ISSN 2063-5346

In vitro study on lytic efficacy of phages against MDR *Escherichia coli*

MAHAK JAIN[1*], DR. HEMANT B. GADEKAR[2], DR. APOORVA TRIPATHI[3], DR. GOPALNATH[4]

[1]Ph.D. SCHOLAR, RKDF MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, SRK UNIVERSITY, BHOPAL, MP

[2]PROFESSOR & DEAN, DEPARTMENT OF MICROBIOLOGY, RKDF MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, BHOPAL, MP

[3]PROFESSOR & HEAD , DEPARTMENT OF MICROBIOLOGY, RKDF MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, BHOPAL, MP

[4]PROFESSOR ,DEPARTMENT OF MICROBIOLOGY, IMS, BHU, VARANASI

***CORRESPONDING AUTHOR:-** :-Ph.D. SCHOLAR, RKDF MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, SRK UNIVERSITY, BHOPAL, MP.

Article History: Received: 12.06.2023 **Revised:** 14.07.2023 **Accepted:** 31.07.2023

ABSTRACT

Introduction: Multi-drug-resistant (MDR) strains of biofilm-producing Escherichia coli are being reported worldwide and are threatening the health of human beings. These species are seen as the highest priority for the development of new phage biocontrol agents. Therefore, we isolated and characterized new and effective lytic phages as biocontrol agents against MDR E. coli.

Aim:-The aim of this study is in vitro lytic efficacy of phages against MDR *Escherichia coli*

Methodology: This study was conducted in department of Microbiology at RKDF Medical College Hospital & Research Centre, SRK University, Bhopal, Madhya Pradesh.This study included 140 *Escherichia coli* .Out of 140 *Escherichia coli* 122 were MDR *Escherichia* coli and rest were normal. Bacteriophages (phages) were isolated from different wastewater samples and subjected for plaque formation.

Result: The result of this study revealed that best plaque formation was seen at 10^{12}pfu/ml concentration.

Conclusion:- In conclusion, it may be suggested bacteriophage therapy might be beneficial for the patient who are suffering with the MDR strains from a long period of time.

Keywords:- MDR, Escherichia coli, Bacteriophage therapy.

DOI:10. 10.48047/ecb/2023.12.si5.363

Introduction

Bacteriophages (phages) are self-replicating viruses, which are capable of infecting and lysing their specific host bacteria (1). They are ubiquitous organisms on Earth estimated to number at 10^{30}-10^{32} (2). Phages are relatively safe, nontoxic, and harmless to animals, plants, and humans (3, 4). They are found in various environments related to their host such as in food, soil, sewage water, feces, and farm environments (2). Several bacterial species such as *Campylobacter*, *Escherichia coli*, *Listeria*, *Salmonella*, *Pseudomonas*, and *Vibrio* species are used as hosts to isolate their specific bacteriophages (5–7). Because of their host specificity and nontoxicity, lytic phages are considered to be an alternative solution to combat antimicrobial-resistant pathogens. Outbreaks of listeriosis and widespread occurrence of multidrug resistance in *E. coli*, *Salmonella*, and *Staphylococcus* species have been reported in South Africa (8–11). However, there has been no attempt to use bacteriophages to control antibiotic-resistant pathogens, in either hospital settings.

Nosocomial infections pose a significant challenge in contemporary healthcare. The increasing prevalence of nosocomial infections can be attributed to the widespread dissemination of antibiotic resistance among microorganisms. This resistance arises from the unrestricted and imprudent utilization of antibacterial medications in medical practices, as well as in different domains of the food industry and agriculture.(12-13). The World Health Organization (WHO) emphasizes that the escalating bacterial resistance to antibiotics has the potential to undermine numerous advancements made in medicine and epidemiology throughout the 20th century in combating infectious diseases.

Most of pathogenic bacteria have become multi–drug resistant to antibiotics (14). Furthermore, there is a concerning inadequacy in the development of new antibiotics to keep pace with the demand, resulting in a shortage of effective replacements for the existing medicines. (15). The economic burden associated with antibiotic resistance continues to rise, with the United States alone facing an annual cost approaching $55 billion (16). In contrast, the future costs of further growth in drug resistance remain uncertain. Therefore, ensuring appropriate treatment and actively participating in programs aimed at combating antibiotic-resistant bacteria are key objectives in modern medicine. Bacteriophages, which are obligate intracellular bacterial parasites, possess promising attributes for controlling pathogens. (17-18)

Therefore the aim of this study is In vitro lytic efficacy of phages against MDR E. coli.

Material & Methods:-

Study Area: This study was conducted in department of Microbiology at RKDF Medical College Hospital & Research Centre, SRK University, Bhopal, Madhya Pradesh.

Study Population: This study included 140 *Escherichia coli* .Out of 140 *Escherichia coli* 122 were MDR *Escherichia* coli and rest were normal.

Study duration: The duration of study was over a period of two years.

Data collection: All specimens were collected and transported to Microbiology Laboratory aseptically for culture and antibiotic susceptibility test. Then MDR were isolated as per CDC guidelines. MDR defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.(19)

Isolation of Environmental isolates:-

a) Collection of water sample from different sources

b) Removal of contamination from water

c) Lawn culture of bacteria on MHA
d) Isolation of bacteriophages
e) Purification of bacteriophages by soft agar overlay method

Collection of Water Samples from different sources:-

The water samples were collected from various water sources i.e. Lake, ponds, and sewer. The Water samples were collected from one foot below surface in the sterile bottle. Then the sample was taken to the laboratory for processing immediately. By using Gupta et al. (20**)** method the samples were processed.

Sources of water sample collection as follows:

- Sewage **(3 samples from different sewage)**
- Pond **(3 samples from different pond)**
- Lake **(3 samples from different lake)**

Fig 1: Pond, Sewage

Fig 2: Collection of water sample in sterile container

Removal of contamination from water:-

By using micropipette, 1.5 ml water had taken in the micro-centrifuge (Eppendorf) tubes from the each bottle. All Eppendorf tubes were treated with the 1 % chloroform. It got mixed thoroughly by shaking for 10 minutes. At 4^0C all Eppendorf tubes were centrifuged at 10000 rpm for 10 min..

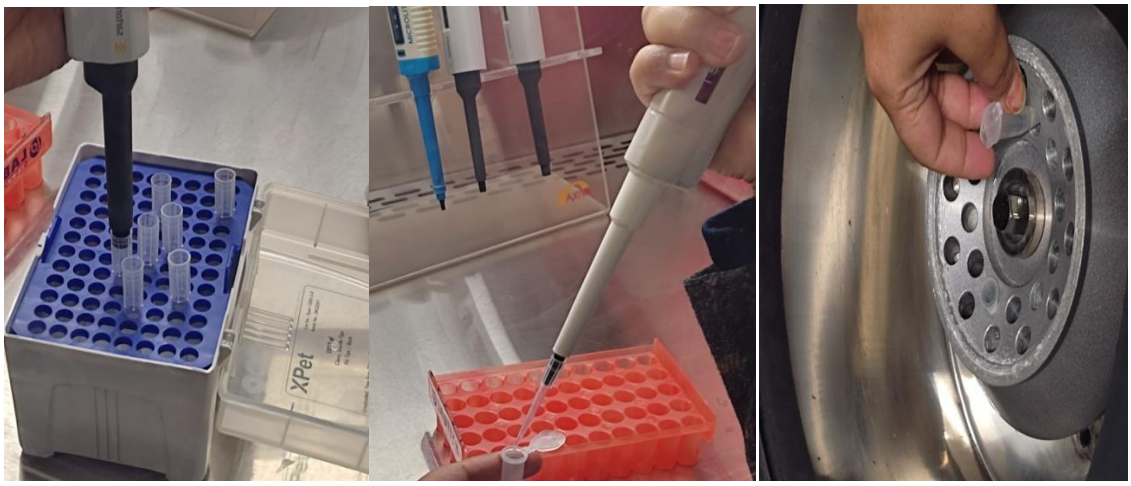

Fig 3: Method for removal of contamination from water

Lawn culture of bacteria on MHA & dropping:-In the normal saline 2 OD bacterial suspension was prepared in the sterile tube. Then Lawn culture was performed on MHA using cotton swabs sticks. The lawn culture plates were subsequently incubated in an incubator set at 37°C for a duration of 4 hours.Then collected supernatant were dropped over the bacterial lawn culture on MHA. Afterthat plates were placed in an incubator for a period of 18-24 hours at 37ºC.

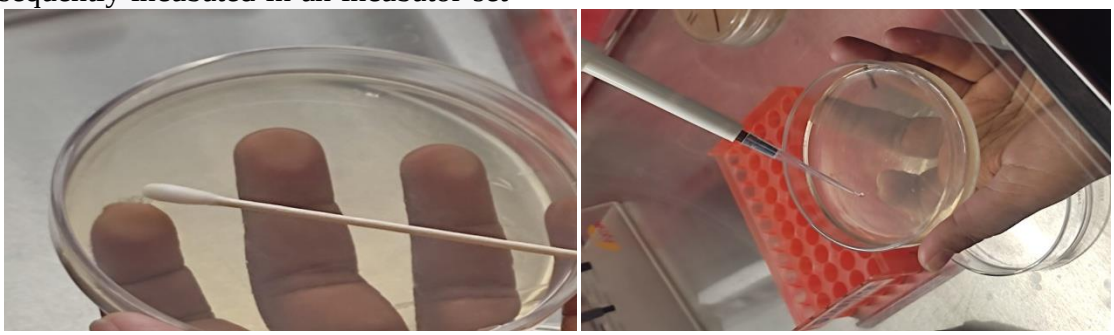

Fig4: Lawn culture of bacteria on MHA & dropping of phage lysate

Harvesting of culture plates followed by Centrifugation:

By using 3 ml TMG (Tris-HCl, magnesium sulfate, and gelatin pH 7.4) buffer solution, culture plates were harvested. Subsequently, suspended mixture of bacteria and lytic bacteriophages collected in Eppendorf tube.

For further processing, 1% chloroform was introduced in Eppendorf tubes .Then uniform mixing was done manually for a period of 10 min. The Eppendorf tubes were subssequently subjected to centrifugation at 10,000 rpm for 10 minutes at a temperature of 4°C. This process was then repeated three times. After that supernatants were collected which contained the phage lysate.

Fig 5: Harvesting of culture plates followed by Centrifugation & collection of phage lysate

Isolation and purification of bacteriophage by Overlay method:-

Double Agar Layer Method (DAL) :-

The collected phage lysates were diluted in TMG buffer (TMG **:**Phage=450µl **:**50 µl) by serial dilution. 100 µl of the diluted phages of different dilutions were added to different micro-centrifuge tubes containing mixture of 890 µl TMG and 10 µl bacterial suspension (>2.0 OD). The mixture was added to a test tube containing 3-4 ml of soft agar maintained at 45°C and mixed well by vortex. The soft agar mixture was poured and spreaded on labeled plates of MHA. After solidification the plates were incubated overnight at 37°C. Plaques of different morphology were observed on next day.[370]

Fig 6: Overlay Method

STATISTICALANALYSIS:

The data was prepared in Microsoft excel windows 10 for statistical analysis. The results were computed and represented as tables or graphs for better description.

Data analysis: Data were analysed by using Microsoft excel.

TABLE:1 Distribution of cases according to isolates

Isolates	No.	%
Normal E. coli	18	12.8%
MDR E. coli	122	87.2%
Total	140	100%

The above pie chart explains that among the 140 E. coli, 87. 2% MDR Escherichia coli found and rest were non MDR E. coli.

TABLE:2 Distribution of cases according to age group

Age	No.	%
<20	4	3.27
21-30	26	21.3
31-40	14	11.4
41-50	18	14.7
51-60	14	11.4
61-70	16	13.1
>70	30	24.5
Total	122	100

TABLE:3 Distribution of samples

Samples	No.	%
Fluid	2	1.6%
Blood	6	6.6%
Pus	12	9.8%
Urine	100	80.3%
Swab	2	1.6%
Total	122	100%

TABLE:4 Distribution of water samples for environmental isolates

SR NO.	Water sample	No.
1	Sewage	3
2	Pond	3
3	Lake	3
Total		9

Table no 4 showed that we were taken 9 samples from different sources for isolation of bacteriophage against the MDR E. coli.3 were collected from sewage,3 from pond & 3 from lake.

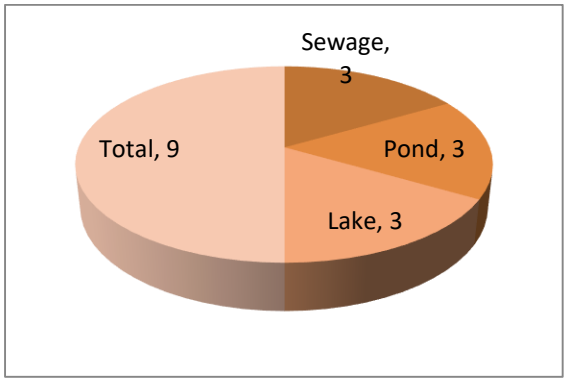

Fig:7 Chart showing sample distribution for bacteriophage

Table:5 Lytic activity on MDR E. coli due to presence of bacteriophage

Sample source	Sample no.	Observed lytic activity
Sewage	1	66
	2	70
	3	42
Pond	4	20
	5	24
	6	4
Lake	7	2
	8	8
	9	0

This table illustrate that the highest lytic activity of bacteriophages seen against the MDR Escherichia coli in sample no. 2 of sewage followed by sewage sample no. 1 and other water samples.

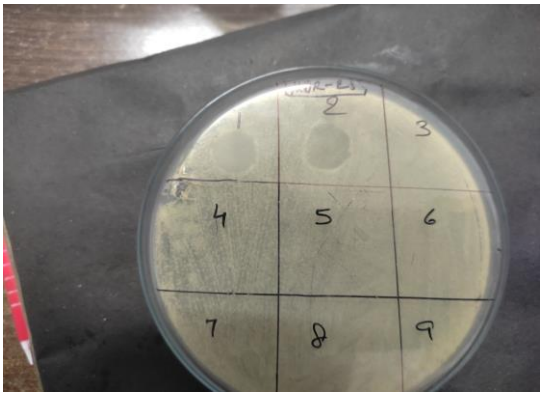

Fig 8: In vitro lytic activity of bacteriophage against MDR Escherichia coli

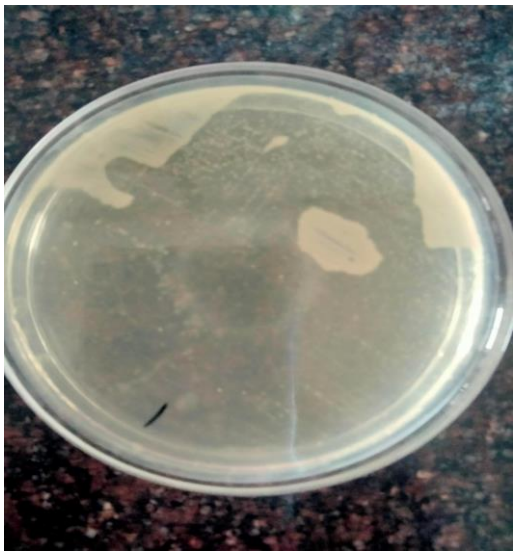

Fig 9: Individual lysis

Table:6 Lytic activity of bacteriophage at different concentration

Concentration of Phage lysate	Lytic activity of bacteriophage on MDR isolates
B1	122
B2	106
B3	98
B4	78
B5	48
B6	41
B7	26
B8	18
B9	12
B10	8
B11	8
B12	2
B13	0
B14	0
B15	0

In the above table showed that the lytic activity is more in the first tube of dilution where the concentration of bacteriophage is more as compared to another tubes.

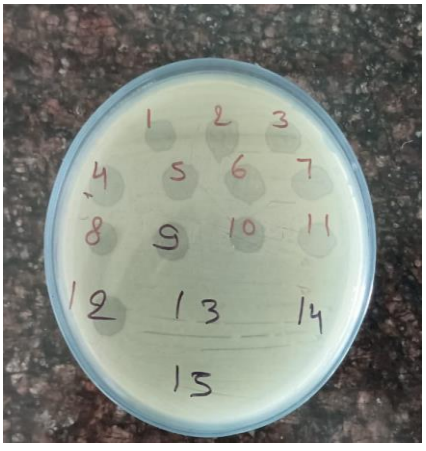

Table:7 Optimal concentration of phage & bacteria for plaque formation

Different dilution	Plaque formation
B1	0
B2	0
B3	0
B4	2
B5	3
B6	3
B7	4
B8	19
B9	32
B10	45
B11	68
B12	77
B13	46
B14	33
B15	26

We have seen the best plaque formation at B12 tube (10^{12} pfu/ml) due to optimum concentration of phage & bacteria followed by tube no. 11 & 10(10^{10} &10^{11} pfu/ml).

Discussion

The present study observed that Escherichia coli among all clinical specimens was found to be 17.8% prevalent. Related result has been found by Tanzina Ak etal **[21]** They found 16.8% prevalence of Escherichia coli in

their observations. Our study's findings indicated that MDR Escherichia coli had a prevalence rate of 87.2%.There are a number of reason of these results which are as follow; irrational use of antibiotics, easy availability of antibiotic on the counter, take incomplete dose of antibiotic & not following the antimicrobial stewardship.

The present study finding has been supported by Ibrahim ME et al, Ibrahim, D.R, Jain, P. et al.**[22-25]**

This study observed that age was vital factor which plays a significant role in Escherichia coli infections. In Our study most of the cases found the Escherichia coli infection in the elderly age group (>70) followed by sexually active group.

There are many factors which are responsible for Escherichia coli infection in elders. These factors include chronic disease, functional abnormalities and specific medications. Among elders asymptomatic bacteuria is more prevalent as compared to symptomatic bacteruria. Though, there is not so much requirement of treatment in the asymptomatic bacteruria.

Moreover, symptomatic UTI requires stringent antimicrobial therapy among the elderly cases. As a result, elderly people are also more likely to develop serious complications.

Roubaud Baudron C et al **[26]** confirmed the results of our study as they found high prevalence of Escherichia coli in elderly population. According to the researcher, in their study this type of result might be due to old age or associated comorbid conditions.

The samples of this study have taken 9 water samples for the isolation of bacteriophages. Out of which, 3 water samples from the sewage,3 water samples from the pond, 3 from the lake. All water samples were treated with the chloroform and supernatant collected & dropped over the MDR E. coli. Then lytic activities were observed over the MDR Escherichia coli.

Our study found that lytic activity of bacteriophage at different concentration. Which were obtained by the serial dilution. Result of this study revealed that the highest lytic activity of bacteriophage seen in the first tube and lytic activity decrease with the increased dilution.Spot test were used for observation of lytic activity over the host i.e. MDR Escherichia coli followed by overlay method (DAL method) for further confirmation.

The result of this revealed that the optimum concentration of bacteria & phage for plaque formation observed at the conc. of 101^2.

A.A. Abou Zeid conducted a study on the efficacy of bacteriophages against MDR Escherichia coli. The researchers collected water samples from various sources, including hospital wastewater, agricultural drainage water and sewage water, to isolate bacteriophages against E. coli. By using spot test and plaque assay they detected bacteriophages and E coli used as a host. **[27]**

The study by **H. Brussow et al [28]**involved collecting water samples from various sources, such as sewer, wastewater, and rivers. They also included human and animal waste, for the isolation of bacteriophages. This is comparable to the approach taken in our study. **[29]**

Z. Lu et al **[29]** used spot test method for evaluation of bacteriophages against Escherichia coli and DAL plate method were used for plaque formation. Plaque formation on DAL plate was evident for presence of bacteriophages. They used titration upto10^{11}pfu/ml. According to their findings, isolated phages had the ability to infect multiple strains of bacteria and demonstrated a broad range of hosts.

J.Uchiyama, M.K. Mirzaei, P.Yuet al suggested that in their study, the cocktail of phages could be more helpful to infect E. coli. Phage cocktail have broader host range and also could infect another spp..**[30-32]**

M.Park et al observed that phage cocktail were capable to lyse different spp. of E. coli.**[33]**

H.Shin et al **[34]** observed that in this study, phages have more potential in liquid culture to decrease of bacterial population. They observed that the host cells were rapidly lysed by the bacteriophages and the complete inhibition was achieved after 8 hours.

Pavel Alexyuk **[35]** conducted a study on the evaluation of bacteriophages against the Escherichia coli and also prepared the phage cocktail. The water sample used in the study was obtained from wastewater and sewage disposal fields. This study found bacteriophages was completely lysed their host. Therefore, it requires 4 hour incubated lawn culture for complete inhibit the bacterial cells by the phages. This study had taken total of nine strains. Against the nine stain the host range lysed by each bacteriophage which varied from two to six bacterial strains. They prepared bacteriophage cocktail from the isolated bacteriophage against the bacterial strain. Their results suggested that phage cocktail was an effective as a treatment for suppressing the growth of E. coli. They suggested that bacteriophage cocktail could be helpful against the Escherichia coli. Bacteriophage cocktail can play an crucial role in the dealing with infectious diseases caused by bacteria and may be helpful for empirical treatment.

Dev Raj Patel et al. **[36]** conducted a study on bacteriophage uses as a therapy in chronic nonhealing wounds. The researchers collected samples from various sources, including hospital sewage, the Ganga river, ponds, and the sewer of the municipal corporation. Then after they isolated the bacteriophages against the Klebsiella pneumonia, which was the infective bacteria in case of several non healing wound in diabetic patients. They applied these isolated phages for the purpose of local phage therapy.In our study we followed the same process but the difference is not utilizing the phages on the patients.

In Prerna Srivastava's study's, titled "Bacteriophages Can Make a Difference in Water Quality: Evidence From a Community-Based Study from North India".In this study suggested that phages were present in the drinking water and could potentially impact water quality. Which were found effective against the Escherichia coliand Klebsiella. In the similar way another researcher **[37-38]** also found the drinking water which is contrast to our study.

Chandan Kishor **[39]** conducted a study in which they focused on phage isolation and purification against Staphylococcus for osteomyelitis cases. They had taken the water samples for bacteriophages from the river, ponds and sewer.They applied these bacteriophages in case of osteomyelitis.They found the isolated phages were effective against the MRSA strain.By using this study they came to conclude that these bacteriophages might be a alternative way in the treatment of MRSA cases.

Nath G et al **[40]** conducted a study on efficacy of bacteriophage as an alternative to antimicrobials. They had done their study on MDR AB in an immunocompromised mouse model. Their observations showed the optimum conc. of bacteiophage cocktail is 1012 pfu/ml for the empirical treatment. But they found that if the conc. of phages decreases than mortality rate were increases in the infected mouse.

In our study also found that the optimum conc. of bacteriophages is 10^{12}pfu/ml. This conc. of bacteriophages considered

as appropriate for the plaque formation which is similar to above study.

Gopal Nath et al **[41]** did the experimental study in mice model; phage therapy as an alternative to antibiotics. They collected the water samples for the isolation of environmental isolate (bacteriophage).They also used double agar overlay method for plaque formation. This is similar to our study. They used 10^{10}pfu/ml conc. of phages in acute infections in vivo. In our study suggested that the 10^{12} pfu/ml is the appropriate conc. for in vitro evaluation.

Conclusion

- In conclusion, it may be suggested bacteriophage therapy might be beneficial for the patient who are suffering with the MDR strains from a long period of time.
- It can also be concluded that bacteriophage therapy as it is low cost, high specificity will be beneficial for replacing antibiotic usage to treat difficult infection of such types of MDR cases.
- Further Recommendations: The impact of the study will be more effective if a larger sample size is used in this geographical area. So it is recommended that a sample size more than be used to detail well defined precise results.

References

1. Akhtar M, Viazis S, Diez-Gonzalez F. Isolation, identification and characterization of lytic, wide host range bacteriophages from waste effluents against *Salmonella enterica* serovars. *Food Control.* (2014) 38:67–74.
2. Huang C, Shi J, Ma W, Li Z, Wang J, Li J, et al. Isolation, characterization, and application of a novel specific *Salmonella* bacteriophage in different food matrices. *Food Res Int.* (2018) 111:631–41.
3. Sillankorva SM, Oliveira H, Azeredo J. Bacteriophages and their role in food safety. *Int J Microbiol.* (2012) 2012:863945.
4. Harada LK, Silva EC, Campos WF, Del Fiol FS, Vila M, Dabrowska K, et al. Biotechnological applications of bacteriophages: state of the art. *Microbiol Res.* (2018) 212–3:38–58.
5. Niu Y, Johnson R, Xu Y, McAllister T, Sharma R, Louie M, et al. Host range and lytic capability of four bacteriophages against bovine and clinical human isolates of Shiga toxin producing *Escherichia coli* O157: H7. *J Appl Microbiol.* (2009) 107:646–56.
6. Akhtar M, Viazis S, Christensen K, Kraemer P, Diez-Gonzalez F. Isolation, characterization and evaluation of virulent bacteriophages against *Listeria monocytogenes*. *Food Control.* (2017) 75:108–15.
7. Yin Y, Liu D, Yang S, Almeida A, Guo Q, Zhang Z, et al. Bacteriophage potential against *Vibrio parahaemolyticus* biofilms. *Food Control.* (2019) 98:156–63.
8. Ateba CN, Bezuidenhout CC. Characterisation of *Escherichia coli* O157 strains from humans, cattle and pigs in the North-West Province, South Africa. *Int J Food Microbiol.* (2008) 128:181–8.
9. Akindolire M, Babalola O, Ateba C. Detection of antibiotic resistant *Staphylococcus aureus* from milk: a public health implication. *Int J Environ Res Public Health.* (2015) 12:10254–75.
10. Dlamini BS, Montso PK, Kumar A, Ateba CN. Distribution of virulence factors, determinants of antibiotic resistance and molecular fingerprinting of *Salmonella* species isolated from cattle and beef samples: suggestive evidence of animal-to-meat contamination. *Environ Sci Pollut Res.* (2018) 5:32694–708.
11. WHO. *Listeriosis – South Africa.* (2018). Available online at: https://www.who.int/csr/don/28-

march-2018-listeriosis-south-africa/en/ (accessed March 07, 2019).

12. Vivas, R.; Barbosa, A.A.T.; Dolabela, S.S.; Jain, S. Multidrug-Resistant Bacteria and Alternative Methods to Control Them: An Overview. *Microb. Drug Resist.* **2019**, *25*, 890–908.
13. Iwu, C.D.; Korsten, L.; Okoh, A.I. The Incidence of Antibiotic Resistance within and beyond the Agricultural Ecosystem: A Concern for Public Health. *MicrobiologyOpen* **2020**, *9*, e1035.
14. A.Sulakvelidze, Z. Alavidze, J.G. Morris, Bacteriophage therapy. Antimicrob Agents Chemother, Vol.45,pp.649-59,2001.
15. M.Kutateladze, R.Adamia, Bacteriophages as potential new therapeutics to replace or supplement antibiotics. Trends Biotechnol;vol. 28(12) , PP.591- 595,2010.
16. R.Smith, J.Coast ,The true cost of antimicrobial resistance. BMJ;vol.346, PP.14-93,2013.
17. Z.Hosseinidoust, N. Tufenkji, T.G. van de Ven, Formation of biofilms under phage predation: considerations concerning a biofilm increase. Biofouling;vol. 29, PP.457–468,2013.
18. J.Morris, N .Kelly, L. Elliott, A. Grant, M. Wilkinson, K. Hazratwala, P. McEwen, Evaluation of Bacteriophage Anti -Biofilm Activity for Potential Control of Orthopedic Implant Related Infections Caused by Staphylococcus Aureus. Surg Infect;vol. 20(1),pp.16-24,2019.
19. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012 Mar;18(3):268-81.
20. Gupta P., Singh H. S., Shukla V. K., Nath G., Bhartiya S. K. (2019). Bacteriophage therapy of chronic nonhealing wound: clinical study. *Int. J. Lower Extrem. Wounds* 18 (2), 171–175.
21. Tanzina Akter et al, Isolation, identification and antimicrobial susceptibility pattern analysis of ESCHERICHIA COLI isolated from clinical samples of Bangladesh: Asian Journal of Biomedical and Pharmaceutical Sciences, 2016,6(54),13-16.
22. Deb P, Das T, Nath C, Ahad A, Chakraborty P. Isolation of multidrug-resistant *ESCHERICHIA COLI*, *Staphylococcus* spp., and *Streptococcus* spp. from dogs in Chattogram Metropolitan Area, Bangladesh. J Adv Vet Anim Res. 2020 Oct 9;7(4):669-677.
23. Ibrahim ME, Bilal NE, Hamid ME. Increased multi-drug resistant ESCHERICHIA COLI from hospitals in Khartoum state, Sudan. Afr Health Sci. 2012 Sep;12(3):368-75.
24. Ibrahim, D.R.; Dodd, C.E.R.; Stekel, D.J.; Meshioye, R.T.; Diggle, M.; Lister, M.; Hobman, J.L. Multidrug-Resistant ESBL-Producing *E. coli* in Clinical Samples from the UK. *Antibiotics* **2023**, *12*, 169
25. Jain, P., Bepari, A.K., Sen, P.K. *et al.* High prevalence of multiple antibiotic resistance in clinical *E. coli* isolates from Bangladesh and prediction of molecular resistance determinants using WGS of an XDR isolate. *Sci Rep* (2021)**11**, 22859.
26. Roubaud Baudron C, Panhard X, Clermont O, Mentré F, Fantin B, Denamur E, Lefort A; COLIBAFI Group. ESCHERICHIA COLI bacteraemia in adults: age-related differences in clinical and bacteriological characteristics, and outcome. Epidemiol Infect. 2014 Dec;142(12):2672-83.
27. H.C.Chang, C.R. Chen, J.W. Lin, G.H .Shen, K.M. Chang, Y.H. Teseng, S.F. Weng, Isolation and characterization of novel giant Stenotrophomonas maltophilia phage phi SMA5. Appl. Environ. Microbiol; 2005:vol. 71, PP.1387-1393.

28. H.Brussow, Phage therapy: The *ESCHERICHIA COLI* experience. Microbiology;vol. 2005:151(7) ,pp.2133–2140.

29. Z.Lu, F .Breidt, V. Plengvidhya, H.P. Fleming, Bacteriophage ecology in commercial sauerkraut fermentations. Appl. Environ. Microbiol;vol. 2003: 69(6) , PP.3192-3202.

30. J.Uchiyama, M. Rashel, Y. Maeda, I .Takemura, S .Sugihara, K. Akechi, A .Muraoka, H .Wakiguchi, S.Matsuzaki ,Isolation and characterization of a novel Enterococcus faecalis bacteriophage EF24C as therapeutic candidate, FEMS Micropiol. Lett;vol. 2008:278, PP.200-206.

31. M.K. Mirzaei, A.S. Nilsson, Isolation of phages for phage therapy: a comparison of spot tests and efficiency of plating analyses for determination of host rage and efficacy. PLOS ONE; 2015:vol. 10(5) , PP.127-606.

32. P.Yu, J .Mathieu, M. Li, Z .Dai, P.J. Alvarez, Isolation of polyvalent bacteriophages by sequential multiple-host approaches. Appl. Environ. Microbiol, 2016:Vol.82, PP.808-815.

33. M.Park, J.H. Lee, H. Shin, M. Kim, J .Choi, D.H. Kang, S .Heu, S. Ryu, Characterization and comparative genomic analysis of a novel bacteriophage, SFP10, simultaneously inhibiting both Salmonella enterica and ESCHERICHIA COLI O157:H7. Appl. Environ. Microbiol; 2012:vol. 78, PP.58-69.

34. H.Shin, N. Bandara, E .Shin, S .Ryu, K.P. Kim, Prevalence of Bacillus cereus bacteriophages in fermented foods and characterization of phage. Res Microbiol; 2011:vol. 162, PP.791-797.

35. Alexyuk P, Bogoyavlenskiy A, Alexyuk M, Akanova K, Moldakhanov Y, Berezin V. Isolation and Characterization of Lytic Bacteriophages Active against Clinical Strains of *E. coli* and Development of a Phage Antimicrobial Cocktail. Viruses. 2022 Oct 28;14(11):2381.

36. Patel DR, Bhartiya SK, Kumar R, Shukla VK, Nath G. Use of Customized Bacteriophages in the Treatment of Chronic Nonhealing Wounds: A Prospective Study. Int J Low Extrem Wounds. 2021 Mar;20(1):37-46.

37. Srivastava P, Mishra C P, Nath Bacteriophages Can Make a Difference in Water Quality: Evidence From a Community-Based Study From North India. Cureus 2022:14(8): e27551.

38. Toribio-Avedillo D, Blanch AR, Muniesa M, Rodríguez-Rubio L. Bacteriophages as Fecal Pollution Indicators. Viruses. 2021 Jun 7;13(6):1089.

39. KISHORE et al: PHAGE THERAPY OF CHRONIC OSTEOMYELITIS Indian J Med Res January 2016:143; pp 87-94

40. Nath G, Janam R, Kumar R, Gangwar M. Bacteriophage therapy: An alternative to antibiotics – An experimental study in mice. Ann Natl Acad Med Sci 2019; 55 : 151-8.

41. Gopal Nath, Ram Janam, Rajesh Kumar Mayank Gangwar, Bacteriophage Therapy in P. aeruginosa Septicaemia: Annals of the National Academy of Medical Sciences (India) :2019;Vol. 55: 3.