16%)	2 (5%)

Data are n or n (%).

Table 3: **Colony-forming unit count per mL of urine culture post-treatment versus pre-treatment in the modified intention-to-treat population**

normalisation of urine culture was achieved in 27 (28%) of 97 patients: five (18%) of 28 patients in the Pyophage group compared with nine (28%) of 32 patients in the placebo group (odds ratio [OR] 1·60 [95% CI 0·45–5·71]; p=0·47) and 13 (35%) of 37 patients in the antibiotic group (2·66 [0·79–8·82]; p=0·11). All patients with microbiological normalisation also showed resolution from the predefined UTI symptoms (post-hoc analysis). An analysis of the full intention-to-treat population is provided in appendix 3, p 2.

58 (60%) of 97 of patients were free from the predefined UTI symptoms at the end of treatment: 16 (57%) of 28 patients in the Pyophage group, 19 (59%) of 32 patients in the placebo group, and 25 (68%) of 37 patients in the antibiotic group. Regarding the microbiological outcomes, a reduction of colony-forming units per mL (post-hoc analysis) was observed in seven (25%) of 28 patients, 12 (38%) of 32 patients, and 18 (49%) of 37 patients (tables 2, 3, appendix 3, p 3).

Adverse events were reported in 22 (23%) of 97 patients and ranged from grade 1 to 3 (table 4). Adverse events were similar in type and incidence between the three groups and were reported in six (21%) of 28 patients in the Pyophage group, 13 (41%) of 32 of patients in the placebo group, and 11 (30%) of 37 patients in the antibiotic group (table 2). Most adverse events were sudden onset of fever (>38°C) and concomitant use of antibiotics. No adverse events resulted in prolongation of hospitalisation. According to the definition of the modified intention-to-treat population, patients lost to follow-up (eight [8%] of 97) were considered as adverse events in the safety analysis. Reasons for withdrawal included signs and symptoms of systemic infection (eg, temperature >38°C, C-reactive protein >100 mg/L), other rapidly progressing disease, immediately life-threatening illness, or withdrawal of informed consent.

	Patients with adverse events	Grade					
		1	2	3	4	5	Lost to follow-up
Pyophage (n=28)	6 (21%)	1 (4%)	1 (4%)	3 (11%)	0	0	1 (4%)
Placebo (n=32)	13 (41%)	1 (3%)	1 (3%)	7 (22%)	0	0	4 (13%)
Antibiotics (n=37)	11 (30%)	0	1 (3%)	7 (19%)	0	0	3 (8%)

Adverse events included intensification of pain not limiting normal postoperative activities, intensification of pain requiring provisional medication to follow normal postoperative activities, use or change of antibiotics due to sudden onset of temperature >38°C, and revision surgery due to prolonged haematuria. Adverse event severity was graded according to the Common Terminology Criteria for Adverse Events by the National Cancer Institute.

Table 4: **Adverse events by treatment group in the modified intention-to-treat population**

Discussion

In this randomised, placebo-controlled, double-blind trial, intravesical bacteriophage therapy was non-inferior to standard-of-care antibiotic treatment, but was not superior to placebo, in terms of efficacy or safety in treating UTIs. Interpretation of these results is multifactorial and the data should be evaluated in the context of the specific clinical indication, trial design considerations, and the study product, and how these reflect on the overarching paradigm of bacteriophage therapy. Although this study does not suggest efficacy for bacteriophage instillation to treat complicated UTIs in patients undergoing TURP, it does highlight important learning points for the future of bacteriophage therapy and subsequent trials.

The study population and clinical indication were chosen for logistical and feasibility reasons so that the

intervention group would be in line with common practice (ie, placing of a catheter). However, the treatment success rate was unexpectedly low for both active treatment groups (ie, antibiotics and bacteriophages). More surprising was the similar primary outcome success for both bacteriophage and placebo instillation, which we hypothesise was due to a therapeutic effect of a mechanical reduction of the bacterial load following bladder irrigation. This is supported by Birkhäuser and colleagues,[23] who reported that daily bladder irrigation with tap water reduces the bacterial load and the subsequent need for antibiotic treatment for UTI in a randomised controlled trial in patients with ileal pouches. The effect of mechanical reduction of the bacterial load would also have been expected for the Pyophage solution, thus bringing into question any inherent direct antibacterial activity of the applied bacteriophages. Ongoing microbiological analyses evaluating post-treatment urine cultures regarding bacteriophage concentrations and their activity against isolated pathogenic strains will provide more insight into their antibacterial activity. Additionally, a short observation and follow-up period in close proximity to a surgical event might have confounded measures for the primary and secondary outcomes.

No significant difference in the type or severity of adverse events, including fever, was observed between any of the treatment groups. This is in line with all reports of bacteriophage therapy to date, including a phase 1 study testing nasal and oral application of the same commercial bacteriophage product in *Staphylococcus aureus* carriers.[15,18,24–26] However, including blood tests and systemic immune and inflammatory markers in this study could have produced a more comprehensive understanding of safety in the patients, and these measures should be included in future studies. Local intravesical application makes systemic reactions to the treatment unlikely, and no septic adverse events occurred during this trial.

Limited data from bacteriophage treatments in humans are available to guide appropriate power calculations and predict effect sizes. None of the three randomised controlled trials completed to date have evaluated UTI, and only one case report was available at the time of study design.[11,18,25,27] This scarcity of data, together with our bacteriophage enthusiasm, probably compounded the power calculation and led to an overestimation of the effect sizes for the Pyophage treatment group. Thus, more conservative assumptions and higher number of patients are needed. Phase 0 observational studies and phase 1 studies in colonised patients would help refine expectations of the effect size, as would data from additional well documented case reports, for future trials.

The study location of the Alexander Tsulukidze National Centre of Urology, where bacteriophage therapy is a long-approved treatment strategy, imposed several limitations on the trial. Treatment times and follow-up were limited due to the large geographical distances of patients from the trial site, which excluded the possibility for extended follow-up and resulted in difficulty differentiating whether symptoms were caused by surgery or infection in the primary outcome measure. Adherence to the study protocol was compromised because of local standard operational procedures in the hospital and limited hospital resources, thus resulting in a high degree of protocol deviation and creating the need for a modified intention-to-treat analysis. To evaluate the clinical plausibility, we did a complete intention-to-treat analysis, which supported the clinical plausibility of the primary analysis (appendix 3, p 2).

According to common practice for commercial bacteriophage cocktails in eastern Europe, the Pyophage cocktail consisted of many different bacteriophages with activity against a range of pathogenic bacteria; the precise concentration and identity of each bacteriophage is not established, but has previously been elaborated through metagenomic analysis and shown to be devoid of genetically harmful elements.[26] During the trial, Pyophage was regularly enriched with new bacteriophages that were adapted to increase activity against local epidemiological strains.[15] This approach aims to increase the efficacy of the bacteriophage cocktail to treat newly emerging pathogens over time.[15,26,28,29] This composition strategy differs largely from well characterised specific combinations—or even personalised selection—of bacteriophages that are the focus of development for European and North American clinical trials.

Regardless of composition, the activity of the investigational bacteriophage product against the patient isolate was verified in vitro. Although previous trials have cited this activity as an essential criterion for successful treatment, in-vitro sensitivity towards bacteriophage or antibiotics did not translate into a therapeutic effect for many patients receiving Pyophage or antibiotic treatment. This discordance between in-vitro results and in-vivo results requires further investigation for companion diagnostics and proper patient selection. Additionally, the low titres of the different bacteriophages in Pyophage might have led to relevantly low in-vivo concentrations due to dissemination in the bladder and dilution with urine, resulting in an insufficient decrease of the bacterial load. The concentration of bacteriophages at the site of infection, physical access to the site, and pathogen load are all important parameters for using and evaluating bacteriophage therapy.

The utility of bacteriophage therapy as an antibacterial treatment strategy is of important medical, scientific, economic, regulatory, and public interest. Bacteriophage therapy has the potential to provide an alternative treatment to antibiotics in consideration of increasing antimicrobial resistance; a treatment that might provide added benefits for microbiome integrity and reduced toxicity compared with antibiotics. However, that potential remains to be validated by significant proof-of-efficacy results from well designed clinical trials.

This pragmatic randomised controlled trial for treating UTIs in patients undergoing TURP revealed little about the efficacy of bacteriophage therapy, but the safety profile of bacteriophage application was reiterated. Improvements for similar trials in future would include studying non-surgical patient populations, the use of better characterised and more highly concentrated bacteriophages, and modifications to the start of therapy and follow-up, such as higher dosing, prolonged administration until microbial response, or measuring of recurrence. The many variables in clinical indications, bacteriophages, and infecting pathogens that complicated the interpretation of these results should be minimised by aiming for a better controlled explanatory trial, with a clear causation and a well defined method of bacteriophage application (either predefined composition or personalised selection of bacteriophages).

In conclusion, our findings are encouraging and provide important stimuli for physicians and authorities to support further large-scale clinical studies using bacteriophages for otherwise virtually untreatable infections, in order to further establish their efficacy. The recent approval by the Food and Drug Administration of a bacteriophage collection for personalised therapies, as well as the Belgian regulatory approach of allowing custom-made bacteriophage medicines for individual patients,[30] shows that personalised approaches, rather than predefined bacteriophage cocktails, could be a better way to use the antibacterial effects of bacteriophages.

Contributors

NC, AC, LMB, WS, and TMK created the study design. AC was the principal investigator of the clinical trial. LMB analysed the data. LL, SM, WS, and TMK drafted the manuscript. AU, NC, MG, AC, GC, MPS, MDL, UM, and LMB critically reviewed the manuscript. NC, AC, and TMK obtained the funding of this study. All the authors read and approved the final manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Individual participant data that underlie the results reported in this Article, after de-identification, as well as study protocol, statistical analysis plan, and clinical study report will be available to researchers who provide a methodologically sound proposal from the publication date until 5 years after Article publication, to achieve aims in the approved proposal. Proposals should be directed to the corresponding author (TMK) and the requested data will be sent if applicable.

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