Sveriges lantbruksuniversitet
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Phage therapy against *Staphylococcus aureus*

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Fagterapi mot *Staphylococcus aureus*

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SAMMANFATTNING

Det söks efter alternativ till antibiotika eftersom dess användbarhet mot resistenta bakterier är begränsad och användningen kan ge negativa biverkningar. Ett alternativ som utforskas är användning av fager. Fagterapi har varit känd i över hundra år och används även i några länder. Det är viktigt att använda lytiska fager då temperata fager kan ge resistens och sprida virulens bland infekterade bakterier. Bakterien *Staphylococcus aureus* (*S. aureus*) är en viktig patogen med ökande antibiotikaresistentproblematik. Syftet med uppsatsen var att undersöka tillgänglig litteratur och besvara frågan: "finns det tillräckligt med vetenskaplig forskning för att använda fager som terapi mot *S. aureus* i klinik"? Det gjordes genom att söka efter relaterade artiklar i databasen PubMed. Där ett antal av 6983 hittades av vilka 990 stycken var review artiklar. Majoriteten av genomgångna artiklar visar på baktericida effekter av fagterapi. De täckte ett brett spektrum av användningsområden med översyn av fagterapi för mastit på kor, biofilm av bakterier, septikemi, *S. aureus* infektion efter ortopedisk kirurgi, bakteriemi, MultiDrug- och Methicillinresistens samt endoftalmit. Försök hade utförts *in vitro* samt *in vivo* på försöksdjur liksom i fältförsök. Fag-cocktails från den ryska marknaden påvisade baktericid effekt på humana stammar av *S. aureus* samtidigt som den baktericida effekten på stammar från gris var begränsad. Behandling med fagterapi av *S. aureus* från mjölkkor med mastit påvisade baktericid effekt vid laboratorietester på möss. Däremot kunde inte fältförsöket på kor med subklinisk mastitinfektion visa att fagterapin hade signifikant ökad baktericid effekt jämfört med kontrollgruppen. Andra försök har gjorts för att utvärdera fagterapi som möjlig del av lösningen mot biofilm i miljö liksom vid ortopediska ingrepp. Försöken utfördes *in vitro*, liksom i möss-, kanin- och fårmodeller. Med resultat som visade på baktericid verkan både vid kombinerad behandling med andra antibakteriella medel och som möjlig intervention för att begränsa bakterietrycket. Sammanfattningsvis har det visats att fagterapi har baktericid effect mot *S. aureus* både *in vitro* och *in vivo* i laboratorieförsök. Även om fältförsöket på kor med subklinisk mastit inte kunde påvisa signifikant baktericid effekt till förmån för fagterpi framför kontrollgrupp. Samtidigt finns det i till exempel Ryssland, Georgien, USA och Kina en marknad med medicinska produkter som gör det möjligt att använda fagterpi i klinik.

SUMMARY

Alternatives to antibiotics are being searched for since their efficacy on resistant bacteria is limited and using it can give negative side effects. One alternative that is being researched is the use of phages. Phage therapy has been known for about one hundred years and is also being used in some countries. It is important to use lytic phages for temperate phages might spread resistance and transfer virulence factors among infected bacteria. The bacteria *Staphylococcus aureus* (*S. aureus*) is an important pathogen with increasing resistance problem to antibiotics. The aim of this study was to investigate the available literature and answer the question: ´is there enough scientifically based research done to use therapy with phages against *S. aureus* in clinic´? The answers were found by searching for related articles in the database PubMed. Where a number of 6983 published articles were obtained of which 990 were review articles. The large majority of included articles in this review showed bactericidal effects from phage therapy. They had a broad coverage on looking into phage therapy against diseases as mastitis in cows, biofilm layers, septicaemia, *S. aureus* infection following orthopaedic surgery, bacteremia, MultiDrug- and Methicillin resistance and endophthalmitis. Trials have been conducted *in vitro* and *in vivo* on laboratory animals as well as in field. Phage cocktails on the market in Russia proved to be bactericidal on human *S. aureus* strains and meanwhile showing limited bactericidal effect on porcine strains. Treatment with phages of mastitis *S. aureus* from dairy cows had bactericidal effects in treating infected laboratory mice. The field trial on cows with subclinical mastitis infection on the other hand could not show that treatment with phage therapy had significantly higher levels of bactericidal effect compared to the control group. Attempts have been made to evaluate if phage therapy might be a part of the solution of biofilm in environment and in orthopaedic surgery. These trials were conducted *in vitro* as well as in mice, rabbit and sheep models. With results showing its efficacy as combined treatment with other antibacterial agents and as a possible intervention to decrease bacterial load. The conclusion drawn from this is that phage therapy has achieved bactericidal effects against *S. aureus in vitro* and *in vivo* on laboratory level. The field trial found on cows with subclinical mastitis could not confirm significant bactericidal effect in favour of phage treatment to controls though. Meanwhile in Russia, Georgia, US and China for example, phages are medical products on the market and available for use in clinic.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium, also known as the ´golden staph´ due to the characteristic yellow pigment production (Lan *et al.*, 2010). It is considered a commensal and opportunist in humans since about 20 - 30 % of the population are carriers (Gorwitz *et al.*, 2008). Nonetheless, *S. aureus* can cause serious infections in both humans and animals with fatal consequences as well as food poisoning (Quinn, 2011). In dairy cows *S. aureus* can be the reason to mastitis, an infection in the udder of the cow. Leading to pain and suffering of the animal along with economic losses for the owner and industry (Anderson *et al.*, 2012).

Antibiotics was first used in large scale in the 1940s and saved millions of lives. Shortly thereafter isolates emerged that were resistant to penicillin. As to *S. aureus*, short after the semisynthetic penicillin methicillin got out on the market, resistant strains emerged and the MultiResistant *S.aureus* (MRSA) spread (Aminov, 2010). Recent studies also raise anxiety on the collateral damage antibiotics can do to the microbiota (Modi *et al.*, 2014).

New antibiotics need to be developed as well as alternative methods in treating bacterial infections (Lee *et al.*, 2013). One of these methods might be ´phage therapy´. Phages, or bacteriophages, are virus that can infect and eventually kill bacteria. They were detected around 100 years ago and have a history of medical use in many countries. The phage therapy has with antibiotic resistance met a renaissance (Abedon *et al.*, 2011). Phages that infect *S. aureus*, are of the order Caudovirales -viruses with tails (Xia & Wolz, 2014). They are DNA viruses of Podovidridae, Siphoviridae or Myoviridae families (Ackermann, 2009). Phages can be virulent (lytic) and kill bacteria or temperate and integrate into the bacterial genome (see Fig. 1). For therapy only virulent phages are suitable to use, the temperate ones may help bacteria to avoid future phage infections and might carry virulence factors making the bacteria more capable of causing disease (Carter, 2013).

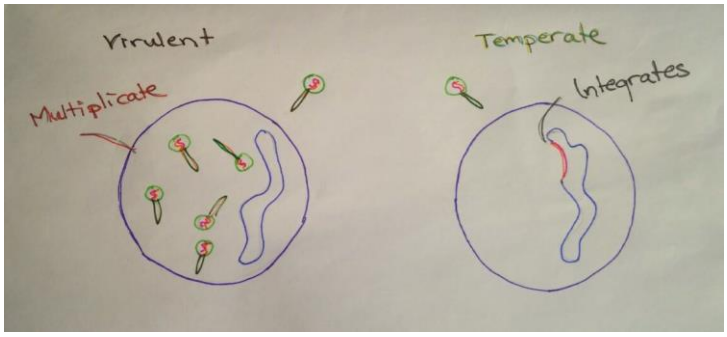

Figure 1. Schematic view on how virulent Caudovirales phage to the left and temperate Caudovirales phage to the right affect S. aureus bacteria.

OBJECTIVE AND MATERIALS

The aim of this paper was to investigate the literature on the subject phage therapy against *S. aureus*. The objective was to eventually answer the question: ´is there enough scientifically based research done to use therapy with phages against *S. aureus* in clinic´?

A research in the database archives of PubMed was made. The search terms bacteriophage OR phage AND *S. aureus* OR *Staph aureus* OR *Staphylococcus aureus* AND therapy OR therapeutics* OR treatment OR therapies OR practice OR treat* with filter "Other animals" and "Humans" gave as a result 6983 items, of which, 990 were review articles. A selection of articles were made by date, topics and based on their ability to answer to the aim of the objective of this paper.

LITERATURE OVERVIEW AND RESULTS

Phage therapy for *S. aureus*

Several studies have proven by *in vitro* models that phages have bactericidal effects against *S. aureus.* For example the two research teams Leskinen *et al.,* (2017) and Rahman *et al.*, (2011) demonstrated this with *in vitro* models. Leskinen *et al.*, (2017) showed that phage a ´cocktail´ named fRuSau02 had lytic impact and the ability to kill 135 *S. aureus* strains of human origin. However this phage ´cocktail´ showed very limited bactericidal effect on the 54 *S. aureus* strains of the porcine origin tested with only 30 % sensitivity and infectivity (Leskinen *et al.*, 2017). Rahman *et al.*, (2011) got the same conclusion of bactericidal reaction on 43 strains of *S. aureus* of human clinical isolates in combination with the antibacterial agent Rifampicin.

Phage ´cocktail´ fRuSau02 came from a producer in Russia. Leskinen *et al.*, (2017) ran laboratory *in vitro* tests on 135 human *S. aureus* strains of which some were MRSA. These strains came directly from infected patients who were in contact with healthcare. On the other hand when this same ´cocktail´ was used on *S. aureus* strains of porcine origin only 30 % (of 54 strains) were sensitive (Leskinen *et al.,* 2017).

Phage SAP26 is of the family Siphoviridae. Rahman *et al.*, (2011) verified its lytic effect on 110 MRSA and 61 MSSA isolates. To further examine its possibility to be used on biofilm formations an experiment comparing phage therapy to other antibacterial agents were run. The antibacterial agents used were Azitromycin, Vancomycin and Rifampicin. These were compared to each other and in combinations with phage SAP26. In addition one group was tested to the phage alone. This test showed that the phage SAP26 alone could kill 28% of the bacteria in 24 h. Thus, when combined with Rifampicin 40 % of the bacterial cells died within 24 h. The combination of SAP26 and Rifampicin was the highest bactericidal effect obtained (Rahman *et al.*, 2011).

Following the *in vitro* trials several laboratory tests have been done using mice as model animals. Mostly researchers conclude phage therapy to be safe and showing bactericidal effects, others are contradictory. For although both Gupta & Prasad (2011) and Singh *et al.*, (2014) conclude that phage therapy can be safely tested against MRSA strains there are other studies showing negative side effects. In a safety trial by Breyne *et al.*, (2017) uninfected and healthy mice were injected with phages in their mammary glands. When evaluating this they found that necrosis had emerged in the tissue and could be seen in histology sections (Breyne *et al.,* 2017).

When Gupta & Prasad, (2011) used phage P-27/HP to show its therapeutic value on Methicillin resistant *S. aureus* 27/HP they used bacteria from human patients. Their test was run in a mouse model. There were three groups of five mice (n=5) in each 1: positive control,

2: experimental group and 3: negative control group. A rate of 100% survival in the experimental group and reduction of splenic *S. aureus* 27/HP advocates the advantage of P-27/HP for this strain (Gupta & Prasad, 2011).

Singh *et al.*, (2014) on the other hand wanted to investigate the possibility to treat purulent eye infections, endophthalmitis, caused by *S. aureus* with phage therapy. They used 10 mice that were infected through infection of their eyes with *S. aureus* strain RN6390. This was a methicillin sensitive strain. Thereafter they were treated with phage Ply187 one single time. Results gave that leukocyte infiltration was reduced in comparison to a control group of mice eyes and that staphylolytic effect was obtained. An additive result from the mice trial was a reduction of retinal cell damage that *S. aureus* bacterial toxins generate. During preparation for this trial *in vitro* study of phage Ply187 on methicillin resistant and several other *S. aureus* strains was conducted, showing staphylolytic activities as well. In conclusion the authors find their result comfortable for future test with Ply187 on endophthalmitis caused by MRSA strains (Singh *et al.*, 2014). All articles reviewed in this paper are schematically demonstrated in Table 1.

Phage therapy and mastitis in cows

Several studies have been arranged to try phage therapy on mastitis caused by *S. aureus* in cows. Iwano *et al*. (2018) have shown, in a mouse model, that phage ΦSA012 and phage ΦSA039 of the family Myoviridae can be effectively used on 93 *S. aureus* strains. The bacteria (*S. aureus*) were found in dairy cows with mastitis. Mammary glands of lactating mice were inoculated with the suspension containing *S. aureus*. The pups were only separated from their mothers during this procedure thereafter they were kept together throughout the time of the experiment. The result was that phage therapy decreased infection and proliferation of *S. aureus* when administered at a multiplicity of 100 compared to the bacterial load of the infection. Treatment with phages gave a statistically significant difference with less bacterial colony-forming units (CFU) in mammary gland compared to controls two days after the therapy. In addition intraperitoneal route proved to be more efficient than intra mammary or intravenous (Iwano *et al.*, 2018).

Yet another study run by Breyne *et al.*, (2017) tried almost the same with *S. aureus* N305 strain and both Myovirus phage ATCC 23361 and BP39. This specific bacterial strain is used in experiments as isolate from dairy cows mastitis. Phage ATCC 23361 were evolved by the laboratory team and phage BP39 came from PhageLux, a company that holds a genebank of phages. Lactating mice were infected and treated with phage as examination group, cefalonium as positive control group (a first generation cephalosporin antibiotics) or phosphate buffer saline as negative control group. The mammary glands were inoculated of lactating mice through the teat canal, a small cut had to be done in each teat for entering the needle. At the end of the experiment the animals were euthanized and examined. Bactericidal effects could be confirmed with phage therapy treated bacterial load at significant levels lower than the negative control group. The numbers of CFU for phage therapy group were at 6,16 $\log_{10}$ CFU/ gram compared to the negative control group at 8,17 $\log_{10}$ CFU/ gram. As a result they made clear that phage therapy could be an alternative to antibiotics (Breyne *et al.*, 2017).

On the contrary from highly bactericidal effects *in vitro* and in laboratory animals models Gill *et al*. (2006) conclude from a placebo-control and multisite study that bacteriophage treatment of subclinical mastitis against *S. aureus* was limited. The animals included were dairy cows from seven different farms. An exclusion criterion was that they had recently been treated with antibiotics. The phage used was bacteriophage K. The treatment group consisted of 13 animals with a total of 18 affected udder quarters and the placebo group included 11 cows with 20 affected udder quarters. Administration route was intramammary infusion directly after milking the udder, followed by massage to circulate the content and transport the solution within the udder. Three udder quarters (3/18) in the bacteriophage treatment group were cured and no *S. aureus* detected four weeks post treatment. The placebo group held no cured udder quarters (0/20). This difference in result did not reach statistically significant levels. As this was a field trial it had a higher value of its results and was closer to clinical work than the others referred to on the subject mastitis by *S. aureus* in cows (Gill *et al.*, 2006).

Phage therapy in septicaemia and bacteraemia

Additional research on phage therapy against *S. aureus* has been made to evaluate its action on septicaemia and bacteraemia. Takemura-Uchiyama *et al.*, (2014) explored if bacteriophage S13´ was accurate to cure lung-derived septicaemia. The strain SA27 of *S. aureus* was used on neutropenic mice, individuals with reduced immune system capacity. This phage is from family Podoviridae and had been tried out to be bactericidal *in vitro* before the clinical trial started. During the *in vivo* part of the test inoculation was made through the intranasal route, which caused suffocation and death to some animals. Surviving individuals were included in the experiment and results. Bacterial concentrations were examined from blood, lungs, spleen and liver. There was a difference in survival rate at 67%, to 10 % in the negative control group, in favour of the phage treatment (Takemura-Uchiyama *et al.*, 2014).

Phage therapy has also been tested against bacteraemia. In one study by Oduor *et al.*, (2016) *S. aureus* of a MultiDrugResistant *Staphylococcus aureus* (MDRSA) strain was injected into mice through their tail vein. The phage was obtained from the environment and sought for in wastewater and sewage. An *in vitro* test was performed before starting the experiment to control the capability of the phages to lysate this MDRSA. There were 30 animals included and five individuals were left uninfected as controls. The groups treated included 1: clindamycin 2: phage therapy, 3: clindamycin and phage therapy 4: untreated negative controls. Within 9 days post treatment of phage therapy bacteraemia in bloodstream had been cured and no bacteria could be detected. This was far better than the clindamycin treated groups that had bacteria in blood samples with MDRSA10 days post treatment. The difference was significant in favour of phage therapy (Oduor *et al.*, 2016).

Phage therapy on biofilm and orthopaedic surgery

Biofilm formation is a problem in hospital environment and *S. aureus* is one part of this problem (Archer *et al.* 2011). This specific bacterium is also a complicating factor after orthopaedic surgery (*Ibid*). Numerous attempts have been made to see if phage therapy might be a component of the solution. These trials have been conducted *in vitro,* as well as in mice, rabbit and sheep models.

While one study by Kaur *et al.*, (2016) showed that phage therapy decreased bacterial load the most when combined with another antibacterial agent. Both Drilling *et al.*, (2014), Lungren *et al.*, (2014) and Hsieh *et al.*, (2011) found that phages alone had impact and bactericidal effect on biofilm and bacteraemia infection derived from such. All trials were conducted in animal models.

In the study by Kaur *et al.*, (2016) phage therapy was used against *S. aureus* ATCC43300 during surgery. This strain of bacteria is methicillin resistant. The orthopaedic surgery was performed on 30 mice where the medullary canal of the thighbone (femur) was introduced with a K-wire. After this was done the bacteria was inoculated, as to simulate an infection post surgery. They compared five groups respectively. By day 10 the bacterial load in joint tissue in groups with combined antibacterial agent and phages had gone down to zero. While groups with naked wire or only hydroxopropyl methylcellulose on the wire still by day 10 carried bacteria in joint tissue. The difference in groups with phages had significantly decreased bacterial load (Kaur *et al.*, 2016).

On the other hand in the study by Lungren *et al.*, (2014) 10 rabbits were included. The bacterium *S. aureus* of strain 46106, a methicillin sensitive strain of human origin was used. It was a randomised trial where all animals had a central venous catheters surgically induced. In the control group (5 individuals) all animals were maintained untreated and they all had biofilm presented at the catheters. Meanwhile, in the experimental group, that was treated with bacteriophage K, only one of five (1/5) exposed biofilm formation. The difference in bacterial load between the two groups was significant. In conclusion they lift that this reduction of biofilm is noteworthy and that future *in vitro* studies must foresee the importance of enough phage density in the target bacteria (Lungren *et al.*, 2014).

In the trial done by Drilling *et al.*, (2014) sheep were the animal model. Their aim was to evaluate and control the safety of phage therapy on biofilm of *S. aureus*. The strain used was ATCC25923 and the phage used was a mixture of *S. aureus* specific bacteriophage. The trial included four groups of three individuals in each treated as follows 1: no treatment, 2: ethylenediaminetetraacetid acid (EDTA), 3: cocktail of *S. aureus* specific phages (CTSA) and 4: CTSA + EDTA. The sheep were inoculated to cause a manifest infection and produce biofilm layer in the frontal sinuses. The conclusion was that the phage cocktail was safe to use and that the treatment reduced the number of bacteria in the biofilm. With no significant inflammation or damage to mucosal cilia between the groups, indicating noteworthy EDTA treatment to be safe and effective as well (Drilling *et al.*, 2014).

Hsieh *et al.*, (2011) performed a contamination test with *S. aureus*. They used isolates from human patients found on endotracheal tubes. The test was run with mice as model animals. The lytic phage (Stau2) was used. Stau2 is a member of the Myoviridae family. The strain S23 of *S. aureus* was induced via intraperitoneal injection. This specific strain is a MRSA isolate. It was studied first *in vitro* together with isolate of the phage to confirm that Stau2 possessed lytic ability on the bacteria. These tests showed that Stau2 had bactericid effect on 80 % of *S. aureus* isolates found on endotracheal tubes from patients. Without treatment the S23 infected mice died. Another 25 mice were treated with the phage Stau2 in different concentrations. The phage treated mice had full protection when treated with a 10 fold dose

(multiplicity of infection 10) of Stau2 to the induced dose of *S. aureus*, when treated immediately after the inoculation. The authors suggest this specific phage to be used against skin infections or in food industry on *S. aureus* including MRSA strains (Hsieh *et al.*, 2011).

Table 1. Overview of included articles: – means limited bactericidal effects, (+) means bactericidal effects, indication of risk and + means bactericidal effects.

Bacterial strain etiology	Model	Results	Authors
Human clinical isolates	In vitro	+	Leskinen *et al.* (2007)
Porcine	In vitro	-	Leskinen *et al.* (2007)
Mastitis cows	Mouse	+	Iwano *et al.* (2018)
Mastitis cows	Mouse	(+)	Breyne *et al.* (2007)
Mastitis cows	Cow	-	Gill *et al.* (2006)
Methillin resistant human isolates	Mouse	+	Takemura-Uchiyama *et al.* (2014)
Human clinical isolate	In vitro	+	Rahman *et al.* (2011)
Methillin resistant human isolates	Mouse	+	Kaur *et al.* (2016)
Human clinical isolate	Rabbit	+	Lungren *et al.* (2014)
Wastewater, MultiDrug resistance	Mouse	+	Oduor *et al.* (2016)
Methillin sensitive and resistant human clinical isolates	Mouse	+	Hsieh *et al.* (2011)
Methillin resistant human isolates	Mouse	+	Gupta & Prasad (2011)
Methillin sensitive and resistant human isolates	Mouse	+	Singh *et al.* (2014)

DISCUSSION

Discussion on results

The problem with phage being temperate and integrating to replicate with the bacteria have been discussed. Leskinen *et al.*, (2017) mentioned this in their report. It might have been a possible reason to why the porcine strains of *S. aureus* were more resistant to the fRuSau02 phage ´cocktail´ than the human strains. Leskinen *et al.*, (2017) suggests that the pig´s environment may have presented temperate phages to the bacteria. That was not being proven; nevertheless it could have been the reason to their resistance. Also Menouni *et al.* (2015) stresses this to be a complicating factor. By remodelling the bacterial genome phages can help them to become more capable of resisting the therapy (Menouni *et al.*, 2015).

Reported studies of phage therapy in mastitis caused by *S. aureus* gave a diverged picture. Both studies on mice quoted here gave bactericidal results (Breyne *et al.*, 2017; Iwano *et al.*, 2018). On the other hand the field trial study by Gill *et al.* (2006) on dairy cows got other results. It showed that the phage effect to decrease bacterial load was limited and did not significantly improve the infection. In their discussion they point out that an immune response from the cows were seen in milk by high levels of somatic cell count. Somehow, the *S. aureus* infected and phage treated udders reacted strongly to this treatment immunologically (Gill *et al.*, 2006). In addition there might have been other aspects of how bacteria could resist phage infections. Abedon *et al.*, (2012) listed in an article some mechanisms of phage-resistance in bacteria. Bacteria can for example change their genome and in following generations process a type of adaptive immunity (Abedon *et al.*, 2012).

In all included articles on phage therapy against *S. aureus* in biofilm the one of Rahman *et al*., (2011), Kaur *et al*. (2016) and Hsieh *et al.* (2011) bactericidal results were given. These results have been fortified lately. In a review Gutiérrez *et al*. (2018) explained how the phage protein affects biofilm. Using lytic proteins from phages, both biofilm on endocarditis and general bacteraemia, might be cured. Another point they made was the possibility to remodel these protein structures using bio techniques. This review also points out that the development of phage-resistance will be a future concern for phage therapy (Gutiérrez *et al.*, 2018).

Several of these experiments had more extensive bactericidal results when phage therapy was combined with antibiotics than used as single treatment. It might be a result of phage therapy and antibiotics killing bacteria laterally. If the antibiotics kill a part of the present bacteria and phage therapy one part they might as well benefit from each other. This was discussed as a possibility to why several of the studies presented lowered bacterial load when phage therapy was combined with antibiotics than from the phage therapy alone. Comeau *et al.* (2007) concluded the same when proving that combined antibiotics and phage therapy were more bactericidal together. They reported this to be the case even when sub-lethal doses of antibiotics and phage therapy united were used (Comeau *et al.*, 2007).

All of the studies reported here with mice as model animals gave positive results on bactericidal effect. The common denominator was defined bacterial strains and known phage strains. This was a notion that Kaźmierczak *et al.* (2014) also made in a review, that it was crucial to select the right phage for the bacteria present. It might have been a critical factor of

success in the reported tests in this essay (Kaźmierczak *et al.*, 2014). Importance in selecting and matching the right phages for certain bacterial strains to be used in therapy was also confirmed by Abedon *et al.*, (2011). Several companies in multiple countries provide phage cocktails on the market. Abedon *et al.*, (2011) mentions that in some institutions, using a lot of phage therapy, the mixtures are continuously updated to meet bacterial changes in genotypes (Abedon *et al.*, 2011).

A somehow complementing result compared to those given by this essay was published in 2016. Chadha *et al.* (2016) then concluded from a test with phage therapy on the bacterium *Klebsiella pneumonie* that ´cocktail´s with several types were more efficient than a single phage strain (Chadha *et al.*, 2016). Some of the tests conducted and referred to in this literature overview used monophage therapy and some used ´cocktail´s.

Discussion on essay

The aim of this study was to, by investigating the literature available, see if phage therapy could be used in clinical treatment against *S. aureus*. Already the search result could tell that there were a lot of studies done on the subject. The large number of articles found on the database PubMed showed that this specific bacteria and phage therapy has been widely observed. The answer from the thesis would be that phage therapy could be used against *S. aureus* under certain defined conditions.

Some of these results can be discussed further though. The experiments with phage therapy to *S. aureus* strains from dairy cows with mastitis on mice all gave bactericidal answers. The field trial on cows on the other hand did not. This might be since they suffered from subclinical mastitis, an indication that the bacterial load was too low for the phage to reach their potential. Another possibility is the difference in quantity and quality of milk in mouse mammary gland and a cow´s udder. There is a difference of how phages can reach a site of infection in a cow´s udder filled with milk and a small mouse with considerably smaller amounts of liquid. Comparing the difference in composition and compounds of protein-, fat- and carbohydrates- ratio in milk from mice and cows, the treatment and phage suspension might differ as well.

The results that are seemingly closest to advancing into field trials of those presented here might be the use of phages against biofilm formation. It could be used on surfaces and on delimited infected locations as in orthopaedic surgery. Possibly the administration on surfaces could be more direct and phages could be administered directly.

The conclusion is that bactericidal effects have been proven by phages against *S. aureus in vitro* and *in vivo* on laboratory level. The one field trial on subclinical mastitis cows referred to could not confirm significant bactericidal effect in favour of phage treatment to controls. Meanwhile in Russia, Georgia, US and China for example phages are a medical product on the market. This means ultimately that even if phage therapy is not an everyday choice in medicine there are several ´cocktail´ choices available for use in clinic.

REFERENCES

Abedon, S. T. (2012). Bacterial ‘immunity’ against bacteriophages. *Bacteriophage* [online], 2(1): 50–54. doi: 10.4161/bact.18609 [2018-02-22]

Abedon, S. T., Kuhl, S. J., Blasdel, B. G. & Kutter, E. M. (2011). Phage treatment of human infections. *Bacteriophage* [online], 1(2): 66–85. doi: 10.4161/bact.1.2.15845 [2018-02-19]

Ackermann, H.W. (2009). Phage Classification and Characterization. In: Clokie M.R., Kropinski A.M. (eds) *Bacteriophages*. *Methods in Molecular Biology*™, vol 501. Humana Press.

Archer N.K., Mazaitis M.J., Costerton J.W., Leid J.G., Powers M.E., Shirtliff M.E. (2011). *Staphylococcus aureus* biofilms: Properties, regulation and roles in human disease. *Virulence* [online] 2(5): 445-459. doi:10.4161/viru.2.5.17724 [2018-03-29]

Aminov, R. I. (2010). A Brief History of the Antibiotic Era: Lessons Learned and Challenges for the Future. *Frontiers in Microbiology*, 2010;1:134. doi: 10.3389/fmicb.2010.00134 [2018-02-26]

Anderson, K. L., Lyman, R., Moury, K., Ray, D., Watson, D. W. & Correa, M. T. (2012). Molecular epidemiology of Staphylococcus aureus mastitis in dairy heifers. *Journal of Dairy Science*, 95(9): 4921–4930.

Breyne, K., Honaker, R. W., Hobbs, Z., Richter, M., Żaczek, M., Spangler, T., Steenbrugge, J., Lu, R., Kinkhabwala, A., Marchon, B., Meyer, E. & Mokres, L. (2017). Efficacy and Safety of a Bovine-Associated Staphylococcus aureus Phage Cocktail in a Murine Model of Mastitis. *Frontiers in Microbiology*, 8.

Carter, J. B. (2013). *Virology: principles and applications*. 2. ed. Chichester: Wiley.

Chadha, P., Katare, O. P. & Chhibber, S. (2016). In vivo efficacy of single phage versus phage cocktail in resolving burn wound infection in BALB/c mice. *Microbial Pathogenesis*, 99: 68–77.

Comeau, A. M., Tétart, F., Trojet, S. N., Prère, M.-F. & Krisch, H. M. (2007). Phage-Antibiotic Synergy (PAS): β-Lactam and Quinolone Antibiotics Stimulate Virulent Phage Growth. *PLoS ONE*, 2(8).

Drilling, A., Morales, S., Boase, S., Jervis-Bardy, J., James, C., Jardeleza, C., Tan, N. C.-W., Cleland, E., Speck, P., Vreugde, S. & Wormald, P.-J. (2014). Safety and efficacy of topical bacteriophage and ethylenediaminetetraacetic acid treatment of Staphylococcus aureus infection in a sheep model of sinusitis. *International Forum of Allergy & Rhinology*, 4(3): 176–186.

Gill, J. J., Pacan, J. C., Carson, M. E., Leslie, K. E., Griffiths, M. W. & Sabour, P. M. (2006). Efficacy and Pharmacokinetics of Bacteriophage Therapy in Treatment of

Subclinical Staphylococcus aureus Mastitis in Lactating Dairy Cattle. *Antimicrobial Agents and Chemotherapy*, 50(9): 2912–2918.

Gorwitz, R. J., Kruszon-Moran, D., McAllister, S. K., McQuillan, G., McDougal, L. K., Fosheim, G. E., Jensen, B. J., Killgore, G., Tenover, F. C. & Kuehnert, M. J. (2008). Changes in the Prevalence of Nasal Colonization with Staphylococcus aureus in the United States, 2001–2004. *The Journal of Infectious Diseases*, 197(9): 1226–1234.

Gupta, R. & Prasad, Y. (2011). Efficacy of Polyvalent Bacteriophage P-27/HP to Control Multidrug Resistant Staphylococcus aureus Associated with Human Infections. *Current Microbiology*, 62(1): 255–260.

Gutiérrez, D., Fernández, L., Rodríguez, A. & García, P. (2018). Are Phage Lytic Proteins the Secret Weapon To Kill Staphylococcus aureus? *mBio*, 9(1).

Hsieh, S.-E., Lo, H.-H., Chen, S.-T., Lee, M.-C. & Tseng, Y.-H. (2011). Wide Host Range and Strong Lytic Activity of Staphylococcus aureus Lytic Phage Stau2. *Applied and Environmental Microbiology*, 77(3): 756–761.

Iwano, H., Inoue, Y., Takasago, T., Kobayashi, H., Furusawa, T., Taniguchi, K., Fujiki, J., Yokota, H., Usui, M., Tanji, Y., Hagiwara, K., Higuchi, H. & Tamura, Y. (2018). Bacteriophage ΦSA012 Has a Broad Host Range against Staphylococcus aureus and Effective Lytic Capacity in a Mouse Mastitis Model. *Biology*, 7(1): 8.

Kaur, S., Harjai, K. & Chhibber, S. (2016). In Vivo Assessment of Phage and Linezolid Based Implant Coatings for Treatment of Methicillin Resistant S. aureus (MRSA) Mediated Orthopaedic Device Related Infections. *PLoS ONE*, 11(6).

Lan, L., Cheng, A., Dunman, P. M., Missiakas, D. & He, C. (2010). Golden Pigment Production and Virulence Gene Expression Are Affected by Metabolisms in Staphylococcus aureus. *Journal of Bacteriology*, 192(12): 3068–3077.

Lee, C.-R., Cho, I. H., Jeong, B. C. & Lee, S. H. (2013). Strategies to Minimize Antibiotic Resistance. *International Journal of Environmental Research and Public Health*, 10(9): 4274–4305.

Leskinen, K., Tuomala, H., Wicklund, A., Horsma-Heikkinen, J., Kuusela, P., Skurnik, M. & Kiljunen, S. (2017). Characterization of vB_SauM-fRuSau02, a Twort-Like Bacteriophage Isolated from a Therapeutic Phage Cocktail. *Viruses*, 9(9).

Lungren, M. P., Donlan, R. M., Kankotia, R., Paxton, B. E., Falk, I., Christensen, D. & Kim, C. Y. (2014). Bacteriophage K Antimicrobial-Lock Technique for Treatment of Staphylococcus aureus Central Venous Catheter–Related Infection: A Leporine Model Efficacy Analysis. *Journal of Vascular and Interventional Radiology*, 25(10): 1627–1632.

Menouni, R., Hutinet, G., Petit, M.-A. & Ansaldi, M. (2015). Bacterial genome remodeling through bacteriophage recombination. *FEMS Microbiology Letters*, 362(1): 1–10.

Modi, S. R., Collins, J. J. & Relman, D. A. (2014). Antibiotics and the gut microbiota. *The Journal of Clinical Investigation*, 124(10): 4212–4218.

Oduor, J. M. O., Onkoba, N., Maloba, F., Arodi, W. O. & Nyachieo, A. (2016). Efficacy of lytic Staphylococcus aureus bacteriophage against multidrug-resistant Staphylococcus aureus in mice. *The Journal of Infection in Developing Countries*, 10(11): 1208–1213.

Quinn, P. J. (2011). *Veterinary microbiology and microbial disease*. 2. ed. Chichester: Wiley-Blackwell.

Rahman, M., Kim, S., Kim, S. M., Seol, S. Y. & Kim, J. (2011). Characterization of induced Staphylococcus aureus bacteriophage SAP-26 and its anti-biofilm activity with rifampicin. *Biofouling*, 27(10): 1087–1093.

Singh, P. K., Donovan, D. M. & Kumar, A. (2014). Intravitreal Injection of the Chimeric Phage Endolysin Ply187 Protects Mice from Staphylococcus aureus Endophthalmitis. *Antimicrobial Agents and Chemotherapy*, 58(8): 4621–4629.

Takemura-Uchiyama, I., Uchiyama, J., Osanai, M., Morimoto, N., Asagiri, T., Ujihara, T., Daibata, M., Sugiura, T. & Matsuzaki, S. (2014). Experimental phage therapy against lethal lung-derived septicemia caused by Staphylococcus aureus in mice. *Microbes and Infection*, 16(6): 512–517.

Xia, G. & Wolz, C. (2014). Phages of Staphylococcus aureus and their impact on host evolution. *Infection, Genetics and Evolution*, 21: 593–601.