RESEARCH ARTICLE

Isolation of Lytic and Lysogenic Bacteriophages Specific to *Pseudomonas* spp.

Dhruvi M. Doshi[1]*, Bharat B. Bhanderi[2]

Abstract

In the present study, total 12 *Pseudomonas* spp. were obtained from 94 dogs suffering from otitis externa, and were characterized and subjected to *in vitro* antibiotic susceptibility test using commonly used antimicrobial drugs. Virulence associated gene *algD* (alginate synthesis) was detected in 91.67% isolates of *Pseudomonas* spp. Out of 12 *Pseudomonas* spp. isolates, six were selected and subjected for the isolation of bacteriophages. Total 12 samples, *i.e.,* eight sewage water and four pond water samples were processed for isolation of the bacteriophages specific to six *Pseudomonas* spp. selected using double agar overlay assay, out of which 50.00% (6/12) samples showed presence of phages in the form of plaque. Out of six samples three each were lytic and lysogenic phages. The bacteriophages were screened for the presence of integron genes (*intI1, intI2* and *intI3*) where *intI1* and *intI2* were detected in two lysogenic phages each. Out of three lysogenic phages, one was responsible for conversion of phenotypically non-mucoid strain of *Pseudomonas* spp. to mucoid. Moreover, a comparative *in vitro* antibiotic susceptibility zone analysis of the wild *Pseudomonas* spp. vs. lysogenic phage affected *Pseudomonas* spp. revealed that lysogenic phage infection altered the antimicrobial susceptibility of lysogenic phage affected *Pseudomonas* spp. isolates at a sub-inhibitory concentration of antibiotics, suggesting that they might be precursory to antimicrobial resistance.

Key words: Antibiotic resistance, Integron genes, Lytic and Lysogenic bacteriophages, Mucoid variation.

Ind J Vet Sci and Biotech (2023): 10.48165/ijvsbt.19.6.13

Introduction

Pseudomonas are Gram-negative, rod-shaped bacteria often isolated from soil, water, animals, plants, decaying organic matter and serves as opportunistic pathogen in otitis (Barnard and Foster, 2017). Day by day there is a rising emergence of multidrug-resistant (MDR) *Pseudomonas* isolates that are becoming resistant to all β-lactams, aminoglycosides and fluoroquinolones drugs which poses severe medical problems in the world (Kirikae *et al.,* 2008). *Pseudomonas* strains can acquire resistance from mobile genetic elements like plasmids, transposons or integrons (Peng *et al.,* 2014). Most *P. aeruginosa* isolates are still susceptible to colistin and polymixin B. To combat antimicrobial resistance bacteriophages are one of the preferred alternatives and is little established in the field of veterinary science.

A bacteriophage is a particle or virion that consists of a single-stranded (ss) or double-stranded (ds) DNA or RNA molecule, encapsulated inside a protein or lipoprotein coat that were recognized in early 20th century because of pioneering work of Twort and D'Herelle. They can be isolated from sea water, pond water, fresh water and sewage water ecosystems (Jensen *et al.,* 1998). They are broadly divided into two categories: Lytic and Lysogenic phages. Lytic phage on entering host bacteria immediately commandeers its replicative machinery for multiplication and encoding structural proteins and enzymes and then releasing progenies on lysis of bacterial cell thus offering an alternative to antibiotics. Lysogenic phage follows an alternative life cycle involving integration of their genome into the host chromosome forming a prophage. During this phase, the phage DNA replicates along with the host cell as lysogen and is maintained in the bacterial population (Davies *et al.,* 2016). *Pseudomonas* genus specific bacteriophages were first described in the middle of 20th century. Currently, 137 completely sequenced *Pseudomonas* phage genomes are found in public databases (Pires *et al.,* 2015). Double agar

[1-2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University (Now in Kamdhenu University), Anand-388 001, Gujarat, India

Corresponding Author: Doshi Dhruvi, Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat- 388001, India, e-mail: drdhruvi127@gmail.com

How to cite this article: Doshi, D. M., & Bhanderi, B. B. (2023). Isolation of Lytic and Lysogenic Bacteriophages Specific to *Pseudomonas* spp. Ind J Vet Sci and Biotech. 19(6), 63-68.

Source of support: Nil

Conflict of interest: None

Submitted: 23/08/2023 **Accepted:** 10/10/2023 **Published:** 10/11/2023

overlay plaque assay is identified as gold standard method for isolation of bacteriophages (Sambrook and Russell, 2001).

Unlike broad-spectrum antibiotics, lytic phages are highly specific and do not elicit resistance towards untargeted bacteria. A controlled clinical trial was carried out using lytic bacteriophage preparation in chronic otitis due to antibiotic-resistant *P. aeruginosa,* which resulted into a significant reduction in phage treated group (Wright *et al.*, 2009). Lysogenic phages were intergrated into *Pseudomonas* spp. as lysogens had the greatest impact on reducing the susceptibility to the antibiotics to sub-inhibitory level, suggesting they might be precursory to antimicrobial resistance (Tariq *et al.,* 2019). Moreover, infection by lysogenic bacteriophages has been proposed as a mechanism for the conversion of *P. aeruginosa* from the non-mucoid to the mucoid phenotype due to alginate synthesis (Miller and Rubero, 1984). Alginate overproduction provides protection to bacteria from external challenges like antimicrobials, accelerates biofilm production and makes them resistant to opsonisation (Everest, 2016). Looking towards the increasing AMR amongst *Pseudomonas* spp. and recognizing phage therapy as an alternative to AMR, this study was undertaken.

Materials and Methods

Sample Collection, Cultural Isolation, Biochemical Characterization and ABST

The study was performed during April 2019 to March 2020. Total 94 ear swabs samples were collected aseptically from dogs suffering with otitis externa. For *Pseudomonas* spp. culture examination, ear swab samples were streaked on Nutrient agar (NA) for primary isolation and characterization. The plates were incubated at 37°C for 24-48 h. The *Pseudomonas* spp. were identified based upon pigment production on NA and further identified based upon oxidase test (Markey *et al.,* 2013). The isolates were subjected to *in vitro* antibiotic susceptibility test (ABST) as per Bauer *et al.* (1966) and interpretation of results was done according to the Clinical and Laboratory Standards Institute (Wayne, 2019) using antimicrobial drugs, *viz.,* ampicillin, amoxicillin-clavulanic acid, gentamicin, amikacin, tobramycin, ceftriaxone, cefotaxime, enrofloxacin, moxifloxacin, ciprofloxacin, ofloxacin, norfloxacin, imipenem, meropenem and polymyxin B.

Isolation of Bacteriophages

The bacteriophages were isolated specific towards six *Pseudomonas* spp. isolates out of 12 selected randomly. For obtaining bacteriophages, sewage and pond water samples up to 100-200 mL were collected in sterilized glass from Anand district over a period of 3 months (Dec-19 to Feb-20). The sewage and pond water samples were brought on ice in a Styrofoam box and were immediately subjected to phage isolation.

For isolation of bacteriophages, 1% underlay Brain Heart Infusion (BHI) agar and 0.3% overlay BHI agar was prepared. For isolation of bacteriophages double agar overlay plaque assay was used where the sewage or pond water samples were centrifuged at 10000 rpm for 10 min, the supernatant was collected and mixed with equal volume of double strength (2x) BHI and 10 mL of overnight culture of *Pseudomonas* spp. was added to the mixture and incubated at 37°C for enrichment of the bacteriophages. On the following day, the enrichment broth was centrifuged at 6000g for 10 min and supernatant was filtered with a membrane pore filter 0.45 µm in size to get clear lysate. Ten-fold serial dilution of phage lysate was performed using BHI broth in 10 Eppendorf tubes. 10 bottom agar plates (1% BHI agar) were taken and marked from 10^{-1} to 10^{-10} after that 200 µL of the phage lysate of the specific dilution was added into the corresponding plate along with 100 µL overnight culture broth of *Pseudomonas* spp., 100 µL 1M $CaCl_2$ and 0.3% of overlay agar were added into all the plates containing 1% bottom agar. Plates were swirled vigorously but briefly so that all the contents get mixed homogenously and were allowed to solidify. The plates were incubated at 37°C overnight with the lid facing upwards. The plaques were obtained on the following day.

PCR

The DNA of *Pseudomonas* spp. isolates was extracted using simple boiling method and subjected to detection of alginate synthesis gene [*algD* (Lanotte *et al.,* 2004)]. The DNA of bacteriophage was extracted as per the method stated by

Table 1: Primer sequence of ARGs and size of amplified products

Target genes		Primer sequence (5'-3')	Size of amplified products (bp)	References
*alg*D (Alginate synthesis)	F	ATGCGAATCAGCATCTTTGGT	1310	Lanotte *et al.* (2004)
	R	CTACCAGCAGATGCCCTCGGC		
intI1 (Class I integron)	F	ACG AGC GCA AGG TTT CGG T	565	Oosterik *et al.* (2014)
	R	GAA AGG TCT GGT CAT ACA TG		
intI2 (Class II integron)	F	GTG CAA CGC ATT TTG CAG G	403	Oosterik *et al.* (2014)
	R	CAA CGG AGT CAT GCA GAT G		
intI3 (Class III integron)	F	CAT TTG TGT TGT GGA CGG C	717	Oosterik *et al.* (2014)
	R	GAC AGA TAC GTG TTT GGC AA		

Table 2: Steps and conditions of thermal cycling for different genes primer pair used in PCR

Primers	Cycling conditions				
	Initial Denaturation	**Denaturation**	**Annealing**	**Extension**	**Final Extension**
intI1	95°C, 5 min	94°C, 1 min	54°C, 1 min	72°C,1 min	72°C, 10 min
intI2	95°C, 5 min	94°C, 30 s	52°C, 30 s	72°C, 2 min	72°C, 7 min
intI3	94°C, 5 min	94°C, 30 s	54°C, 30 s	72°C, 2 min	72°C, 10 min
*alg*D	94°C, 5 min	94°C, 45 s	62°C, 1 min	72°C. 1 min	72°C, 7 min

Note: Annealing was repeated for 30 cycles each for each primer.

Sambrook and Russell (2006) and PCR was carried out to detect integron genes [*intI1, intI2* and *intI3* (Oosterik *et al*., 2014)] in bacteriophage genome. The primer sequence and PCR conditions were as mentioned in Table 1 and 2. Horizontal transfer ARGs (antibiotic resistance genes) in bacteria are always facilitated by integrons and bacteriophages. Agarose gel electrophoresis was carried out to confirm the targeted amplification where PCR products using DNA molecular weight marker (Thermo Fisher) on 2.0 % agarose (low EEO, SeaKem, USA) gels containing 0.5 µg/mL ethidium bromide (Sigma-Aldrich, USA) at 80 V in 0.5X TBE buffer. The amplified product was visualized as a single compact band of expected size under UV light and documented by gel documentation system (Genetix Biotech Pvt. Ltd. Delhi).

Results and Discussion

Out of the 94 otitis samples, 12 were positive for *Pseudomonas* spp. by cultural isolation and they produced diffusible pigmentation on NA, *viz.,* 58.33% (7/12) isolates produced pyoverdin (greenish yellow), 33.33% (4/12) isolates produced pyocyanin (blue green) and 8.33% (1/12) isolate produced pyomelanin (brownish-black) pigments and were positive for oxidase. The isolates were found susceptible to antibiotics in descending order which was meropenem (100.00%); tobramycin, imipenem and polymyxin (91.67% each); gentamicin, amikacin and moxifloxacin (83.33% each); ceftriaxone (75.00%), enrofloxacin, ofloxacin, ciprofloxacin and norfloxacin (66.67% each); amoxicillin clavulanic acid (16.67%); cefotaxime (8.33%), and showed 100% resistance towards ampicillin.

In the present study, total 12 samples *i.e.,* eight sewage water and four pond water samples were processed for isolation of the bacteriophages specific to six *Pseudomonas* spp. obtained from otic ears of dogs. Out of eight sewage samples, 50.00% (4/8) were found positive for the presence of bacteriophages where two each were lytic and lysogenic. Out of four pond water samples, 50.00% (2/4) were found positive for the presence of bacteriophages where one each was lytic and lysogenic. For six sewage/pond water positive samples, each phage sample was isolated specifically towards the particular strain of *Pseudomonas* spp. isolated from otic ears of dogs.

The nomenclature of the bacteriophages was done on the basis of the system proposed by Kropinski *et al.* (2009) with slight modifications, where (a) The first part, *i.e.,* 'vB' which signifies that the phage is a bacterial virus, (b) The second part comprises of the host bacteria used to isolate the phages, e.g., for *Pseudomonas* spp. it was designated with the acronym 'Pae' and (c) The third part is made up of the type of sample from which the phage is isolated, e.g., 'S' for sewage water sample and 'P' for pond water sample followed by the number of the strain of *Pseudomonas* spp. specific to which the phage is isolated. Thus, if the phage is named vB_Pae_S1, then it means that the phage is a bacterial virus specific to *Pseudomonas* spp., isolated from the sewage water specific to strain one of *Pseudomonas* spp. (Table 3).

In the present study, the lytic phages isolated from sewage water, *i.e.,* vB_Pae_S7R and vB_Pae_S45 gave clear plaques ranging from 1-3 mm in size and vB_Pae_P34 obtained from pond water showed clear plaques of size 4 mm. The plaques obtained did not possess any halo or turbidity (Fig. 1). It was in agreement with previous studies done by Harada *et al.* (2022) who identified lytic bacteriophages as clear plaque ranging 0.5-3 mm in diameter.

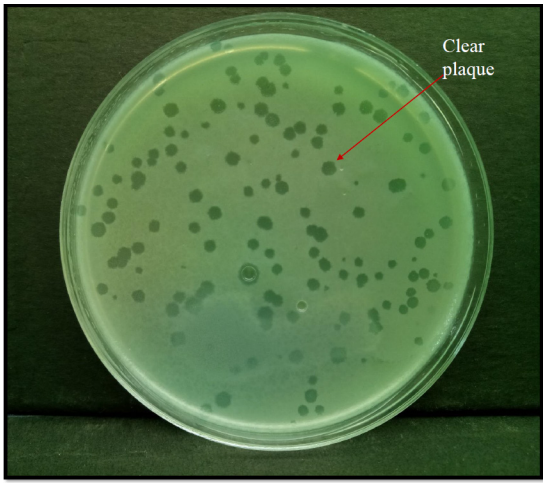

Fig. 1: Clear plaques produced by Lytic phages of *Pseudomonas* spp.

In the present study, the lysogenic phages (vB_Pae_P9, vB_Pae_S20 and vB_Pae_S51) that were isolated from the sewage water and pond water gave the plaques (area of lysis) surrounded by halo or sometimes by mucoid growth of bacterial colonies, ranging from 2-4 mm in diameter or sometimes as turbid plaques (Fig. 2). The results were in agreement with Miller and Rubero (1984).

Table 3: Sample wise isolated bacteriophages of *Pseudomonas* spp.

Sr. no.	Type of Sample	Date of collection	Place of collection	Bacteriophages specific to *Pseudomonas* spp.	Type of Bacteriophage
1.	Sewage water	23/12/2019	Anand	vB_Pae_S7R	Lytic
2.	Sewage water	1/01/2020	Anand	Negative	-
3.	Pond water	7/01/2020	Anand	vB_Pae_P9	Lysogenic
4.	Sewage water	9/01/2020	Anand	vB_Pae_S20	Lysogenic
5.	Sewage water	15/01/2020	Anand	Negative	-
6.	Pond water	21/01/2020	Anand	vB_Pae_P34	Lytic
7.	Sewage water	24/01/2020	Anand	Negative	-
8.	Sewage water	27/01/2020	Anand	Negative	-
9.	Pond water	3/02/2020	Anand	Negative	-
10.	Sewage water	5/02/2020	Anand	vB_Pae_S45	Lytic
11.	Pond water	10/02/2020	Anand	Negative	-
12.	Sewage water	12/02/2020	Anand	vB_Pae_S51	Lysogenic

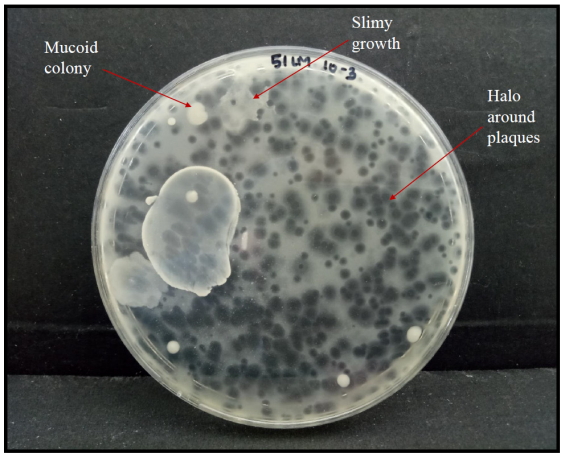

Fig. 2: Halo around plaques, slimy growth and mucoid colonies produced by Lysogenic phages of *Pseudomonas* spp.

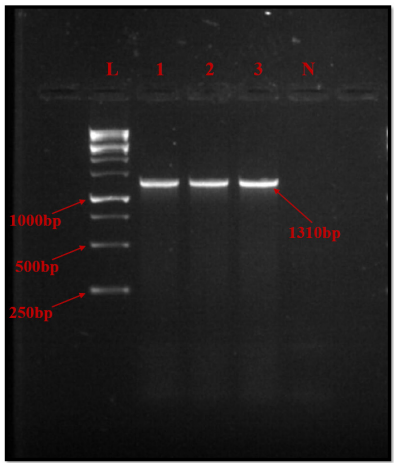

Fig. 3: Agarose gel showing amplified product for algD (1310bp) for bacterial isolates L- 250-25000bp N-Negative, 1-3- bacterial isolates

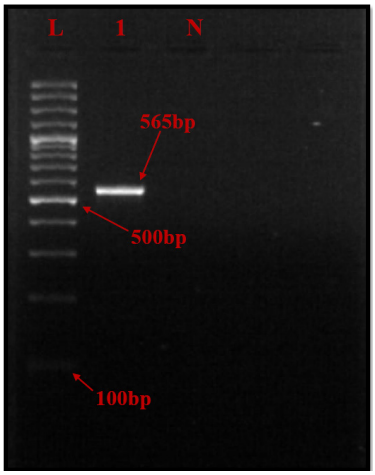

Fig. 4: Agarose gel showing amplified product for intI1 (565bp) for bacteriophage L-100-1500bp N-Negative, 1- bacteriophage sample

PCR Based Detection of Virulence Associated Gene *algD* in *Pseudomonas* spp. Genome

The extracted DNA of *Pseudomonas* spp. isolates were subjected to PCR for detection of *algD* gene where it was detected in 91.67% (11/12) isolates having size 1310 bp (Fig. 3). The similar findings were reported earlier where *algD* was detected in 100.00% and 95.30% isolates, respectively (Sambrook and Russell, 2006; Tahmasebi *et al.,* 2017).

PCR Detection of Integron Genes in Bacteriophage Genome

For integron genes, *intI1* was detected in lysogenic phage vB_Pae_P9 having size 565 bp (Fig. 4) and *intI2* was detected in vB_Pae_S51 having size 403 bp (Fig. 5). The gene *intI3* with size 717 bp was not detected in any of the phages. This was in partial agreement with Anand *et al.* (2016), who detected integron genes *intI1* and *intI2* in 10.9 % of the bacteriophages isolated while *intI3* in 9.1% of the bacteriophage genome. None of the lytic phages contained any of the integron genes.

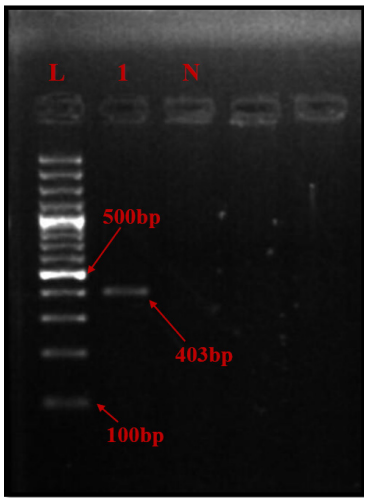

Fig. 5: Agarose gel showing amplified product for intI2 (403bp) for bacteriophage L-100-1500bp N-Negative, 1- bacteriophage sample

Induction of Mucoid Variation in *Pseudomonas* spp. by Lysogenic Bacteriophage

In the present study, out of three lysogenic phages isolated, one of the phage vB_Pae_S51 resulted into conversion of the non-mucoid strain 51 of *Pseudomonas* spp. from otitis externa into mucoid. But when it was subjected for the isolation of bacteriophages using sewage water, the plaques obtained on the bacteriophage culture plate were surrounded by a halo along with slimy and mucoid growth of bacterial colonies around the plaque. When this slimy and mucoid growth was sub-cultured on another BHI agar plate, they gave the mucoid colonies of *Pseudomonas* spp. (Fig. 6). To test this mucoid strain for the presence of lysogenic phage, once again the plaque assay was carried out using those mucoid strains. The mucoid strain 51 was inoculated in the BHI broth and grown over-night at 37°C temperature. Next day the overnight culture was centrifuged at 5000 rpm to remove the bacterial cell debris and the supernatant was filtered and was subjected to plaque assay against the same 51 wild non-mucoid strain. Next day the same results *i.e.,* the presence of plaques surrounded by halo and the presence of slimy and mucoid growth around the plaques confirmed that the lysogenic phage mediated the conversion of the strain from the non-mucoid to mucoid phenotype. Also, *intI2* was detected in lysogenic phage vB_Pae_S51. Thus, due to induction of phage encoded genes into the bacterial genome, it might have resulted into the conversion of the phenotypically non-mucoid strain 51 of *Pseudomonas* spp. into mucoid (Miller and Rubero, 1984).

Alginate production gene (*algD)* was detected in both mucoid and non-mucoid strain 51. Similar results were shown by Martin *et al.* (1993) where they reported that a cluster of three tightly linked genes *algU, mucA* and *mucB* controlled development of mucoid phenotype in *Pseudomonas* spp. Gene *algU* is required for activation of *algD* that causes over-production of alginate. However, genes *mucA* and *mucB* counteracts the activity of *algU* thus inhibiting alginate production. Inactivation of *mucA* or *mucB* causes mucoid phenotype. Thus, it can be concluded that the genes responsible for alginate production are present mostly in all the *Pseudomonas* spp. either they are phenotypically mucoid or non-mucoid. But a factor is required for the activation of alginate synthesis in *Pseudomonas* spp. and here phage may be serving as that factor causing inactivation of *mucB* thereby activating *algU* which in turn activated *algD* resulting into mucoid phenotype.

Fig. 6: Conversion of non-mucoid 51 wild strain of Pseudomonas spp. into mucoid 51PMU by lysogenic phage vB_Pae_S51

Comparative *In Vitro* Antibiotic Susceptibility Zone Analysis of the Wild *Pseudomonas* spp. vs. Lysogenic Phage Affected *Pseudomonas* spp.

In the present study, ABST was carried out for phage infected *Pseudomonas* strain 51PMU (phage induced mucoid strain 51 of *Pseudomonas* spp.), 9PA (phage affected strain 9 of *Pseudomonas* spp.) and 20PA (phage affected strain 20 of *Pseudomonas* spp.). For 51 wild vs. 51PMU of *Pseudomonas* spp., a significant difference was noticed in the zone diameter ranging from 3 to 10 in ciprofloxacin followed by tobramycin, meropenem, moxifloxacin, norfloxacin, imipenem, amikacin and polymyxin B. For 9 wild vs. 9PA strains, a significant difference was noticed in zone diameter ranging from 3 to 8 in ceftriaxone, amoxicillin clavulanic acid, cefotaxime and ciprofloxacin. Lysogenic phage infection reduced the antimicrobial susceptibility of both isolates to sub-inhibitory levels suggesting that they might be precursory to antimicrobial resistance. For 20 wild vs. 20PA strains, no significant difference could be noticed. This was in agreement with study carried out by Tariq *et al.* (2019), who had experimentally infected PAO1 lab strain of *P. aeruginosa* with 20 temperate/lysogenic bacteriophages, out of which 7 phages decreased the antibiotic susceptibility of PAO1 towards antibiotics tested, *i.e.,* ceftazidime, colistin, meropenem and piperacillin tazobactam to the sub-inhibitory level suggesting that lysogenic phages are responsible for antibiotic resistance.

Conclusion

In the present study, we have recognized phage therapy as an alternative to antibiotics treatment due to increasing antimicrobial resistance, but it is essential that prior to going for phage therapy one should identify whether the phage is lytic or lysogenic because only lytic phage serves the therapy purpose. The lysogenic phages might cause conversion of the non-mucoid strain to mucoid due to alginate production or can reduce the antimicrobial susceptibility of *Pseudomonas* spp. to sub-inhibitory concentrations of antibiotics, and enhance the antimicrobial resistance amongst the isolates.

Acknowledgement

Authors are thankful to the Dean of the College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand for providing necessary facilities to carry out the work.

References

Anand, T., Bera, B.C., Vaid, R.K., Barua, S., Riyesh, T., Virmani, N., ... & Tripathi, B.N. (2016). Abundance of antibiotic resistance genes in environmental bacteriophages. *Journal of General Virology*, *97*(12), 3458-3466.

Barnard, N., & Foster, A. (2017). *Pseudomonas* otitis in dogs: A general practitioner's guide to treatment. *In Practice*, *39*(9), 386-398.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, *45*(4), 493-496.

Davies, E.V., Winstanley, C., Fothergill, J.L., & James, C.E. (2016). The role of temperate bacteriophages in bacterial infection. *FEMS Microbiology Letters*, *363*(5), 1-38.

Everest, F.L.C. (2016). Characterising the role and evolution of temperate bacteriophages in chronic respiratory infections including cystic fibrosis and bronchiectasis. *Doctoral Dissertation,* University of Northumbria at Newcastle (United Kingdom).

Harada, L.K., Silva, E.C., Rossi, F.P., Cieza, B., Oliveira, T.J., Pereira, C., ... & Balcão, V.M. (2022). Characterization and *in vitro* testing of newly isolated lytic bacteriophages for the biocontrol of *Pseudomonas aeruginosa*. *Future Microbiology*, *17*(2), 111-141.

Jensen, E.C., Schrader, H.S., Rieland, B., Thompson, T.L., Lee, K.W., Nickerson, K.W., & Kokjohn, T.A. (1998). Prevalence of broad-host-range lytic bacteriophages of Sphaerotilus natans, *Escherichia coli*, and *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology*, *64*(2), 575-580.

Kirikae, T., Mizuguchi, Y., & Arakawa, Y. (2008). Investigation of isolation rates of *Pseudomonas aeruginosa* with and without multidrug resistance in medical facilities and clinical laboratories in Japan. *Journal of Antimicrobial Chemotherapy*, *61*(3), 612-615.

Kropinski, A.M., Prangishvili, D., & Lavigne, R. (2009). Position paper: The creation of a rational scheme for the nomenclature of viruses of Bacteria and Archaea. *Environmental Microbiology*, *11*(11), 2775-2777.

Lanotte, P., Watt, S., Mereghetti, L., Dartiguelongue, N., Rastegar-Lari, A., Goudeau, A., & Quentin, R. (2004). Genetic features of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients compared with those of isolates from other origins. *Journal of Medical Microbiology*, *53*(1), 73-81.

Markey, B., Leonard, F., Archambault, M., Cullinane, A., & Maguire, D. (2013). *Clinical Veterinary Microbiology*. e-book. Elsevier Health Sciences, pp. 275-288.

Martin, D.W., Schurr, M.J., Mudd, M.H., & Deretic, V. (1993). Differentiation of *Pseudomonas aeruginosa* into the alginate-producing form: inactivation of mucB causes conversion to mucoidy. *Molecular Microbiology*, *9*(3), 497-506.

Miller, R.V., & Rubero, V.J. (1984). Mucoid conversion by phages of *Pseudomonas aeruginosa* strains from patients with cystic fibrosis. *Journal of Clinical Microbiology*, *19*(5), 717-719.

Oosterik, L.H., Peeters, L., Mutuku, I., Goddeeris, B.M., & Butaye, P. (2014). Susceptibility of avian pathogenic *Escherichia coli* from laying hens in Belgium to antibiotics and disinfectants and integron prevalence. *Avian Diseases*, *58*(2), 271-278.

Peng, Y., Bi, J., Shi, J., Li, Y., Ye, X., Chen, X., & Yao, Z. (2014). Multidrug-resistant *Pseudomonas aeruginosa* infections pose growing threat to health care–associated infection control in the hospitals of Southern China: A case-control surveillance study. *American Journal of Infection Control*, *42*(12), 1308-1311.

Pires, D.P., Vilas Boas, D., Sillankorva, S., & Azeredo, J. (2015). Phage therapy: a step forward in the treatment of *Pseudomonas aeruginosa* infections. *Journal of Virology*, *89*(15), 7449-7456.

Sambrook, J., & Russell, D.W. (2001). *Molecular Cloning - Laboratory Manual*. New York, Cold Spring Harbor Laboratory Press.

Sambrook, J., & Russell, D.W. (2006). *The Condensed Protocols from Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press.

Tahmasebi, H., Adabi, J., Shahraki Zahedani, S., & Zeyni, B. (2017). Comparison of two methods of Direct PCR and PCR with DNA extracted by kit for detection of OPrL, ExoA, and algD genes in clinical isolates of *Pseudomonas aeruginosa*. *Qom University of Medical Sciences Journal*, *11*(3), 11-21.

Tariq, M.A., Everest, F.L., Cowley, L.A., Wright, R., Holt, G.S., Ingram, H., ... & Smith, D.L. (2019). Temperate bacteriophages from chronic *Pseudomonas aeruginosa* lung infections show disease-specific changes in host range and modulate antimicrobial susceptibility. *MSystems*, *4*(4), 1110-1128.

Wayne, P.A. (2019). Clinical and laboratory standards institute. *Performance standards for antimicrobial susceptibility testing. CLSI document M100-S17.*

Wright, A., Hawkins, C.H., Änggård, E.E., & Harper, D.R. (2009). A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*: A preliminary report of efficacy. *Clinical Otolaryngology*, *34*(4), 349-357.