

NATURAL COCKTAIL PHAGE THERAPY COULD PROVIDE AN EFFICACIOUS TREATMENT FOR RESPIRATORY INFECTIONS

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ABSTRACT

Cocktail of phages present in the Ganga river were administered to volunteers suffering from various conditions. We allowed the phages in the cocktail to self-select the bacteria that are present and target them. We have collected water from upper reaches of the Ganga river where the water is reported to be of drinking water quality. Volunteers were enlisted by messages on social media. They were asked to score their respiratory status on Likert scale 1-10 at the beginning of the treatment and periodically thereafter. They were asked to administer Ganga water to themselves by oral, nasal or topical routes. The change in score as reported by the volunteers was noted. Regression analysis was undertaken to assess the contribution of a number of independent variables to the reported change. It was found that an improvement in score of respiratory status of 1.5 on Likert scale is obtained in 150 days treatment. Higher level of initial morbidity provides greater improvement. Females are likely to obtain better improvement. Age did not show any impact on the results. The numbers of co-morbidities did not give a significant coefficient. This could be because the cocktail of phages in the Ganga water works simultaneously on multiple morbidities hence the co-morbidities do not affect the results. A major limitation of this study is the absence of a control sample. We tried to partially overcome this limitation by comparing the results of four chronic volunteers—defined as suffering from respiratory conditions for 2 years or more—and 41 non-chronic volunteers. Chronic volunteers reported an improvement of average 1.5 on Likert scale while the non-chronic volunteers reported an improvement of 0.98 which suggests that Ganga water contributed to the improvement in the condition of the volunteers. Our study is complemented by another study that found benefits in respiratory conditions for chronic patients when Ganga water was administered under medical supervision.

INTRODUCTION

Phages have been used to treat infections of *Streptococcus* ^[1], *Acinetobacter*, *Enterobacteriaceae*, *Escherichia*, ^[1] *Klebsiella*, *Pseudomonas aeruginosa* ^[1-3] and *Staphylococcus aureus* ^[2,5]. A cocktail of phages can be more effective. A cocktail was successfully used against *P. aeruginosa* ^[6]. Another cocktail of five phages that specifically targeted *S. aureus*, *S. pyogenes*, *P. mirabilis*, *P. vulgaris*, *P. aeruginosa*, and *E. coli* was used successfully ^[1]. The administration of a single phage could lead to the development of resistance hence a cocktail of multiple phages is the preferred method of treatment ^[2]. Each phage in a cocktail targets a different surface receptor. This requires the bacterium has to develop mutations to several genes in order to become resistant, the chances for which happening are less ^[1].

The use of a phage cocktail requires the availability of a phage display ^[6,7]. The costs of creating a phage display are enormous. In one reported case phages were contributed by 15 different institutions to successfully treat a patient of *Acinetobacter baumannii*. The use of Phage Therapy (PT) has

been hindered because the stability of each phage has to be assessed both singly and in a cocktail ^[1].

Anti-bacterial activity of water of the Ganga and Yamuna rivers was noted by British bacteriologist Ernest Hankins as early as 1896 and by French microbiologist Felix D'Herelle in the 1930s ^[8,9]. In 2018 the National Environment Engineering Research Institute, Nagpur and the Institute of Microbial Technology, Chandigarh found about 200 isolates of phages in the water of Ganga river. They also reported specific bactericidal activity of the phages against "*Mycobacterium*, *Streptococcus*, *Pseudomonas*, *Yersinia*, *Salmonella*, *Staphylococcus*, *Klebsiella*, *Vibrio*, *Shigella*, *Clostridium*, *Acinetobacter*, *Erwinia*, *Aeromonas*, *Escherichia*, *Cronobacter*, *Enterobacteria*, and *Campylobacter*" ^[10,11].

We make a novel advance by administering naturally available cocktail of phages of water in the Ganga river as a treatment. The efficacy of this phage cocktail is more because the large numbers of isolates of phages in the Ganga water self-select the bacterium that are present and attack them simultaneously without first having to identify and isolate the phages. The cost of this therapy is less because the water of Ganga river is available aplenty. It is a perennial and plentiful resource.

MATERIALS AND METHODS

Safety, Collection and Administration of Ganga water

The Central Pollution Control Board has found water at Haridwar to meet with the criteria of pH, DO and BOD for drinking water^[12,13]. The water at Rudra Prayag located upstream of Haridwar also meet these criteria^[14]. However, the water collected from Rudra Prayag had coliforms at 9300 MPN per 100 ml against the requirement of less than 50 MPN per 100 ml for drinking water^[13]. Therefore, we stored the water for one month at room temperature. We found no coliforms thereafter indicating that the water had become fit for drinking. It has been reported that the ability of the Ganga water to kill coliforms is increased with concentration^[15]. However, we decided not to concentrate the pages by centrifuging because the effect of centrifugation on the phage quality and diversity was not tested.

Water was collected along with sediments in 1-liter plastic bottles. The bottles were first rinsed by alcohol and then by Ganga water. Then water and sediments were collected in the bottles. The bottles were sealed using adhesive to prevent leakage during transport.

The volunteers were given the following instructions: ⁽¹⁾ Do not put bottle in direct sunlight. ⁽²⁾ you may take Ganga water in any of the following three ways: Take 2 tea spoons of Ganga water and swirl in the mouth, then take breath with Ganga water in the mouth and then drink it. Take it 2 times in a day. Take cold steam of Ganga water with nebulizer 2 times a day; then drink the water remaining in the container. Apply this water on the affected part to treat skin related problems 2 times a day. Ganga water should be taken 1 hour before or after meals.

Selection of Volunteers

We sent messages on social media and WhatsApp that we were undertaking experiment of treatment of various ailments Ganga water. About 75 volunteers spread across India volunteered and were enlisted. The volunteers were informed that this was an experimental project, that there was no guarantee of benefits. Informed consent was taken from them. Ganga water was packed in bottles along with a small amount of sediment and supplied to the volunteers by post. Telephonic interviews were done with them before start of treatment and periodically thereafter. Credible data was available 45 volunteers as reported in this study. This sample size, though small, compares favorably with one study of the use of a phage cocktail against *P. aeruginosa* and *S. aureus* that was undertaken with

nine patients^[6].

Inclusion criteria was the provision of assessment of the respiratory condition before- and after the treatment with Ganga water by the volunteer. Exclusion criteria was irregular treatment and irregular reporting of the condition.

Method of Assessment

We have used a 1-10 Likert Scale^[16,17], with “5” and less representing a “morbid” situation, and “6” to “10” representing normal situation.

We recognize that the scores given by different volunteers are not comparable. However, the scores given by the same volunteer at different points of time would be comparable^[18].

Some volunteers were using other medication regimens in parallel and the results may get confounded^[19,20]. However, we have statistically isolated the effect of such confounding medications.

Collection of data

Following data was collected by telephonic interviews initially: Present condition of digestive, urological, insomnia, metabolism, mental health, respiratory, cardiological, diabetes, dermatological, arthritis, or other (specify) on Score: “1” as worst and “10” as best. Confounding medicine. Status of chronic conditions along with years since suffering;

Following data was collected periodically thereafter. Question above was repeated. Additional questions asked were: Method of taking Ganga water (oral, topical or nasal). How many times a day? Have you consumed the prescribed quantity of Ganga water regularly?

Regression Analysis

Regression analysis was undertaken to assess the contribution of different factors to the change in respiratory condition reported by the volunteers. The dependent variable was change in respiratory condition on Likert Scale as reported by the volunteer. The independent variables were as follows:

Days’ log: Number of days of administration of Ganga water, log. We found log gives a better fit than days-squared.

Respiratory Start Level: The level of respiratory condition on Likert Scale 1-10 at the time of start of the treatment as reported by the volunteer.

Volunteers were asked to assess their conditions of specified diseases in the initial survey as at Question No 1 above. The numbers of conditions on which a volunteer assessed his/her situation on Likert Scale at “5” or less was considered to be “numbers of comorbidity.”

RESULTS

Regression Analysis

The results are given at Table 1 below.

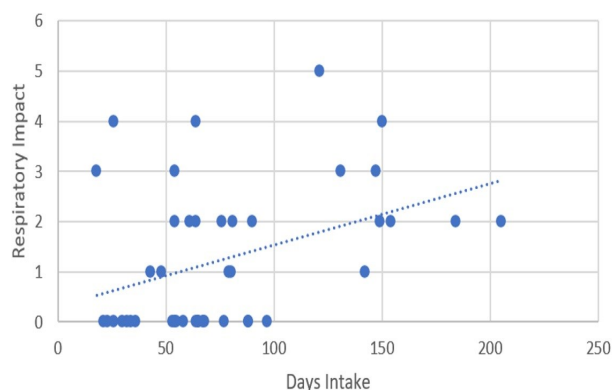
Sl No	Adjusted R-squared	Intercept	Days Log	Respiratory Start Level	Female	Co-morbid No.	Other Medicine	Age Years	Nebulizer	Oral	Topical
1	0.774	5.094 (0.00)	0.688 (0.11)	-0.629 (0.000)	0.360 (0.110)	-0.028 (0.578)	-0.200 (0.385)	0.002 (0.749)	-0.433 (0.407)	-0.469 (0.44)	-0.381 (0.118)
2	0.784	5.016 (0.00)	0.659 (0.11)	-0.615 (0.000)	0.332 (0.118)		-0.204 (0.334)		-0.400 (0.429)	-0.397 (0.49)	-0.392 (0.099)
3	0.792	4.698 (0.00)	0.613 (0.12)	-0.617 (0.000)	0.328 (0.115)		-0.201 (0.328)				-0.404 (0.082)
4	0.792	4.604 (0.00)	0.614 (0.11)	-0.615 (0.000)	0.313 (0.131)						-0.442 (0.054)

Regression No 1. All nine independent variables were included. The “Co-morbid Numbers” and “Age” showed P-value greater than 0.150 and an insignificant coefficient at less than 0.1. These were dropped.

Regression No 2. “Nebulizer” and “Oral” gave P-values greater than 0.150 and were dropped.

Regression No 3. “Other medicine” gave P-value greater than 0.150 and was dropped.

Regression No 4. The remaining four independent variables gave P-value of less than 0.150 and Adjusted R-squared was at 0.792. We used this result for assessing the effect of Ganga water.



The existing NCS evaluation data from two earlier years were separated on certain parameters and evaluated [21].

Illustrative Case Studies

Case Study 1 (VS). Male, 61 years. Volunteer was having difficulty in breathing for the last nine years. He was not able to climb to his flat on the third floor in one stretch. He used to rest for 10-15 seconds on each floor. After 20 days of treatment nasally he was able to climb up to third floor in one go. He had ranked his status at “3” on the Likert Scale in the beginning which he assessed at “6” after the treatment.

Case Study 2 (SM). Female, 47 years. She used to get breathless in climbing up to her flat on the second floor. Now she can climb easily. She assessed her respiratory status as “5” in the beginning which she assessed at “10” after the treatment.

Complementary Study under Medical Supervision

A complementary study of use of PT for the treatment of respiratory conditions was undertaken by Bagdi, Niwane and Jhunjhunwala [22]. Three patients suffering from chronic allergic rhinitis were administered Ganga water under medical supervision. Patients were asked to take 5 ml Ganga water in morning and evening by nasal route through nebulizer and 10 ml orally in the afternoon along with prescribed allopathic, ayurvedic and homeopathy medicines that they were taking previously.

As assessed by the medical practitioner, the patients got 70 percent relief in infections in upper upper-respiratory tract-, nose and nasal passages; and 30 to 60 percent relief in lower respiratory tract-trachea, bronchial tree and alveoli.

DISCUSSION

The use of bacteriophages for treating respiratory infections has been reported previously [23, 24]. However, this is the first case with the use of cocktail of naturally available bacteriophages. The improvement of the respiratory conditions increases with the duration of intake of Ganga water. The negative coefficient of “Respiratory Start Level” indicates that higher level of initial morbidity leads to greater improvement. Females are likely to obtain better improvement possibly due to different physiology. This requires more study.

The numbers of co-morbidities did not give a significant coefficient. This could be because Ganga water works simultaneously on multiple morbidities hence the co-morbidities do not affect the re-

sults of respiratory condition.

Age did not give a significant coefficient suggesting that elder volunteers befitted as much as younger volunteers.

The negative coefficient of “Topical application” defies explanation. This requires more study.

The small size of the sample is a limitation. However, sometimes experimental treatments based on theoretical considerations alone may lead to major breakthroughs [23]. Therefore, there is a need to consider natural cocktail phage therapy with Ganga water as a treatment for respiratory diseases. Further study is required on a number of issues. One, the numbers of isolates of phages in the Ganga water vary at different seasons of the year and different stretches of the river. Two, a more robust protocol for intake by oral-, nasal- and topical routes needs to be developed. Three, the effect of centrifugation on potency and stability of the phages requires more study. Another limitation of this study is the reliance on the assessment of the condition made by the volunteers. However, the patient, not the doctor, is the primary stakeholder in health and health care [24]. Thus, the benefits as assessed by the volunteers may be taken seriously and future pathways may be explored to explore natural cocktail phage therapy.

Conclusion

The cocktail of natural phages in the Ganga river may provide an efficacious treatment of respiratory conditions. This applies specifically to the Ganga water in view of the presence of 200 isolates of phages therein. This therapy can be tried initially as an adjunct treatment.

Data Availability

The data used in this study is unique and was collected by the authors. It can be accessed by writing to the corresponding author at bharatjj@gmail.com.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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REFERENCES

- Chang RY, Wallin M, Lin Y, Leung SS, Wang H, Morales S, Chan HK. Phage therapy for respiratory infections [Internet]. *Advanced drug delivery reviews*. 2018 Aug 1; 133:76-86.
- Wunderink RG. Turning the phage on treatment of antimicrobial-resistant pneumonia [Internet]. *American journal of respiratory and critical care medicine*. 2019 Nov 1;200(9):1081-2.
- El Bcheraoui C, Mokdad AH, Dwyer-Lindgren L, Bertozzi-Villa A, Stubbs RW, Morozoff C, Shirude S, Naghavi M, Murray CJ. Trends and patterns of differences in infectious disease mortality among US counties, 1980-2014 [Internet]. *Jama*. 2018 Mar 27;319(12):1248-60.
- Pires DP, Vilas Boas D, Sillankorva S, Azeredo J. Phage therapy: a step forward in the treatment of *Pseudomonas aeruginosa* infections [Internet]. *Journal of virology*. 2015 Aug 1;89(15):7449-56.
- Merabishvili M, Pirnay JP, Verbeken G, Chanishvili N, Tediashvili M, Lashkhi N, Glonti T, Krylov V, Mast J, Van Parys L, Lavigne R. Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials [Internet]. *PloS one*. 2009 Mar 20; 4(3): 4944.
- Rose T, Verbeken G, De Vos D, Merabishvili M, Vanechoutte M, Lavigne R, Jennes S, Zizi M, Pirnay JP. Experimental phage therapy of burn wound infection: difficult first steps [Internet]. *International journal of burns and trauma*. 2014;4(2):66.
- Karimi M, Mirshekari H, Basri SM, Bahrami S, Moghoofoei M, Hamblin MR. Bacteriophages and phage-inspired nanocarriers for targeted delivery of therapeutic cargos [Internet]. *Advanced drug delivery reviews*. 2016 Nov 15;106:45-62.
- Hankin ME. The bactericidal action of the waters of the Jamuna and Ganges rivers on Cholera microbes [Internet]. *Ann. Inst. Pasteur*. 1896;10:511-23.
- Duckworth DH. Who discovered bacteriophage? [Internet]. *Bacteriological reviews*. 1976 Dec;40(4):793-802.
- National Environment Research Institute, Untitled [Internet]. 2018; page 5-44. Available from: URL: <https://nmcg.nic.in/writereaddata/fileupload/NMCGNEERI%20Ganga%20Report.pdf>.
- Institute of Microbial Technology. *Gangiomics: Unravelling the Unseen Phage Diversity in the Sediments of the Holy River Ganges through metagenomic approach* [Internet]. 2018.
- Central Pollution Control Board. *Water Quality Criteria* [Internet]. 2019. Available from <https://cpcb.nic.in/water-quality-criteria/>.
- Central Pollution Control Board. *Pollution Assessment: River Ganga* [Internet]. July 2013. Available from <https://cpcb.nic.in/wqm/pollution-assessment-ganga-2013.pdf>.
- Central Pollution Control Board, *Biological Water Quality Assessment of the River Ganga (2017-18)* [Internet]. 2018. Available from https://ueppcb.uk.gov.in/files/bio_mointering_by_cpcb.pdf.
- Ganga Today. Ganga water have ability to kill the coliform [Internet]. 2021. Available from <https://gangatoday.com/articles/298-ganga-water-have-a-ability-to-kill-the-coliform.html>.
- Versta Research. How to Label Your 10-Point Scale [Internet]. Available from: URL: <https://verstaresearch.com/blog/how-to-label-your-10-point-scale/>.
- Decker, Fred., How to Interpret Likert Surveys, *Sciencing*. March 2018. Available from: URL: <http://sciencing.com/interpret-likert-surveys-8573143.html>.
- Chidambaram AG, Josephson M. Clinical research study designs: The essentials [Internet]. *Pediatric investigation*. 2019 Dec 1;3(04):245-52.
- Sessler DI, Imrey PB. Clinical research methodology 2: observational clinical research [Internet]. *Anesthesia & Analgesia*. 2015 Oct 1;121(4):1043-51.
- Tripodis Y, Ziropiannis N. Dynamic factor analysis for multivariate time series: An application to cognitive trajectories [Internet]. *International journal of clinical biostatistics and biometrics*. 2015;1(1)
- .Jump Projects and Mimeconsulting. In the Mix with NCS [Internet]. 2020. Available from <https://wearencs.com/sites/default/files/202009/In%20The%20Mix%20with%20NCS-Sub-Group%20Analysis%20Report.pdf>.
- Bagdi VB, Niwani SS, Jhunjunwala B. Observational Case Studies of Gangajal Therapy [Internet]. *International Ayurvedic Medical Journal online*. 2021.
- VinodKumar C.S, Srinivasa H, Kumar CA, Kalasuramath S, Prasad BS, Jayasimha V.L, Nachimuthu R, Manohar P. Therapeutic effectiveness of bacteriophage in the treatment of pneumonia caused by NDM-4 producing *Klebsiella pneumoniae* in a mouse model. *IP Indian J Immunol Respir Med* 2022;7(2):78-84.
- VinodKumar C.S, Swamy AV, Shamanur S, Venkatarreddy A, Jayasimha VL, Reddy SN, Dsouza SJ, Srinivasa H. Nebulization as the mode to administer therapeutic bacteriophages to resolve *Acinetobacter pneumonia* in rabbits. *IP Indian J Immunol Respir Med* 2022;7(3):1-8.
- Górski A, Międzybrodzki R, Łobocka M, Głowacka-Rutkowska A, Bednarek A, Borysowski J, Jończyk-Matysiak E, Łusiak-Szelachowska M, Weber-Dąbrowska B, Bagińska N, Letkiewicz S. Phage therapy: what have we learned? [Internet]. *Viruses*. 2018 May 28;10(6):288.
- Truog RD. The United Kingdom sets limits on experimental treatments: the case of Charlie Gard [Internet]. *Jama*. 2017 Sep 19;318(11):1001-2.

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