RESEARCH ARTICLE Indian Journal of Animal Research, Volume 56 Issue 7: 873-879 (July 2022)

Isolation and Characterization of Lytic Bacteriophage against Methicillin Resistant *Staphylococcus aureus* from Pyoderma in Dog

Archana, P. Kaushik, Anjay, A. Kumar[1], S. Kumari[2], P. Kumar[2], P. Shekhar[3]

10.18805/IJAR.B-4869

ABSTRACT

Background: Pyoderma in dog poses complexity owing to involvement of multi drug resistant bacterial infection particularly *Staphylococcus* species. This may result into failure of antibiotic therapy in pyoderma. Therapeutic use of lytic bacteriophage (phage) may be an alternative to deal antimicrobial resistance in veterinary medicine. There is no report available on isolation and characterization of bacteriophage from clinical cases of pyoderma in animals, hence the study was performed with aim to isolate and characterize lytic phage against methicillin resistant *S. aureus* (MRSA).

Methods: The phage was isolated from pus sample of dog suffering from severe pyoderma. The phage was isolated using MRSA as a bacterial host and was named as *Staphylococcus* phage BVC1 (SPBVC1). The morphology of the phage was determined using TME and was characterised by determining the host range and lytic potential of the phage at range of temperature and pH.

Result: The morphology of phage revealed an icosahedral head of diameter 81.31 nm. with sheath and a central tube and a tail of 92.08 nm. It showed strong lytic activity against Methicillin resistant *S. aureus* and was stable under a range of temperature varying from 4°C to 45°C and pH from 4 to 11. The phage has shown lytic activity against the MRSA however no lytic activity against the MSSA was shown by the phage. The high specificity of the phage for MRSA indicated its potential use as an alternative therapeutic approach against multidrug resistant staphylococcal infections.

Key words: Bacteriophage, MRSA, Pyoderma, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus is a gram-positive cocci present as commensal skin microflora, in the nostrils and respiratory tract (Lee *et al.,* 2009). It is an opportunistic pathogen commonly associated with humans and animals, capable of causing serious diseases and death including sepsis, pneumonia, meningitis in humans and mastitis, keratitis, osteomyelitis, pyoderma in animals (Mavrogianni *et al.,* 2007; Pintado *et al.,* 2019 and González *et al.,* 2020). The occurrence of *Staphylococcus pseudintermedius* as an opportunistic pathogen on the skin is very common in dogs and the studies show 46 to 92% prevalence of *S. pseudintermedius* in dogs (Kawakami *et al.,* 2010; Rubin and Chirino, 2011; Bannoehr *et al.,* 2012 and Morris *et al.,* 2017; Lynch and Helbig, 2021), however involvement of *S. aureus* in canine pyoderma has also been reported (Reddy *et al.,* 2016 ; González *et al.,* 2020). The increased incidence of methicillin resistant *S. aureus* (MRSA) has become a major cause of concern as *S. aureus* is one of the most common causes of nosocomial infections (Chambers *et al.,* 2009 ; Dulon *et al.,* 2011). Methicillin is a β-lactamase stable antibiotic which was introduced in 1959 to combat penicillin, drug used for the treatment of Gram positive bacterium, resistance. However, methicillin-resistant strains of *S. aureus* were isolated soon after its introduction (Jevons 1961; Knox 1961). The development of antibiotic resistance in bacteria to different groups of antibiotics has resulted into the search of alternative approach for treatment of such cases (O'Flynn *et al.,* 2004; Kutter *et al.,* 2015) and bacteriophage has been used as one of the alternative therapy against bacterial strain resistant to antibiotics (Watanabe *et al.,* 2007; Lin *et al.* 2017). Bacteriophages (phages) are diverse and ubiquitous non living biological entities consisting of DNA or RNA enclosed in a protein capsid which are capable of infecting and replicating within bacterial cells, although they can not reproduce independently. They are widely distributed in nature and have been reported from sea and freshwater

Department of Veterinary Public Health and Epidemiology, Bihar Veterinary College, Bihar Animal Sciences University, Patna-800 014, Bihar, India.

[1]Department of Veterinary Biochemistry, Bihar Veterinary College, Bihar Animal Sciences University, Patna-800 014, Bihar, India.

[2]Department of Veterinary Microbiology, Bihar Veterinary College, Bihar Animal Sciences University, Patna-800 014, Bihar, India.

[3]Department of Veterinary Medicine, Bihar Veterinary College, Bihar Animal Sciences University, Patna-800 014, Bihar, India.

Corresponding Author: P. Kaushik, Bihar Veterinary College, Bihar Animal Sciences University, Patna-800 014, Bihar, India. Email: drkaushikvet@gmail.com

How to cite this article: Archana, Kaushik, P., Anjay, Kumar, A., Kumari, S., Kumar, P. and Shekhar, P. (2022). Isolation and Characterization of Lytic Bacteriophage against Methicillin Resistant *Staphylococcus aureus* from Pyoderma in Dog. Indian Journal of Animal Research. 56(7): 873-879. DOI: 10.18805/IJAR.B-4869.

Submitted: 17-01-2022 **Accepted:** 15-04-2022 **Online:** 28-05-2022

throughout the globe including hypersaline environments, the soil, deserts, polar regions and on as well as within other organisms (Díaz-Muñoz and Koskella, 2014). Phages typically bind to specific receptors on the bacterial cell surface, inject their genetic material into the host cell and then either integrate this material into the bacterial genome ("temperate" phages) and reproduce vertically, or hijack the bacterial replication machinery to produce phage progeny and lyse the bacterial cell ("lytic" phages) (Clokie *et al.,* 2011). These lytic phages are used for conventional phage therapy to kill bacterial pathogens. Host specificity varies among phages, some of which are strain-specific, whereas others have a host range across the bacterial strains and even genera (Lin *et al.,* 2017). The use of bacteriophage in the treatment of staphylococcal infections were first described by Bruynoghe and Maisin (1921) by injecting the phage preparation around surgically opened lesions with regression of infection within 24-48 hours. Keeping these facts and observation in mind the present study was conducted to isolate and characterize lytic bacteriophage against methicillin resistant *S. aureus* from pus of a dog with chronic skin disease.

MATERIALS AND METHODS

Isolation of host *S. aureus* from pus

The *S. aureus* was isolated from pus sample by selective plating on mannitol salt agar (MSA). A loopful of pus sample from dog skin surface was inoculated in tryptone soya broth (TSB) containing 10% sodium chloride salt (TSB-S) for enrichment at 37°C for 24 h. The positive sample was streaked on mannitol salt agar, incubated at 37°C for 24 h and observed for mannitol fermentation. Presumptive *S. aureus* colonies were identified on the basis of colony morphology and mannitol fermentation and were confirmed by biochemical test including the catalase activity tests and tube coagulase tests with human plasma to confirm *S. aureus*. To further verify the isolate, genomic DNA was extracted with phenol-chloroform and PCR was performed targeting 16S-rDNA using specific Forward-GTAGGTGGCAAGCGTT ATCC and Reverse- CGCACATCAGCGTCAG primer for *S. aureus* (Karmakar *et al.,* 2016). The catalase-positive, coagulase-positive, mannitol-fermentation and PCR-positive *S. aureus* isolates were also screened for methicillin resistance both phenotypically and genotypically as per Braoios *et al.,* (2009).

Bacteriophage isolation

The pus sample from dog skin was inoculated into 25 ml TSB with 10% NaCl. For enrichment of phage 0.5 ml of overnight grown culture of *S. aureus* isolate was added in TSB before incubation of sample at 37°C for 24 h. After enrichment, 1 ml of sample was transferred into 1.5 ml pre-sterilized microfuge tube and centrifuged at 5000 rpm for 10 min. The supernatant was filtered with 0.22 µm syringe filter and phage filtrate was preserved at 4°C. The presence of phage was confirmed by spot assay against the host bacteria. In brief, 100 µl of overnight grown culture of *S. aureus* was mixed with 3 ml of LB top layer agar (0.7% agar) and poured onto pre-dried nutrient agar (3%) plates and left to dry. Further 10 µl of phage filtrate was spotted over plates and allowed to be absorbed onto the plate. Once dried, plates were incubated at 37°C for 12-24 h and examined for presence of complete lysis/plaque produced. Sample was considered positive for lytic phage on observation of complete lysis/clear plaque/confluent or opalescent lysis, while appearance of turbid zone in the spot was considered negative.

Purification of bacteriophage

The phage which produced clear plaques/lysis was purified by serial dilution and plating on soft agar overlays as per the method described by Adams (1959). The complete lysis zone/plaques formed was extracted using a sterile pipette-tip, re-suspended in 1 ml salt magnesium buffer (NaCl, 5.8 g; $MgSO_4 7H_2O$, 2 g; 1 M Tris Cl pH 7.5, 50 ml; 2% gelatin, 5 ml; add ddH_2O to 1,000 ml) and incubated overnight at 4°C for eluting the phage in buffer. The phage suspension filtered through 0.22 µm syringe filter, was 10 fold serially diluted. 100 µl of diluted phage and 100 µl host bacterium were mixed with 3.0 ml molten soft agar (0.7% agar) and poured quickly on top of solidified nutrient agar plate (3% agar). The plates were gently rotated and incubated overnight at 37°C. The single plaque was passaged three times to obtain a pure phage.

Host range determination

The host range of the bacteriophage was determined against a number of methicillin resistant (62) and methicillin sensitive (42) *S. aureus* isolates as per the methods described by Jamalludeen *et al.,* (2007). Lawn of a single bacterial isolate was inoculated on a BHI agar plate and 10 µl of phage suspension (10^9 pfu/ml) was dropped in the centre of bacterial lawn after the plate dried up. Following incubation at 37°C for 24 h the plates were examined for lysis. A clear zone in the bacterial lawn was recorded as complete lysis.

Transmission electron microscopy (TEM)

Purified bacteriophage was visualized by TEM (Jeol JEM-1011, Japan) at the division of plant pathology, Indian Agricultural Research Institute, New Delhi, India. In order to visualize the phage by TEM, the bacteriophage suspension was concentrated as per the method described by Davis *et al.,* 1986 with some modifications. In brief, 10 ml of high titre bacteriophage filtrate was mixed gently with 10 ml of TM buffer and incubated for 15 minutes at room temperature. After incubation, 2 ml of 5 M NaCl and 2.2 g of solid PEG-8000 was added to the mixture and dissolved completely in a centrifuge tube. The tube was kept at 4°C for 2 h followed by centrifugation at 12000 g at 4°C for 45 min. The supernatant was poured off and pellets were dissolved in 300 µl of TM buffer which were further treated with equal volume of chloroform and centrifuged at 12000 rpm for 5 min after proper mixing. The supernatant was

stored into different aliquot at 4°C and processed for transmission electron microscopy.

Determination of thermal and pH stability of phage

The thermal tolerance of bacteriophage was evaluated at five different temperatures 4, 25, 37, 45 and 65°C in SM (Salt Magnesium) buffer. 100 µl of the bacteriophages suspension (6.2×10^8 PFU/ml) was added to a microfuge tube containing 900 µl SM buffer and placed in water bath at 25, 37, 45 and 65°C and at 4°C in refrigerator. The tubes were incubated at the required test temperature for 60 min and then placed on ice for 10 min before titration by double-layer agar plate method. A ten-fold serial dilution was prepared and 100µl phage from each dilution was added with 100 µl host bacterium and were mixed with 3.0 ml molten soft agar (0.7% agar, w/v) and poured quickly on top of solidified nutrient agar plate (3% agar, w/v). The plates were gently rotated and left to dry at room temperature for 20 min. The plates were incubated overnight at 37°C. The number of plaques formed was recorded and plaque forming units (pfu) per ml in the bacteriophage suspension was calculated. To evaluate the stability of phage at different pH conditions, it was incubated in SM buffer with different pH value. The pH of buffer was adjusted with 4 N HCl (Hi-media) or 2 N NaOH (Hi-media) to a pH range of 1-12 and the phage was treated with each SM buffer solution. After incubation at 37°C for 12 h, phage titration was determined by double-layer agar plate method as described above. All experiments were conducted in duplicate.

RESULTS AND DISCUSSION

Pyoderma in dogs is very common clinical presentation globally. The treatment efficacy of canine pyoderma using antimicrobial agents has become limited and challenging with the emergence of multidrug resistant staphylococci, including resistance to the semi-synthetic penicillinase-resistant penicillins such as methicillin (Jones *et al.,* 2007; Kawakami *et al.,* 2010 and Morris *et al.,* 2017). Also the rate of emergence of resistant pathogens is not proportionate to the rate and economics involved in the discovery of new antibiotics. Therefore, under these circumstances, to overcome the problem of evolving antibiotic resistance in staphylococci, bacteriophage may act as a potent alternative (Kwiatek *et al.,* 2012; Han *et al.,* 2013). Different studies have indicated the systemic and topical use of antimicrobial agents, with varying efficacy in the therapeutic management of pyoderma in dogs (Tomlin *et al.,*1999; Morris *et al.,* 2006; Loeffler *et al.,* 2011; Bryan *et al.,* 2012 and Beco *et al.,* 2013), however very limited reports are available about characterization of *S. aureus* specific phages that has been isolated from canine pyoderma. There is no report available on characterisation of *S. aureus* specific phage isolated from canine pyoderma or dermatitis cases.

Although *S. pseudintermedius* has been reported primarily as the cause of canine pyoderma (Rubin and Chirino 2011; Bannoehr *et al.,* 2012 and Morris *et al.,* 2017),

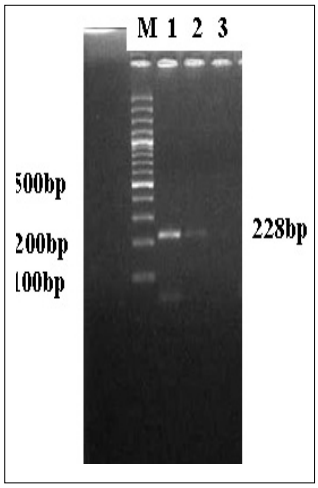

Fig 1: PCR amplification of *16S rRNA* gene with a product size 228 bp, M: 100 bp DNA ladder.

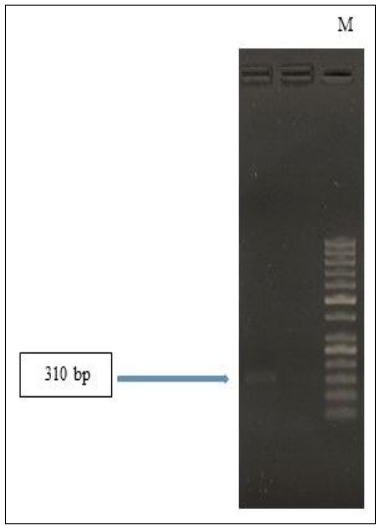

Fig 2: PCR amplification of *mecA* gene with a product size 310 bp, M: 100 bp DNA ladder.

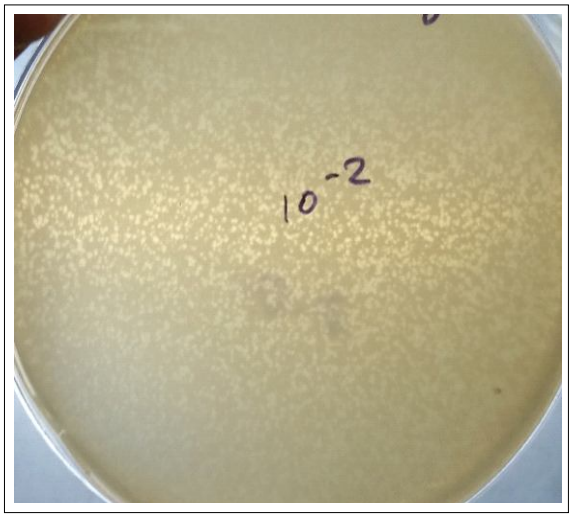

Fig 3: Plaques produced by bacteriophage SPBVC1.

we have isolated *S. aureus* from pus sample of dog in the present study and was confirmed by biochemical test and PCR amplification (228 bp) of 16S rDNA (Fig 1). On further characterization of bacterial isolate, it was found to be methicillin resistant *S. aureus* (MRSA), showing resistance to oxacillin and detection of *mecA* gene of size 310 bp (Fig 2) in the isolate. This confirmed the involvement of MRSA in the case under current study which may have resulted in the treatment failure of antibiotic response. Earlier reports also indicate the involvement of multidrug resistant staphylococci including *S. aureus* and MRSA in canine and human patient suffering from pyoderma globally (Qekwana *et al.,* 2017; Gagetti *et al.,* 2019; González *et al.,* 2020 and Kengne *et al.,* 2020). The confirmed bacterial isolate was used as host for isolation of bacteriophage. The phage was isolated and named as SPBVC1 and was purified for further characterization. The purified phage produced clear medium sized plaques (2-2.5 mm in diameter) when propagated on MRSA host as shown in Fig 3.

The morphology of the SPBVC1 phage under transmission electron microscopy (TEM) showed an icosahedral head of diameter 81.31 nm. and a tail of 92.08 nm. Based on the International Committee on Taxonomy of Viruses (Murphy *et al.,*1995), TEM characteristic of SPBVC1 phage was suggestive of the order *Caudovirales* (Fig 4). The ultrastructure of phage showing a contractile tail consisting of a sheath and a central tube suggested it to be a member of family Myoviridae (Deghorain *et al.,* 2012).

The host range of the phage SPBVC1 was determined using a range of methicillin sensitive and methicillin resistant staphylococci. The lytic pattern of phage showed lysis of 38.8 percent of MRSA isolates only (Table 1), however none of the staphylococcal isolates other than MRSA were lysed by the phage under the study indicating the specificity of phage for MRSA only which is an important characteristic of bacteriophage intended for therapeutic or biotechnological use (Deghorain and Van, 2012). Although the isolated phage SPBVC1 had a limited host range, it had shown lytic activity against MRSA strains which are multidrug resistant staphylococci. Yazdi *et al.* (2018) also reported the isolation of Phage vB-SsapS-104 with a narrow host range for *S. saprophyticus* clinical isolates only. The use of methicillin resistant *S. aureus* as host during isolation of phage or the high density of the methicillin resistant staphylococci population in the pus sample could be one of the possible reason (Richard *et al.,* 2008) resulting in the high specificity of the phage SPBVC1 in the present study. These findings suggest that the SPBVC1 phage reported here may be a useful candidate for phage cocktails intended for therapeutic or biocontrol use in future.

The stability study of phage for their resistance to heat and pH will be helpful in minimizing the phage loss and maintenance of phage viability under various conditions that has a role in various aspects of phage therapy (Li and Zhang, 2014). Therefore, biological stability of the phage SPBVC1 at different temperature and pH was evaluated in the present

Table 1: Lytic potential of bacteriophage SPBVC1 against MRSA strains isolated from different origin.

Methicillin resistant *S. aureus* isolates	Source of bacterial isolate	Lysis by bacteriophage SPBVC1
17B(N)	Buffalo	-
189B(N)	Buffalo	-
190B(N)	Buffalo	-
207B(N)	Buffalo	+
218B(N)	Buffalo	-
226B(N)	Buffalo	+
243B(N)	Buffalo	-
253B(N)	Buffalo	+
19B(S)	Buffalo	-
116B(S)	Buffalo	-
198B(S)	Buffalo	-
199B(S)	Buffalo	-
210B(S)	Buffalo	+
214B(S)	Buffalo	-
244B(S)	Buffalo	+
250B(S)	Buffalo	-
10C(N)	Cattle	-
174C(N)	Cattle	-
176C(N)	Cattle	+
178C(N)	Cattle	+
180C(N)	Cattle	-
46 C(S)	Cattle	+
165C(S)	Cattle	-
171C(S)	Cattle	+
6 D(N)	Dog	-
8D(N)	Dog	-
11D(N)	Dog	+
20D(N)	Dog	-
21D(N)	Dog	+
28D(N)	Dog	+
33D(N)	Dog	+
35D(N)	Dog	-
38D(N)	Dog	-
43D(N)	Dog	+
51D(N)	Dog	-
3D(S)	Dog	+
7D(S)	Dog	-
13D(S)	Dog	+
16D(S)	Dog	-
29D(S)	Dog	+
32D(S)	Dog	+
39D(S)	Dog	-
44D(S)	Dog	-
49D(S)	Dog	+
52D(S)	Dog	-
58D(S)	Dog	-
95D(S)	Dog	-
101	Dog	-

Table 1: Continue...

Table 1: Continue...

4D(W)	Dog	-
42D(W)	Dog	-
18(H)	Human	-
47(H)	Human	+
159(H)	Human	+
183(H)	Human	-
231(H)	Human	-
15D(H)	Human	+
27D(H)	Human	+
30D(H)	Human	-
37D(H)	Human	-
40D(H)	Human	+
41D(H)	Human	-
53D(H)	Human	-
Total MRSA lysed by the bacteriophage SPBVC1 (%)		24/62 (38.70%)

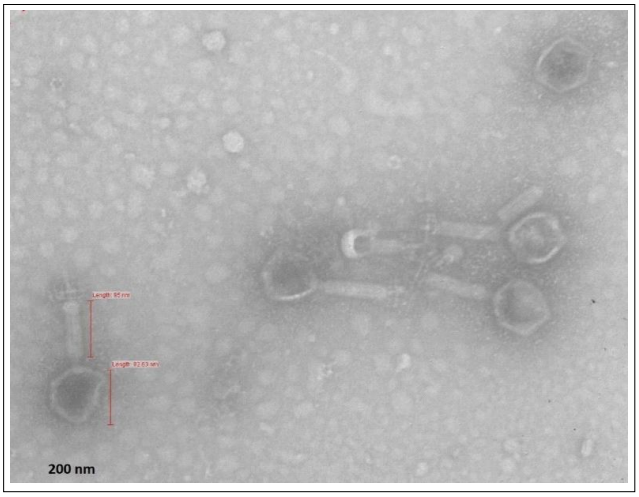

Fig 4: Transmission electron micrograph of bacteriophage, SPBVC1. 120000X.

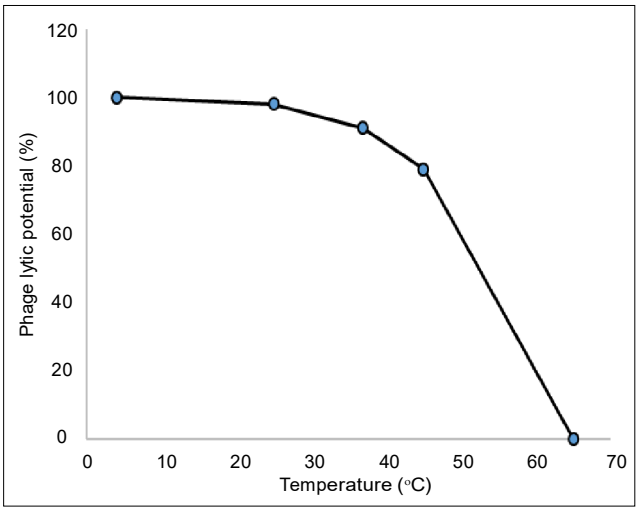

Fig 5a: SPBVC1 Phage thermos stability assay.

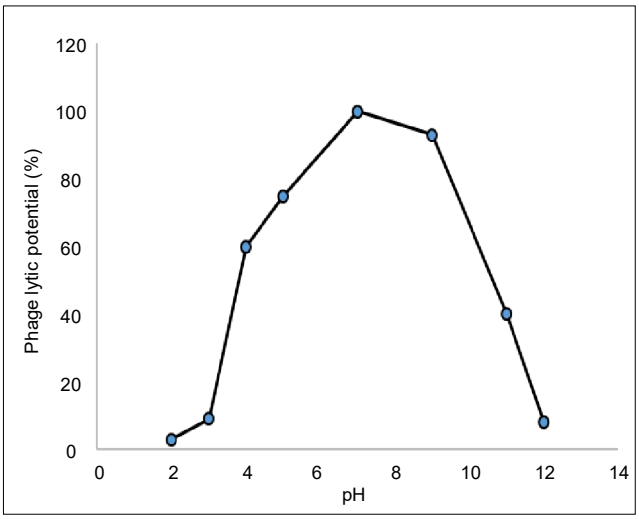

Fig 5b: SPBVC1 Phage stability at varied pH.

study which showed that the phage remained lytic at a range of temperature varying from 4°C to 45°C and became completely inactive at 65°C (Fig 5a). The phage was able to lyse in a range of acidic and alkaline pH value from 4 to 11 however the lysis potential declined significantly below pH 5 (Fig 5b). This indicated that the phage was stable in a pH range from 5 to 11. The results of bio stability of phage are in concordance with earlier observations by different workers (Cui *et al.*, 2017; Ganaie *et al.*, 2018 and Yazdi *et al.,* 2018). The study concludes that the isolated phage (SPBVC1) may be used as a potential alternative against MRSA infections and can be exploited for therapeutic management of pyoderma caused by MRSA in pets however, a detail insight into the phage genome and interaction of phage and bacteria would expand their applicability in phage therapy.

CONCLUSION

The study concludes that the isolated phage (SPBVC1) may be used as a potential alternative against MRSA infections and can be exploited for therapeutic management of pyoderma caused by MRSA in pets however, a detail insight into the phage genome and interaction of phage and bacteria would expand their applicability in phage therapy.

Conflict of interest: None.

REFERENCES

Adams, M.H. (1959). Bacteriophages. Inter Science Publishers, Inc., New York. 1959.

Bannoehr, J. and Guardabassi, L. (2012). *Staphylococcus pseudintermedius* in the dog: Taxonomy, diagnostics, ecology, epidemiology and pathogenicity. Veterinary Dermatology. 23: 253-266, e251-e252. 2.

Beco, L., Guaguère, E., Lorente, M., Noli, C., Nuttall, T. and Vroom, M. (2013). Suggested guidelines for using systemic antimicrobials in bacterial skin infections: Antimicrobial choice, treatment regimens and compliance. Veterinary Record. 172: 156-160.

Braoios, A., Fluminhan, A., Pizzolitto, A.C. (2009). Multiplex PCR use for *Staphylococcus aureus* identification and oxacillin and mupirocin resistance evaluation. Revista de Ciências Farmacêuticas Básica e Aplicada. 1: 303-307.

Bruynoghe, R. and Maisin, J. (1921). Essais de therapeutique au moyen du bacteriophage du Staphylocoque. Comptes Rendus Social Biology. 85: 1120-1121.

Bryan, J., Frank, L.A., Rohrbach, B.W., Burgette, L.J., Cain, C.L. and Bemis, D.A. (2012). Treatment outcome of dogs with methicillin resistant and methicillin susceptible *S. pseudintermedius* pyoderma. Veterinary Dermatology. 23: 361-368.

Chambers, H.F. and Deleo, F.R. (2009). Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nature Reviews Microbiology. 7(9): 629-641.

Clokie, M.R., Millard, A.D., Letarov, A.V. and Heaphy, S. (2011). Phages in nature. Bacteriophage. 1(1): 31-45.

Cui, Z., Feng, T., Gu, F., Li, Q., Dong, Ke., Zhang, Y., Zhu, Y., Han, L., Qin, J. and Guo, X. (2017). Characterization and complete genome of the virulent Myoviridae phage JD007 active against a variety of *Staphylococcus aureus* isolates from different hospitals in Shanghai, China Journal of Virology. 14: 27. doi: 10.1186/s12985-017-0701-0.

Davis, L.G., Dibner, M.D. and Battey, J.F. (1986). Growth and Preparation of Bacteriophage. In: Basic Methods in Molecular biology. New York; Elsevier Science Publishing Co. Inc. 158-160.

Deghorain, M. and Van, M.L. (2012). The Staphylococci phages family: An overview. Viruses. 4: 3316-3335.

Díaz-Muñoz, S.L. and Koskella, B. (2014). Bacteria-phage Interactions in natural environments. Advances in Applied Microbiology. 89: 135-183. doi: 10.1016/b978-0-12-800259-9.00004-4.

Dulon, M., Haamann, F., Peters, C., Schablon, A. and Nienhaus, A. (2011). MRSA prevalence in European healthcare settings: A review. BMC Infectious Diseases. 11: 138.

Gagetti, P., Wattam, A.R., Giacoboni, G., Paulis, A.D., Bertona, E., Corso, A. and Rosato, A.E. (2019). Identification and molecular epidemiology of methicillin resistant *Staphylococcus pseudintermedius* strains isolated from canine clinical samples in Argentina. BMC Veterinary Research. 27: 264.

Ganaie, M.Y., Qureshi, S., Kashoo, Z., Wani, S.A., Hussain, M.I., Kumar, R., Maqbool, R., Sikander, P., Banday, M.S., Malla, W.A., Mondal, P. and Khan, R.I.N. (2018). Isolation and characterization of two lytic bacteriophages against *Staphylococcus aureus* from India: Newer therapeutic agents against Bovine mastitis. Veterinary Research Communication. 42(4): 289-295.

González Domínguez, M.S.G., Carvajal, H.D., Echeverri, D.A.C. and Cárdenas, D.C. (2020). Molecular detection and chara cterizationofthe *mecA* and *nuc* genes from staphylococcus species (*S. aureus, S. pseudintermedius* and *S. schleiferi*) isolated from dogs suffering superficial pyoderma and their antimicrobial resistance profiles. Frontiers in Veterinary Science. 7: 376.

Han, J.E., Kim, J.H., Hwang, S.Y., Choresca, C.H., Shin, S.P., Jun, J.W., Chai, J.Y., Park, Y.H. and Park, S.C. (2013). Isolation and characterization of a Myoviridae bacteriophage against S*taphylococcus aureus* isolated from dairy cows with mastitis. Research in Veterinary Science. 95(2): 758-763.

Jamalludeen, N., Johnson, R.P., Friendship, R., Kropinski, A.M., Lingohr, E.J. and Gyles, C.L. (2007). Isolation and characterization of nine bacteriophages that lyse O149 enterotoxigenic *Escherichia coli*. Veterinary Microbiology. 124: 47-57.

Jevons, M.P. (1961). Celbenin-resistant staphylococci. British Medical Journal. 1: 124-125.

Jones, R.D., Kania, S.A., Rohrbach, B.W., Frank, L.A. and Bemis, D.A. (2007). Prevalence of oxacillin- and multidrug-resistant staphylococci in clinical samples from dogs: 1,772 samples (2001-2005). Journal of American Veterinary Medical Association. 230: 221-227.

Karmakar, A., Dua, P. and Ghosh, C. (2016). Biochemical and molecular analysis of *Staphylococcus aureus* clinical Isolates from hospitalized patients. Canadian Journal of Infectious Diseases and Medical Microbiology. http://dx.doi.org/10.1155/2016/9041636.

Kawakami, T., Shibata, S., Murayama, N., Nagata, M., Nishifuji, K., Iwasaki, T. and Fukata, T. (2010). Antimicrobial susceptibility and methicillin resistance in *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* isolated from dogs with pyoderma in Japan. Journal of Veterinary Medical Science. 72: 1615-1619.

Kengne, M., Fotie, H.B.N., Nwobegahay, J.M., Achiangia, P.N., Tamoufe, U., Goon, D.T., Mboua, J.B., Tchanana, G., Fualefac, A., Echelibe, H., Djonkam, R.K. and Nkeza, A. (2020). Antibiotic sensitivity profile of *Staphylococcus aureus* isolated from HIV/AIDS patients presenting with pyoderma, at the Yaounde Central Hospital, Cameroon. Pan African Medical Journal. 37: 185.

Knox, R. (1961). Celbenin-resistant staphylococci. Br. Med. J. 1: 125-126.

Kutter, E.M., Kuhl, S.J. and Abedon, S.T. (2015). Re-establishing a place for phage therapy in western medicine. Future Microbiology. 10: 685-688.

Kwiatek, M., Parasion, S., Mizak, L., Gryko, R., Bartoszcze, M. and Kocik, J. (2012). Characterization of a bacteriophage, isolated from a cow with mastitis, that is lytic against *Staphylococcus aureus* strains. Achieves of Virology. 157(2): 225-234.

Lee, G.M., Huang, S.S., Rifas-Shiman, S. L., Hinrichsen, V.L., Pelton, S.I., Kleinman, K., Hanage, W.P., Lipsitch, M., McAdam, A.J. and Finkelstein, J.A. (2009). Epidemiology and risk factors for *Staphylococcus aureus* colonization in children in the post-PCV7 era. BMC Infectious Disease. 9: 110. DOI: 10.1186/1471-2334-9-110.

Li, L. and Zhang, Z. (2014). Isolation and characterization of a virulent bacteriophage SPW specific for *Staphylococcus aureus* isolated from bovine mastitis of lactating dairy cattle. Molecular Biology Reports. 41(9): 5829-5838. doi: 10.1007/s11033-014-3457-2.

Lin, D.M., Koskella, B. and Lin, H.C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. World Journal of Gastrointestinal Pharmacology and Therapeutics. 8(3): 162-173.

Loeffler, A., Cobb, M.A. and Bond, R. (2011). Comparison of a chlorhexidine and a benzoyl peroxide shampoo as sole treatment in canine superficial pyoderma. Veterinary Record. 169: e249.

Lynch, S.A. and Helbig, K.J. (2021). The Complex Diseases of *Staphylococcus pseudintermedius* in Canines: Where to Next?. Veterinary Science. 8(1): 11. doi: 10.3390/vetsci8010011.

Mavrogianni, V.S., Cripps, P.J. and Fthenakis, G.C. (2007). Bacterial flora and risk of infection of the ovine teat duct and mammary gland throughout lactation. Preventive Veterinary Medicne. 79(2-4): 163-73.

Morris, D.O., Rook, K.A., Shofer, F.S. and Rankin, S.C. (2006). Screening of *Staphylococcus aureus*, *Staphylococcus intermedius* and *Staphylococcus schleiferi* isolates obtained from small companion animals for antimicrobial resistance: A retrospective review of 749 isolates (2003-04). Veterinary Dermatology. 17: 332-337.

Morris, D.O., Loeffler, A., Davis, M.F., Guardabassi, L. and Weese, J.S. (2017). Recommendations for approaches to methicillin-resistant staphylococcal infections of small animals: Diagnosis, therapeutic considerations and preventative measures. Veterinary Dermatology. 28: 304-369.

Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarvis, A., Martelli, G.P., Mayo, M.A. and Summers, M.D. (1995). Virus Taxonomy. In: Sixth Report of the International Committee on Taxonomy of Viruses. Springer Verlag, Vienna and New York.

O'Flynn, G., Ross, R.P., Fitzgerald, G.F. and Coffey, A. (2004). Evaluation of a cocktail of three phages for biocontrol of *E. coli* O157: H7. Applied Environmental Microbiology. 70: 3417-3424.

Pintado, V., Pazos, R., Jiménez-Mejías, M.E., Rodríguez-Guardado, A., Díaz-Pollán, B., Cabellos, C., García-Lechuz, J.M., Lora-Tamayo, J., Domingo, P. Muñez, E. and Domingo, D. (2019). *Staphylococcus aureus* meningitis in adults: A comparative cohort study of infections caused by meticillin-resistant and meticillin-susceptible strains. Journal of Hospital Infection. 102(1): 108-115.

Qekwana, D.N., Oguttu, J.W., Sithole, F. and Odoi, A. (2017). Burden and predictors of *Staphylococcus aureus* and *S. pseudintermedius* infections among dogs presented at an academic veterinary hospital in South Africa (2007-2012). Peer Journal. 13: e3198.

Reddym,S.B., Kumaria, N.K. and Sivajothi, S. (2016). *Methicillin-resistant Staphylococcus aureus* (MRSA) isolated from dogs with recurrent pyoderma. Journal of Dairy Veterinary and Animal Research. 3(2): 62-65.

Richard, H.H., Springman, R. and James, J.B. (2008). Optimal Foraging by Bacteriophages through Host Avoidance. The American Naturalist. 171: e149-157.

Rubin, J.E. and Chirino-Trejo, M. (2011). Prevalence, sites of colonization and antimicrobial resistance among *Staphylococcus pseudintermedius* isolated from healthy dogs in Saskatoon, Canada. Journal of Veterinary Diagnostic Investigation. 23: 351-354.

Tomlin, J., Pead, M.J., Lloyd, D.H., Howell, S., Hartmann, F., Jackson, A.and Muir, P. (1999). Methicillin resistant *Staphylococcus aureus* infections in 11 dogs. Veterinary Record. 144: 60-64.

Watanabe, R., Matsumoto, T., Sano, G., Ishii, Y., Tateda, K., Sumiyama, Y., Uchiyama, J., Sakurai, S., Matsuzaki, S., Imai, S. and Yamaguchi, K. (2007). Efficacy of bacteriophage therapy against gut-derived sepsis caused by *Pseudomonas aeruginosa* in mice. Antimicrobisl Agents and Chemotherapy. 51(2): 446-52.

Yazdi, M., Bouzari, M. and Ghaemi, E.A. (2018). Isolation and characterization of a potentially novel Siphoviridae phage (vB_SsapS-104) with lytic activity against *Staphylococcus saprophyticus* isolated from urinary tract infection. Folia Microbiologica. https://doi.org/10.1007/s12223-018-0653-9.