JOURNAL OF THE Selva Andina Animal Science
Official Journal of the Selva Andina Research Society.

Review Article

ISSN 2311-3766 (print edition)
JSAAS
ISSN 2311-2581 (online edition)

Bacteriophage cocktail as a substitute for antimicrobials in companion animal dermatology

Cóctel de bacteriófagos como sustituto de antimicrobianos en dermatología de animales de compañía

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Keywords:

Antibiotics,
skin diseases,
phage therapy,
antimicrobial resistance,
Staphylococcus,
Pseudomonas,
veterinary.

J. Selva Andina Anim. Sci.
2022; 9(2):97-117.
Article ID: 113/JSAAS/2022

Article history

Received May 2022.
Returned August 2022.
Accepted September 2022.
Available online, October 2022.

Edited by: Selva Andina Research Society

Palabras clave:

Antibióticos,
enfermedades de piel,
terapia de fagos,
resistencia antimicrobiana,
Staphylococcus,
Pseudomonas,
veterinaria.

Abstract

The present study focuses on the use of phage cocktails as a substitute for antibiotics in companion animal dermatology. For this purpose, a systematic search was carried out in the Scopus database, with the search criteria: "veterinary" and "bacteriophage" and "dermatology" in article title, abstract and keywords during the period 2010-2021. Seven in vitro studies and one in vivo study in companion animals, for which those carried out in laboratory animals were added. In this review, the use of non-transducing lytic phage cocktails as therapeutics for pyodermas is discussed and projected, as well as the resistance to phages and the strategies to overcome it, the comparison with antibiotics, the use of cocktails in other animal species, as well as the use of individual phages and cocktails in veterinary dermatology, and autochthonous phages as a strategy when phage collections from previous studies do not have the desired effects. It is concluded that non-transducing lytic autophage cocktails are an alternative against antimicrobial resistance in companion animal dermatology. Finally, it is recommended to compare the use of these cocktails with other antibiotic substitutes and evaluate their possible synergism to reduce pathogenic bacteria on the skin.

Resumen

El presente estudio se enfoca en el uso de cocteles de fagos como sustituto de antibióticos en dermatología de animales de compañía. Para este propósito, se realizó una búsqueda sistemática en la base de datos de Scopus, con el criterio de búsqueda: "veterinary" and "bacteriophage" and "dermatology" en título de artículo, resumen y palabras clave durante el periodo 2010-2021. Siete estudios *in vitro* y un estudio *in vivo* en animales de compañía, por lo cual se añadieron aquellos realizados en animales de laboratorio. En esta revisión se discute y proyecta la utilización de cócteles de fagos líticos no transductores como terapéuticos de piodermas, asimismo, se revisa la resistencia a fagos y las estrategias para superarla, la comparación con los antibióticos, el uso de cócteles en otras especies animales, así como, la utilización de fagos individuales y cócteles en dermatología veterinaria, y los fagos autóctonos como estrategia cuando las colecciones de fagos de estudios previos no tienen los efectos deseados. Se concluye que los cócteles de autofagos líticos no transductores son una alternativa contra la resistencia antimicrobiana en dermatología de animales de compañía. Finalmente, se recomienda comparar el uso de estos cócteles con otros sustitutos de antibióticos y evaluar su posible sinergismo para reducir bacterias patógenas en piel.

Introduction

In numerous instances, bacterial skin infections are the main reason for consultation in the veterinary practice of small animals[1,2]. These are mostly caused by *Staphylococcus pseudintermedius*, which is part of the skin microbiota of dogs[1], along with *S. aureus*[3,4].

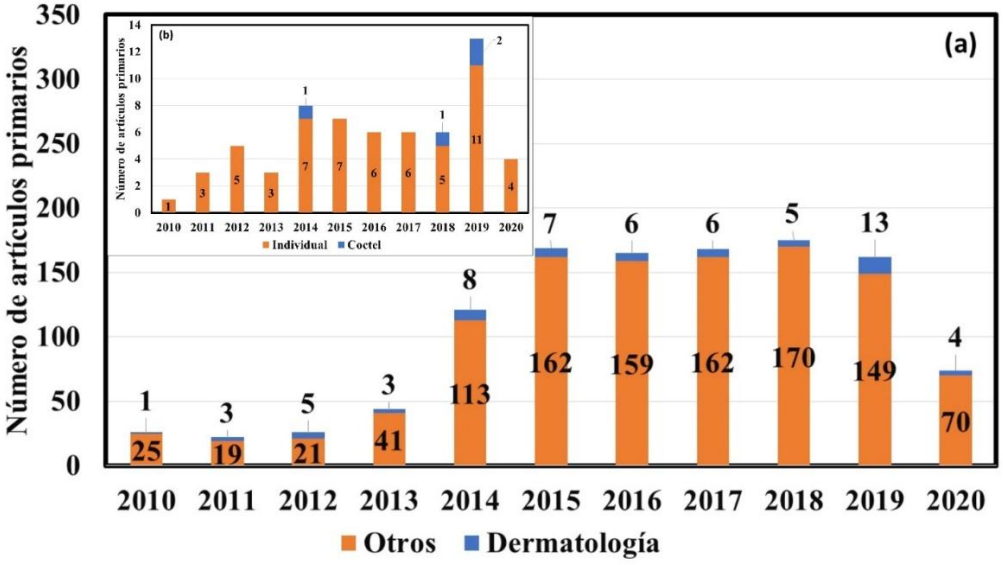
Broad-spectrum systemic and local antibiotics are frequently used to eliminate them[5,6], while they can be supplied by different routes, topical is the most used one, through various presentations such as shampoos, creams, and gels[7,8]. However, its inappropriate use has caused bacteria to develop antimicrobial resistance in companion animals[9-12]. Therefore, it has become a concerning public health problem since staphylococci, which are part of the human and animal microbiota, can act as a reservoir of resistance genes[13].

At the same time, many of these skin conditions can be considered secondary and commonly related to intestinal pathologies[14].

For this reason, in dermatology, oral probiotics[15-17], essential oils[18-20] and, sodium hypochlorite[21] have been postulated as alternative treatments. However, bacteriophages, which are the natural predators of bacteria, are emerging as an excellent option, due to their lytic properties, even in multidrug-resistant bacteria. Likewise, its ability to multiply at the site of infection[22] and its high specificity stand out as potential advantages, so it does not affect beneficial bacteria[23]. Despite these advantages, it is known that these viruses can transmit virulence and antimicrobial resistance genes through transduction[24,25]. Hence, the phages to be used as therapy must be genetically characterized.

On the other hand, autophages are an ongoing trend, especially when commercial products do not have the desired effect[26]. These types of bacteriophages are often isolated from environments in which the target bacteria are found, with the purpose of ensuring that this specific microorganism is part of their spectrum.

Figure 1a Publications of primary scientific articles on bacteriophages in veterinary dermatology. b Publications of scientific articles on bacteriophages in dermatology that used phages cocktails. Data collected from Scopus database (Selection criteria: Article title, abstract, and keywords: "veterinary" "bacteriophage" and "dermatology" during the period 2010-2021)



In the last decade, many scientific articles regarding the use of bacteriophages for bacterial control in veterinary medicine have been published, nonetheless, there are only a few works in the dermatology field (Figure 1a). Similarly, there are few studies made on phage cocktails (Figure 1b).

The present work focuses on the use of non-transducing lytic bacteriophage cocktails as substitutes for antimicrobials to combat skin diseases of bacterial origin, the routes of application in dermatology, their comparison with antimicrobials and the use of autochthonous phages.

Materials and methods

A search was conducted in Scopus database from January 2010 to December 2021. The selection criteria were that the article title, abstract, and keywords had to contain the following terms: "veterinary", "bacteriophage", "dermatology" and " cocktail". The data obtained were classified according to animal species and the administration route studied. For the development of this article, studies that used phages for the detection of pathogenic bacteria, reduction of bacterial load in the carcass, and disinfection of facilities, in the same way, basic investigations (genotypic and phenotypic characterization) were excluded.

Development

Bacteriophages. Also known as phages, these are viruses that can infect and lyse bacteria[27]. Phages were reported[28] and isolated for the first time[29] at the beginning of the 20th century, giving rise to phage therapy (PT) four years after their discovery. Despite that, these viruses were displaced by antibiotics because the latter had a broader spectrum and affordable price[30]. At the present time, due to antimicrobial resistance and new discoveries in PT, phages have regained popularity and are being used to combat bacterial diseases, as the first report of PT in companion animals in 2006[31] exhibits. These viruses are abundant in nature[32] and notorious for being very specific. Even though its specificity may occur at the strain level[33-35], phages that infect more than one bacterial genus have been reported[36].

Due to their infective cycle, they can be classified as virulent or lytic (PhL) and temperate (PhT). The virulent ones prevent bacterial multiplication, in contrast, PhT allows it when there is a low bacterial population[37,38]. Likewise, PhT are involved in the transmission of virulence and antimicrobial resistance genes[27,39,40].

Lytic infection begins with the recognition of phage receptors on the bacterial surface, such as antigens[41], pili[42], glucan[43], polysaccharides[44], proteins[45], and other structures, inducing viral adsorption, subsequently, the phage implants its genetic material (RNA or DNA) in bacteria and destroys the genetic material of the host bacteria by enzymatic action, proceeds to assemble and replicates. Finally, the new phages produce holins and endolysins, the former are carrier proteins that allow endolysins (enzymes) to cross the membrane to their site of action, degrading the peptidoglycan[30] and thus forming pores, causing depolarization that produces bacterial lysis, thereby releasing new phages[46]. However, the lytic cycle can fail by destroying the bacterial genetic material and assembling phages with fragments of it, a phenomenon called transduction. Transduction can be generalized or specialized, the first mentioned, due to er-

rors in the assembly of the new phages, produces viruses with exclusively bacterial and viral genetic material[24,25], the latter is caused when the bacterial and viral genome is mixed, resulting in phages with both genomes[24]. If the bacterium has resistance and virulence genes, these will be transmitted in the next cycle[25], therefore, non-transducing PhL should be used.

Phage resistance. Phages and bacteria have co-evolved, with bacteria developing strategies to evade and outcompete phages. Several cases of bacterial phage-resistance have been reported in veterinary medicine[33,47-49], studying its different mechanisms such as loss of phage-receptors, modification of phage-receptors, CRISPR-Cas system, abortive system, and production of polysaccharide matrix[50].

Some bacterial strategies focus on avoiding phage adsorption by modifying phage receptors, losing them, and producing polysaccharides. In response, phages can change their tail fibers to find newly altered receptors[51] and produce depolymerases[52]. Likewise, bacteria can attack the genetic material of the phage using the CRISPR-Cas system, yet, some phages prevent the degradation of their genetic material by using a protein coat[53]. Finally, when the previous strategies are not enough to avoid viral infection, the bacteria resort to the abortive system[54].

On the other hand, in cases of resistance to individual phages, phage cocktails[49,55] and quorum quenching[56-58]. Can be used. In spite of that, phage resistance to phage cocktails has also been reported[33,47,48], in these situations, quorum quenching is most likely the best alternative, but the composition of the phage cocktail could be changed as well.

While it is true that phage resistance is a problem for PT, phage-resistant bacteria have been reported to reduce their ability to grow and absorb nutrients[33]. Furthermore, phage-resistant bacteria were reported to exhibit sensitivity to antibiotics to which they had previously shown resistance and lower virulence[59,60].

Bacteriophages versus antibiotics. Although topical antibiotics are commonly used on localized and superficial wounds[61], they can generate an imbalance in the skin microbiota due to their broad spectrum[62]. In contrast, the specificity of bacteriophages allows other bacteria outside of their range to remain unaffected, ensuring that the beneficial microbiota proliferates without problem[23,63].

Moreover, topical drugs can be diluted or inactivated by enzymes or other inflammatory mediators[64]. Unlike phages that, due to their continuous multiplication, penetrate tissues in the presence of active bacteria, which is particularly useful in the treatment of infections in tissues with less blood supply[65].

It should be noted that antimicrobial resistance (AMR) is the main factor that has driven the search for other therapeutic alternatives. Systemic antibiotics tend to generate greater resistance than topical ones[66,67], which are prescribed more frequently in canine dermatopathies[68,69].

However, this has normalized empiric treatments[70,71], carried out without the pertinent microbiological sensitivity tests, which can lead to an increase in AMR. This resistance, in turn, has been partially increased by poor practices in the daily veterinary clinic, highlighting its preventive use in cases such as vaccinations, sterilizations, among others[72]. On the contrary, it has been reported that the use of bacteriophages as

a preventive measure produces better results. In challenged mice with *E. coli* CVCC193, those who were inoculated with phages 24 h previously, showed a survival rate of 80-100 %, compared to those who were administered 3 h later (40-50%)[73].

In general, chronic infections are difficult to treat successfully because of AMR, increasing the duration of treatment and putting the patient's life at risk[74]. Enteral antimicrobials are needed in higher concentrations to reach the skin due to the poor irrigation that these tissues have, contributing to the presentation of side effects in pets exposed to these drugs. The adverse effects presented by undergoing treatment on animals are varied and can affect their quality of life. Gastrointestinal problems, and in rarer cases, hemolytic anemias, and acute kidney damage[75].

The use of viruses (phages) in pets can be ethically controversial because animal welfare could be compromised[76,77], hence few studies have used phages as the sole treatment for bacterial infection in companion animals since it is not a common practice in Veterinary Medicine of small species. Still, a study indicated a positive perception of this alternative therapy, both from veterinarians and owners, so this could be an indicator of its future mass use[12].

Despite this, bacteriophage-based therapies have been performed in canines with chronic external otitis, which received previous antimicrobial treatment, with a positive resolution after treatment[31,78], hence, its use to treat persistent conditions can be considered a viable alternative without serious side effects.

At the same time, economically, medical costs increase in patients with resistant bacterial infections, while PT is believed to be less expensive than antibiotic therapy if a specialized center is available[79].

Regarding administration, bacteriophages multiply logarithmically over antibiotics, hence they would need fewer applications[80], thus reducing the treatment period.

Similar to antibiotics, PT can also be affected by bacterial resistance[81,82], despite that, resistance to phages can be anticipated and used as part of a therapeutic strategy[83]. Among the strategies, the decrease in the virulence of phage-resistant strains[60,84,85], and varied attenuation according to whether the therapy is performed with single phages, or cocktails, the latter presents better results[86].

From a practical perspective, for phages to be widely used in the treatment of bacterial infections, they will have to be effective in combination with antibiotics[87]. It has been pointed out that phages can reduce the minimum inhibitory concentration (MIC) of drug-resistant bacterial strains, although this arises from the class of antibiotics and the concentration of bacteriophage-antibiotics that are used together[88]. Thus, phages could positively influence the sensitivity of multidrug-resistant bacteria[89].

However, some authors have justified that PhL may be capable of horizontally transmitting AMR genes to other bacteria through generalized transduction[90], which would be considered counterproductive for the use of PhL transducers therapeutically. In opposition to these findings, it was stated that antimicrobial resistance genes are rarely encoded in phages since this process rarely occurs in the phage lytic cycle[91]. At present, the role of phages in the transduction of AMR genes continues to generate debate, and more studies are needed in this regard.

Regarding the comparison of phages and antibiotics in vivo, one study showed that phages ($1x10^9$ PFU/animal) had a similar effect to vancomycin (15

mg/kg) and a better effect than clindamycin (20 mg/kg) in reducing skin lesions in laboratory mice with *S. aureus* ATCC 25923 ($6x10^9$ CFU)[92].

Another study of a similar nature was made in groups of mice inoculated with *P. aeruginosa* and treated with phage ZCPA1 ($1x10^9$ PFU/mL) in single doses (reduction of 4 log10 of the total bacterial count) and multiple (>4 log10), they showed a 100 % resolution of the wounds and optimal regeneration of the skin, while the group treated with topical gentamicin (2 log10) presented expansion and enlargement of the affected area, which led to purulent wounds that did not heal[93]. Ultimately, the use of phages, either alone or with antibiotics, will reveal superior results than traditional antibiotic therapy.

Bacteriophage cocktails in veterinary medicine. As mentioned above, phages are very specific, which limits the spectrum of individual phages. For this reason, individual phages are combined to broaden the spectrum, this mixture is known as a phage cocktail, it can be simple or mixed[26], the former can infect bacteria of the same genus and the latter several bacterial genera. Phage cocktails were extensively studied in production animals to combat pathogenic bacteria in different animal species, with excellent results (Table 1). Different routes of administration, oral and immersion, were tested as alternatives in veterinary dermatology. The first can be used to maintain intestinal health and indirectly protect the skin; phage titers can be reduced by changes in the pH of the gastrointestinal tract if they are not protected[94-96], while the second can be used to directly treat skin lesions.

On the other hand, veterinary cocktails were used in liquid (water) or solid (food) media, with better results in liquid media (Table 1, 2, and 3).

Bacteriophages in companion animal dermatology. There are few studies on the use of phages in dermatology, either in veterinary or human medicine, however, they suggest phages could be useful to treat pyoderma[4,97-100]. This skin disease could be caused by a wide variety of microorganisms, such as *S. aureus*, susceptible to phage ΦSA012, when applied intravenously or intraperitoneally in a mouse suffering from mastitis caused by said bacteria[97].

In addition, another report carried out *in vivo* in mice, indicated the efficacy of ΦDMSA-2 bacteriophage against methicillin-resistant *S. aureus* (MRSA). It was applied topically on a wound generated by infected surgical excision; in a period of 12 to 16 days, a complete re-epithelialization of the lesion and eradication of the infection was achieved[100], indicating that phages are effective for infections caused by *S. aureus*.

On the other hand, phage VB_SauS_SH-St 15644 caused the lysis of 32 % of MRSA strains *in vitro* and was able to reduce the progress of the infection *in vivo* when applied subcutaneously in mice[98]. The low percentage of lytic activity could be due to the specificity of the phage, hence cocktails could be useful to avoid this problem. Similarly, topical applications of SaGU1 phage to mice were effective in preventing the aggravation of *S. aureus* infection, reducing the presence of bacteria[99].

In addition, phages were useful in reducing the defense mechanisms of bacteria, such as phage phiIPLA-RODI, together with lytic protein CHAPSH3b, were able to reduce the formation of *S. aureus* biofilm, a reduction in viable bacteria was also observed after its application[101].

Table 1 Effect of Phage cocktail usage in veterinary

Bacteria Host	Phage	Phage Family	Phage Dose	Provenance of phage	Route of administration	Animal	Result	References
C. pertringens	CPAS-7, CPAS-12, CPAS-15, CPAS-16, CPTA-37, CPLV-42	*Siphoviridae*	$2.5x10^9$ UFP/ Animal	Poultry farms	Buffer SM	Chickens	Reduction of mortality from 66.67 a 18.00 %	104
					Water		Reduction of mortality from 66.67 a 3.33 %	
					Food		Reduction of mortality from 66.67 a 5.33 %	
Salmonella gallinarum	ST4, L13, SG3	*Siphoviridae*	$1.0x10^8$ UFP/ kg	Sewage	Food	Chickens	Reduction of mortality from 40.00 a 25.00 %.	105
Salmonella typhimurium ATCC 14028	SEP-1, SGP-1, STP-1, SS3eP-1, SalTP-2, SChP-1, SAP-1, SAP-2	-	$5.0x10^9$ UFP/ Animal	Sewage and stool	Food	Piglets	Reduction of Salmonella in stool	106
E. coli APEC	TM1, TM2, TM3, TM4	*Siphoviridae*	$1x10^{10}$ UFP/animal	Sewage	I.V.	Japanese quail	Reduction of mortality from 46.60 a 13.30 %	107
Aeromonas hydrophila	50AhydR13PP, 60AhydR15PP. 25AhydR2PP	*Myoviridae* *Podoviridae*	$1x10^5$ UFP/mL	-	Inmersion	European anguilla	Reduction of mortality from 60.00 a 20.00 %	108
Pseudomonas fluorescens	22PfluR64PP, 67PfluR64PP, 71PfluR64PP, 98PfluR60PP.	*Podoviridae*						

I.P.: intraperitoneal, I.V.: intravenosa.

However, *in vitro* they verified the efficiency of phages to eliminate MRSP, and control the biofilm present, the phages used belonged to the families*Myoviridae* y *Siphoviridae*, of these vB_SpsS-SN8, vB_SpsS-SN10, vB_SpsS-SN11, vB_SpsS-SN13, phiSA012, ph 0044 and ph 0045showed lytic activity. On the other hand, pSp-J and pSp-S prevented the formation of biofilm a dose low and the degraded it a higher dose[34]. However, these MRSPs were *in vitro*, and more studies are required to determine its effectiveness *in vivo*.

Pseudomonas aeruginosa is another bacteria frequently related with pyoderma, especially in canine otitis[102]. A case was reported[31] of apatient of the Saint Bernard breed who suffered from *P. aeruginosa*,and when treated with a phage, presented an improvement without secondary effects9 months after of the application of the phage, and the presence of the bacteria was no longer observe. Likewise, the phages ΦS12-1 Y ΦR18, of the families*Myoviridae* and *Podoviridae*, respectively, were found to have activity *in vitro* lytic against various strains of *P. aeruginosa* isolated from the skin of canines[103].

Since *P. aeruginosa is* on the WHO priority list of multiresistant bacteria[109], these studies are extremely important as it is an alternative to combat bacterial resistance.

Lastly, FAL has also been used against *Klebsiella pneumoniaein vivo*, the phage ZCKP8, of the *Siphoviridae family*, was applied bytopical treatment on infected open wounds in mice. It was possible to closethe injury by 99 % after 17 days, compared to the group control, in which the lesion was closed by 79.76 %, showing the re-epithelializationin those treated with phages[110].

This suggests that there is great potential for the use of phages within the clinical medicine of small animals. In addition, its use has several advantages, like the facility to obtain them, because they have various provenances (Table 2 y 3).

Likewise, there also exists a variety of pathways of application (Table 2), facilitating its use according to the area to be treated. Knowing that 36 % of owners they prefer the topical route and 1% the parenteral routes[111], the topical route can be used through creams or baths, facilitating the application for homeowners. However, it may be difficult to control the viral dose and many times pets could lick themselves, interfering with treatment, so it would be appropriate to recommend the use of Elizabethan collar. The intradermal and subdermal pathways would be adequate for veterinarians, also, it would allow tohave to control of the applied dose more accuratelyand protect the phages from external factors such as licks, UV rays, etc.

Cocktails in veterinary dermatology of small animals. While it is true that phages are highly specific, which reduces its range of infection, without embargo, phage cocktails can eradicate *P. aeruginosa*; there even existreports showing lytic activity against multidrug-resistant bacterial strains (MDR), extensive drug resistant (XDR) and pandrugresistant (PDR)[112].

The use of phage cocktail for treatment in dogs diagnosed with otitis by *P. aeruginosa*,used six phages (BC-BP-01 to BC-BP-06), showed lytic activity, with no apparent side effects, eradicating the disease[78].

Regarding other bacteria that cause lesions in the skin, like *E. coli*, *P. aeruginosa* and *S. aureus*, a cocktail was applied by topical route of three different phages for each of these, managing to eradicate the infection in an approximate of 9 to 13 days. In the case of *E. coli*, 16.70 % of the lesions healed in 9 days, and the remaining in 13 days. Regarding *P. aeruginosa*, 55.50 % of the lesions were free of bacteria in 9 days, and 45.50 % in 13 days, lastly, in those lesions generated by *S. aureus*, 60 % healed in 9 days, and the remaining in 13 daysafter of the application of the phage cocktail[113].

Regarding MRSA, the use of a phage cocktail has been reported, with 3 different phages of the family *Myoviridae* applied topically, achieving to decrease the bacterial load, being equal to or evenmore efficient than vancomycin[114], it should be noted no cases of mortality or side effects were reported in the mice treated with phages.

The use of a phage cocktail is usually more effective compared to an individual phage; in lesions by *K. pneumoniae* in mice were treated with 5 individual phages, and a cocktail of 5 phages. The cocktail was more efficient to remove the bacterial charge and decreased the healing time ofthe wound, a difference of the individual phages[121].

The use of phage cocktails to treat infections points to the need for phage banks, which collect, characterize, and conserve these viruses. However, to date there are very few establishments[122]. A worldwide network of such banks would drastically reduce the possibility of a bacterial outbreak difficult to deal with, however, at present, it is still a long process and complicated to assign phages for determined necessities[123]. Veterinary centers could choose to isolate bacteriophages from the residual water from medicated baths, or from physiological samples (skin and stool) of the patients, to create a phage bank belonging to the clinicwith therapeutic purposes.

Table 2 Spectrum of phages used for fight bacteria pathogenic in veterinary dermatology *in vitro*

Host bacteria	Phage	Family of phage	Provenance of phage	Resultado	References
S. pseudintermedius (41 cepas) *S. pseudintermedius* (47 cepas)	pSp-J pSp-S	*Siphovirus*	Floor and water from animal parks	Lysis plates	34
S. pseudintermedius E133 *S. pseudintermedius* E140	vB_SpsS-SN8, vB_SpsS-SN10, vB_SpsS-SN11, vB_SpsS-SN13	*Siphoviridae*	Dog stool	Lysis plates	40
S. schleiferi, S. intermedius y S. pseudintermedius	PhiSA012	*Myoviridae*	Sewage	Lysis plates	115
S. pseudintermedius SP015, SP017, SP197, SP251, SP253.	ϕDP001	*Siphoviridae*	Dog saliva	Lysis plates	
S. pseudintermedius SP015, SP017, SP070, SP145, SP188, SP195, SP197, SP251, SP253, SP276.	ϕSA039	*Myoviridae*	Sewage	Lysis plates	116
S. pseudintermedius SP015, SP017, SP070, SP197, SP251, SP253, SP276.	ϕSA012	*Myoviridae*	Sewage	Lysis plates	
P. aeruginosa	BrSP1	*Myoviridae*	Sewage	Lysis plates	117
S. pseudintermedius 625, 2854, CCM 2885, CCM 7315, CCM 7829, CCM 7830, 33, 35, 259, 621.	QT1	*Siphoviridae*	Félix d'Hérelle Collection	Lysis plates	118
Staphylococcus spp.	W15, W17, W33, W31, W36	*Myoviridae*	Sea water	Lysis plates	119
P. aeruginosa	pPa_SNU-ABM_DT01	*Myoviridae*	Water samples	Lysis plates	120

Autochthonous phages or autophages. From a practical point of view, commercial products and phage collections from universities and various research centers could be used, where the use of products commercials of phages has been reported[118]. However, its high specificitycould limit the effect expected, because it is possible that the bacteria present in patients are not susceptible to these. Facing this scenario, phages can be obtained or isolated from the patient where the pathogenic agent is found, calling this virus autochthonous phage or autophage[26,126]. In addition, the exogenous phage is also considered autophage, which is applied in an individual so it can later be reisolated[127].

Autochthonous phages can be used as a cocktail to reduce the proprobability of phage resistance and enlarge its spectrum. As described previously, phages can be obtained from the skin and feces (Table 2), and it is considered one of the main sources of autophages in dermatology of small animals.

In dogs, several phages have been reported like T4virus, Jerseyvirus, T5virus, Phix174microvirus, N4virus, T7virus, Bppunalikevirus, Bxz1virus, likewise, bacteriophages belonging to the families *Myoviridae*, *Podoviridae*, *Siphoviridae* and others not identified in the virome fecal of healthy dogsand those with enteropathy[128,129].

Table 3 Effect of the utilization of phages against pathogenic bacteria in dermatology *in vivo*

Bacteria host	Phage	Phage dose (UFP/ animal)	Phage provenance	Via	Nnmber of dosis	Especie animal	Result	References
P. aeruginosa	BC-BP-01, BC-BP-02, BC-BP-03, BC-BP-04, BC-BP-05, BC-BP-06.	$6x10^5$	-	Topica	1	*Canis familiaris*	Reduction of *P. aeruginosa*	78
S. aureus ATCC 25923	F1, F4, F7, F8, F9, F10.	$1x10^9$	Nasal and pharyngeal swab and sewage	SC	14	*Mus musculus*	Reduction of clinical signs and clinical cure.	92
K. pneumoniae B5055	Kpn5	$2x10^{10}$	Sewage	Topica	1	*Mus musculus*	Reduction of *K. pneumoniae*	124
S. aureus SA325	JD007	$5x10^8$	Chicken stool	ID	1	*Mus musculus*	Prevention and reduction of abscesses.	125

UFP: plate forming units. SC: subcutaneous. ID: intradermal.

Similarly, it was reported that the tick harbors a low amountof phages of the families: *Myoviridae, Podoviridae, Siphoviridae, Sphae-rolipoviridae* and *Microviridae*, which could be absorbed during the feeding moment or even arise in the same tick[130].

Regard *in vitro* studies, the autophages vB_SpsS-SN8, vB_SpsS- SN10, vB_SpsS-SN11, vB_SpsS-SN13 were isolated from the skin and mucous membranes of a canine patient, autophages with lithic activity against S. *pseudintermedius* E133 and E140[40], similarly, the autophage ϕDP001, found in the saliva of dog had lysed *S. pseudointermedius*[116].

Regarding *in vivo study*[92] a cocktail of autophages was used (F1, F4, F7, F8, F9 Y F10), which were obtained from nasal and pharyngeal swabs, and from sewage waters with lytic character against *S. aureus* in mice applied via subcutaneous. Similarly, autochthonous phages have been used in bovines, such as the phage SAvB14 that was isolated from the secretion of the gland mammary of cows with mastitis, with high activity lithic against *S. aureus* var. Bovis[131].

The autophages have the advantage of being able to isolate them directly from the affected environment and prepare for its application in the future, being more specific and effective than a cocktail commercial[126], emphasizing that the autophages will be more selective and more efficient due to the effect they have in the infection zone, allowing us to classify autophages as an alternative therapy.

Conclusion

Bacteriophages are an excellent substitute for antibiotics, since they are more specific and do not lyse beneficial bacteria, and have lithic activity against bacteria resistant to antimicrobials. There are bacteria resistant to phages, being unfavorablefor phage therapy, however it has been reportedmany times that by acquiring this resistance, its virulence is reduced, and they become more sensitive to antibioticsthat they used to be resistant to. Also, if the bacteriakeeps its virulence and resistance to antimicrobials, phage cocktails or quorum quenching can be used.Regarding the routes of application, topical and parenteral are the optimal way for treating pyodermas in company animals.

In the present study, we emphasize that FaL should be used instead of tempered, to ensure bacterial lysis even when the bacteria are in low density. Similarly, these FaL must not possess resistance and virulent genes, so that the objective bacteria don't acquire said genes through transduction.Likewise, the appropriate presentation is in the form of a cocktail, since it increases the lytic spectrum and decreases the risk of phage resistance. In short, autochthonous phages can be used when commercial phage cocktails of commercial phages or from previous studies do not have the effect desired. Thus, it can be concluded that the cocktails of lithic autophages without transduction are against antimicrobial resistance in small animal dermatology. Finally, it is recommended to compare the use of these cocktails with other antibiotic substitutes and assess their potential synergism to reduce bacteria pathogenic in the skin.

Source of financing

The authors declare that they received no specific funding for this article.

Conflicts of interest

There is no conflict of interest in this research.

Acknowledgments

The authors would like to thank the Universidad Privada Antenor Orrego de Trujillo.

Ethical considerations

The authors declare that the writing of the article is developed by carefully using previous studies in the literature and acknowledge them through the respective cited authors and sources.

Authors' contribution to the article

Vallenas-Sánchez Yhann Pool Angelo, contributed to the conception and design of study, acquisition, and analysis of data, discussion of results, writing of the manuscript, approval of the final version of the manuscript. *Bautista-Valles Maria Fernanda*, *Llaque-Chávarri Fabiana*, *Mendoza-Coello Martin Enrique*, contributed to the acquisition and analysis of data, discussion of the results, drafting of the manuscript, approval of the version final of the manuscript.

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