COMPLETE GENOME ANALYSIS OF LYTIC PHAGES AND IDENTIFICATION OF HYPOTHETICAL PHAGE PROTEINS TARGETING MAJOR PROTEIN COMPLEXES OF *Staphylococcus aureus*

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A thesis submitted in fulfillment of the requirement for the award of the degree of Doctor of philosophy in Virology at the KAVI – Institute of Clinical Research, in the College of Health Sciences of University of Nairobi.

24 June, 2021

DECLARATION

This thesis is my original work and it has not been presented a degree in any other university.

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DEDICATION

This work is dedicated to my parents (Mr. John Oduor and Mrs. Phelister Akoth) and to any person who has supported me throughout the entire period of this work.

And for the greater glory of God!!!

ACKNOWLEDGEMENT

My heartfelt gratitude to the All Mighty (God) for by His grace this work has been done despite all the challenges faced. I sincerely wish to thank my supervisor Prof. Mikael Skurnik and the entire 2017-2018 Skurnik lab members (Human Microbiome Research Program, Faculty of Medicine-University of Helsinki -Finland) for their support throughout this work. Also, wish to thank Ermir Kadija (University of Shkodra "Luigj Gurakuqi", Shkodra-Albania) for facilitating phage sample isolation (Stabs) in his country.

Sincere gratitude to my academic and research funders; Finnish National Agency for Education (CIMO), the International Society for Infectious Diseases and the European Society of Clinical Microbiology and Infectious Diseases (ISID/ESCMID), the Academy of Finland, Jane and Aatos Erkko Foundation, and PhageBiotics (through Dr. Elizabeth Kutter, Evergreen State College-USA).

I also wish to acknowledge my Kenyan supervisors Dr. Marianne W. Mureithi and Dr Atunga Nyachieo for their support during this study.

Great thanks to my parents (Mr. John Oduor and Mrs. Phelister Akoth) for their mental and financial support.

Thank you very much (Asante sana / Kiitos paljon) to any person who contributed to this work, I do appreciate each of you out there.

God bless / Jumalan siunausta!!!

Table of Contents

List of tables

List of Figures

List of Appendices

List of Abbreviation

ATCC:	American Type Cell Collection
CRISPR:	Clustered regularly interspaced short palindromic repeats
CFU:	Colony forming unit
DNA:	Deoxyribonucleic acid
EDTA:	Ethylenediamine tetra-acetic acid
ENA:	European Nucleotide Archive
EOP:	Efficiency of plating
HUSLAB:	Helsinki University Hospital Laboratory
ICTV:	International Committee on Taxonomy of Viruses
KNBS:	Kenya National Bureau of Statistics
LC-MS/MS:	Liquid chromatography tandem mass spectrometry
MDR:	Multi-drug resistant
MDRSA:	Multi-drug resistant *Staphylococcus aureus*
MRSA:	Methicillin Resistant *Staphylococcus aureus*
NaCl:	Sodium Chloride
NCBI:	National Centre for Biotechnology Information
NGS:	Next generation sequencing
PBS:	Phosphate buffered saline
PFU:	Plaque forming unit
RNA:	Ribonucleic acid
RNAP:	Ribonucleic acid polymerase
RpoE:	RNA polymerase extracytoplasmic E
SDS:	Sodium dodecyl sulphate
U.S.S.R:	Union of Soviet Socialist Republics
USA:	United States of America
Vol/Vol:	Volume by volume
W. H. O:	World Health Organization

Abstract

Background: Bacteriophages (phages) are obligate parasites of bacteria. Phages are grouped according to their life cycle as lytic, temperate (lysogenic), pseudo-lysogenic and chronic phages. Lytic phages have been applied efficiently (phage therapy) against human infections caused by pathogens such as *Staphylococcus aureus* or *Escherichia coli*. In addition, the viruses destroy antibiotic-resistant bacteria. Genome analysis of phages is significant since it helps in selection of safe phages from harmful ones. The analysis further facilitates the identification of phage genes with unknown functions from ones whose purpose are yet to be unravelled. These strange genes are also known as hypothetical genes and they encode hypothetical proteins. Some of these hypothetical genes have homologs in the GenBank. However, others are non-identical with the GenBank deposited genes and consequently referred as novel genes.

Objective: To explore novel lytic phages and compare them with the current known phages. Thereafter, establish functions of their hypothetical proteins against multi-drug resistant *S. aureus* complex target proteins such as wall teichoic acid (WTA), lipoteichoic acid (LTA) and multidrug resistant efflux pumps associated with quinolones and linezolids.

Methods: Highly potent *Staphylococcus* lytic phages were isolated using *S. xylosus* sausage fermentor isolate. Thereafter, characterized through morphological, genomical and proteomic means. The phages' host range were determined by spot and double layer agar assays against numerous clinical samples of *S. aureus* that are MSSA or MDRSA (including MRSA).

Results: Four lytic phages; Stab20, Stab21, Stab22 and Stab23, were identified as *Kayviruses.* In addition, genomic analysis showed that these viruses are possessed numerous hypothetical proteins. Genome work further indicated inability of the Stabs to shuttle lethal genes like antibiotic resistance encoding genes and chromosomal point mutations associated with drug resistance or virulence. Proteomic outcome displayed the close similarity of the phages. Genomic and proteomic analysis showed that the Stabs are closely related with other phages such as Sb-1 and ISP which are

useful therapeutic agents. Stab20 and Stab21 phages had broad host range with high relative EOP. However, two other isolates were active against a few isolates.

Conclusion: Efficacy of these phages against human and livestock *Staphylococcus* bacteria isolates depicts their capability as good candidates for therapeutic and bio-control phage cocktails.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Bacteriophages (phages) have been used as therapeutic or bio-control agents against bacterial infections since 1919 (Summers, 2012). However, phages' bactericidal properties were first observed in 1896 by British Chemist E. H. Hankin while studying the effects of the water from the Ganges and Jumma rivers of India against *Vibrio cholerae* pathogen (Stone, 2002). Years later a British bacteriologist Frederick W. Twort made a similar observation (Sulakvelidze *et al.,* 2001). However, Felix d'Herelle, a French-Canadian microbiologist was the one who finally made use of phages as therapeutic and bio-control agents. Thus, he is considered as the "Father" of phage therapy (Summers, 2012; Wittebole *et al.*, 2014).

d'Herelle's first work involved controlling avian typhosis (*Salmonella gallinarum*) (Summers, 2012). He later treated a 12 year old boy who had severe Shigellosis with "anti-*Shiga*" phages (Sulakvelidze *et al.,* 2001). Thereafter, in 1921 d'Herelle performed a major mass treatment of persons suffering from Shigellosis and had them cured within 24 - 26 hours after receiving an oral dosage of shiga-phage. This work was at the Infants' Hospital in Paris under the supervision of Professor Hutinel (Dublanchet & Bourne, 2007). The success of this first mass phage therapy caught the attentions of many bacteriologists across Europe, North and South America who tried to replicate the work but, failed (Dublanchet & Bourne, 2007). However, d'Herelle continued with phage research and even helped establish a phage therapy institute in Georgia which is now the global centre of phage therapy (Summers, 2012).

Phages are the most trusted therapeutic and bio-control agents in the entire former Union of Soviet Socialist Republic (U.S.S.R). In Georgia and Russia phages are currently sold even over the counters in most pharmacies, besides being prescribed in hospitals (Brüssow, 2012). However, phage therapy is not being practiced in other parts of the world due to political and ideological difference amongst the earliest scientists (Summers, 2012). The advent of antibiotics and lack of knowledge on

phage biology also played a role in discouraging phage therapy in the western world (Oliveira *et al.,* 2015; Adhya *et al.,* 2014). However, the frequent use of antibiotics in hospitals and at home has resulted to the emergence of drug resistant bacterial strains (Spellberg *et al.,* 2008). In addition, the issue of drug resistance has been exerbeited by excessive use of drugs in livestock husbandry. Most of the main antibiotics are used as animal feed additives to enhance rapid growth and for prophylactic purposes (Graham *et al.,* 2007). The outcome of these human activities is the presence of excess antibiotic wastes in the environment which has selected for antibiotic resistant bacterial strains (Larsson, 2014; Phillips *et al.,* 2004). However, some bacteria are just naturally resistant to certain antibiotics (Martínez, 2012). These include bacteria like *Enterococcus* spp which are naturally resistant to aminoglycosides and β-lactams (Hollenbeck & Rice, 2012; Miller *et al.,* 2014). *Staphylococcus* spp such as *S. aureus* have been documented to be naturally non-responsive to β-lactams (Brown & Reynolds, 1980). Furthermore, *S. aureus* strains have been noted to possess drug resistant genes that enable them to develop resistance against multiple antibacterial agents (Hiramatsu *et al.,* 2014; Kaatz *et al.*, 2005).

Staphylococcus spp are gram positive bacteria. However, *S. aureus* appear as bunch of grapes and berries when viewed under a microscope after Gram staining unlike other species of staphylococci bacteria (Licitra, 2013). They form gray to golden yellow colonies on nutrient agar medium and can tolerate salt concentration of 10%. These bacteria are catalase and coagulase positive but oxidase negative (Gnanamani *et al.,* 2017). The microbes can grow aerobically and anaerobically (facultative microbes) at temperatures ranges between 18 – 40 ºC but does best at 35 ºC (Taylor & Unakal, 2020). *S. aureus* are commensal microorganisms of humans, birds and animals (Heaton *et al.,* 2020). However, they become pathogenic when the body's immune system gets compromised (Silva *et al.,* 2020).

Some strains of *S. aureus* have been identified to be resistant to almost all classes of antibiotics currently available and these are the multi-drug resistant *S. aureus* (MDRSA) (Howden *et al.,* 2011). Subsequently the WHO has currently ranked *S.*

aureus bacteria as one of priority 2- high risk pathogens (World Health Organization, 2017b). The bacterium is of high economic significance as it is pathogenic to humans and livestock, and is acquired from either humans or livestock (Fair & Tor, 2014; Mehndiratta & Bhalla, 2014; Smith, 2015). In human *S. aureus* causes infections which include skin and soft tissue infections (SSTIs) such as boils and mastitis. In addition, the bacteria are associated with diseases such as necrotizing pneumonia, and osteomylitis (Thomer *et al.,* 2016). Some of these *S. aureus* human illness such as SSTIs like mastitis and exudative epidermitis; arthritis and pneumonia are also common in pets, livestock and poultry (Heaton *et al.,* 2020). The mentioned effects of MDRSA on humans and their domesticated animals makes the pathogen a suitable tool for biological weapon and thus it also a threat to the global security.

However, there are only a few antibiotics available against the MDRSA strains and some of them have serious adverse effects on the users. These antibacterial agents include vancomycin, teicoplanin, rifampicin, fusidic acid, lincosamides (clindamycin and lincomycin), linezolid and quinupristin/dalfopristin (Rayner & Munckhof, 2005). Though, they pose major harm to patients such as nephrotoxicity, allergies, diarrhoea and thrombocytopenia (Marinho *et al.,* 2011; Rafii *et al.,* 2008). In addition, a number of these drugs are expensive and are out of reach for many people. Furthermore, only a few pharmaceutical firms are currently concerned with the production of new antimicrobials molecules as the venture is less profitable (Conly & Johnston, 2005). Another challenging factor is that the rate at which bacterial pathogens develop resistance to antibiotics is relatively faster compared to antibiotics' development and production pace (Carlet *et al.,* 2012; Spellberg *et al.,* 2008). The result of these has been the shortage of new antibacterial agent against the ever-emerging multi-drug resistant bacteria such as MDRSA. Currently biological agents such as phages and phage products like lysin proteins are being explored for therapeutic purpose. In addition, phages' "hypothetical proteins" and protein-protein interactions are being looked into in the quest for developing new antibacterial molecules against multi-drug resistant (MDR) bacteria such as MRSA. Hence this project focuses on the characterization of novel phages and exploration of new

'hypothetical proteins' from their genomes. The identified proteins might of great value in the development of effective drugs against various MDRSA currently circulating in the communities and hospitals around the world.

1.2 Problem statement

Emergence of multi-drug resistant bacteria such as MDRSA threatens the global public health and food security (Cheng *et al.,* 2016; World Health Organization, 2017b). The WHO estimates that 64% of persons infected with MDRSA such as methicillin resistant *Staphylococcus aureus* (MRSA) are more likely to die than those infected with drug sensitive strains of *S. aureus* (World Health Organization, 2017b). However, only a few pharmaceutical companies are currently involved in the development of new antibiotics (Bartlett *et al.,* 2013). This is due to the fact that research and development of new antibiotics is a non-profitable venture especially when coupled with stringent government regulations (Renwick & Mossialos, 2018). Another major dissuading factor is that bacteria manage to develop rapidly resistance even against the new drugs (Liljeqvist *et al.,* 2012; Nathan & Goldberg, 2005). Consequently, there is scarcity of safe and effective antibiotics against MDRSA strains. Thus, the current major dependence on the old antibiotic or their modified molecules as antibacterial agents. These drugs often target the same sites which the bacteria can alter through mutations thus making them resistant to all the antibiotics in a similar antibiotics. The production of numerous generic antibiotics has also contributed to disincentive in the research and development of new antibiotics (Jose, 2010).

1.3 Justification for the study

Phages have been used for a century in some parts of the world as antibacterial agents and their efficacy and safety proved beyond doubt (Dedrick *et al.,* 2019; Oduor *et al.,* 2016; Schooley *et al.,* 2017). These viruses are capable of mutating to overcome phage or antibacterial resistance staged by the bacteria (Maxwell, 2016).

In addition, phages and their products such as lysin and tails are currently being used as antibacterial agents for food sanitization (Drulis-Kawa *et al.,* 2012). Besides, the viruses are readily available in the environment for easy exploration. This expedites development and production of new therapeutic regimen against phage-resistant bacterial strains.

In biocontrol and therapeutic application of phages, only lytic phages are of value. However, under certain conditions where no obligate lytic phages are available siphoviruses may be use but after deletion of lysogen (integrase) genes (Dedrick *et al.,* 2019). Thus, this work was designed to explore obligate novel lytic staphylococcus phages with antibacterial significance. In addition, the work only targeted staphylococcus pathogens due to limited time frame and funds that were available for the study.

1.4 Research questions

i. What are the physico-chemical properties of the isolated lytic *Staphylococcus* phages?
ii. What are the classification groups of the isolated lytic *Staphylococcus* phages?
iii. Do the isolated lytic *Staphylococcus* phages possess novel genes?
iv. Are there "super-spreader" *Staphylococcus* phages?
v. How potent would the isolated novel proteins from the lytic *Staphylococcus* phages be against clinical and wild *Staphylococcus* strains?

1.5 Null hypothesis (H_0)

Lytic phages and their protein products are not effective antibacterial agents as compared to conventional antibiotics.

1.6 Objectives

1.6.1 General objective

To contribute towards development of new antibacterial agents against MDRSA using lytic phages and their novel protein products that target specific complex structures of these bacterial strains.

1.6.2 Specific objectives

i. To determine the appropriate classification of the isolated lytic phages.
ii. To establish the physico-chemical and growth properties of the isolated lytic phages.
iii. To evaluate the host range of the isolated lytic *Staphylococcus* phages.
iv. To establish whether the phages are “super spreaders”.
v. To identify genes encoding novel hypothetical proteins of unknown function within the genomes of the isolated lytic phages to be searched for host-toxic proteins.

CHAPTER TWO: LITERATURE REVIEW

2.1 Description of phages

Bacteriophages (phages) are prokaryotic viruses that attack bacterial cells (prokaryotes) only and not animal cells (eukaryotes). They are obligate intracellular parasites of eubacteria with either a DNA or RNA genome that might be single or double stranded (Krupovic *et al*, 2011). In addition, phages exist in different morphological features with varied structures. Some have spindle/helical (filamentous), icosahedral and complex morphology. A complex morphology is a combination of an icosahedral-head attached to a spindle (tail) structure that may be present or not. In some cases, the spindles might possess structures like the base plate, tail fibres and whiskers. Filamentous phages includes phage f1, Fd and M13 of *Escherichia coli*; Pf1, Pf4 and Pf5 of *Pseudomonas aeruginosa* and CTXφ of *V. cholerae* (Rakonjac *et al.,* 2011). ΦX174 phages of *Escherichia coli* are examples of icosahedral phages (McKenna *et al.,* 1992) while complex phages are like Twort-like phages of *S. aureus* (Nováček *et al.,* 2016).

These viruses form the largest life form on the earth's biosphere and are estimated to be 10^{30}-10^{31} in number on the planet (Bar-On *et al.,* 2018; Hendrix *et al.,* 1999). Phages are responsible for keeping in check the bacterial population on the earth (Ackermann, 2007). In addition, phages maintain the bacterial balance on super-organisms (such as humans and other animals) to prevent disease outbreaks due to bacteria (Letarov & Kulikov, 2009). Phages exist wherever their bacterial host are found (Breitbart & Rohwer, 2005) and as such they are found even in hostile environments which includes deep-sea hydrothermal vent, the cold and hot deserts (Borriss *et al.,* 2003; Ji *et al.,* 2015; Prestel *et al.,* 2013; Yoshida-Takashima *et al.,* 2012).

2.2 Phage characterization

The International Committee on Taxonomy of Viruses (ICTV) has used the genomic content status and morphological appearance of phages to classify them into various

groups. Generally most phages belong to order *Caudovirales* which has nine main families that includes *Ackermannviridae, Autographiviridae, Chaseviridae, Demerecviridae, Drexlerviridae, Herelleviridae, Myoviridae, Podoviridae* and *Siphoviridae* (International Committee on Taxonomy of Viruses, 2020). These families have been classified based on the tail morphology (Fokine & Rossmann, 2014). *Caudovirales* are tailed phages with icosahedral capsids filled with double-stranded DNA (ds-DNA) and they constitute 96% of all the observed phages (Ackermann, 2007). The sizes of the *Caudovirales* genomes range from 11.6 kb of P1 phage infecting mycoplasmas to 500 kb of *Bacillus* phage G (Salmond & Fineran, 2015; Tu *et al.,* 2001). Each family that form these orders are group into subfamilies that are further divided into genera that in turn has species. However, there are phages with neither genera nor subfamily like *Brochothorix virus A9, Lactobacillus virus* Lb338-1 and *Lactobacillus virus* LP65 of the *Herelleviridae* family (International Committee on Taxonomy of Viruses, 2020).

2.3 Life cycle of phages

Phages display numerous life cycles that have been used in differentiating them as lytic, lysogenic, pseudo-lysogenic and chronic phages (Clokie *et al.,* 2011). The life cycles are important to individual phage group for their survival. Lytic life cycle involves the infection and later destruction of the host bacteria after the phages have multiplied within them. The process ensures that the bacterial population is under control, besides facilitating long term evolution of the host bacteria through transduction (Weinbauer & Rassoulzadegan, 2004). The evolution of bacteria has made them resistant to predatory phage by acquiring immune systems to prevent phage adsorption and block invader DNA entry (Shabbir *et al.,* 2016). However, in order to ensure their survival phages have also had to evolve to counteract resistance from host bacteria by developing features such as anti-CRISPR systems (Maxwell, 2016). In the lysogenic life cycle after infection of the host bacterium the phage genome integrates as part of the host genome or resides as a plasmid in the cytoplasm. Such a form of phage is referred to as a prophage that establishes a long

mutual association between the phage and the host bacterium. Therefore, the bacterium is not killed and its survival might be enhanced if the phage carries host-beneficial genes such as antibiotic resistant genes (Colomer-Lluch *et al.,* 2011; Muniesa *et al.,* 2013). The host bacterium might also turn pathogenic as in the case of *V. cholerae* that becomes pathogenic after acquiring CTXφ (Das *et al.,* 2011). These genes are inheritable and can be passed to a thousand generations from the host bacterium (Davis *et al.,* 2000).

Pseudo-lysogenic life cycle is unstable existence of phage in a host bacterium while waiting for the right environmental conditions for it to assume lytic or lysogenic life cycle. The phages might be waiting for nutrient enrichment, temperature fluctuation or exposure to sunlight (Baugher *et al.,* 2014; Wilson *et al.,* 1996). The chronic infection life cycle is found in archaeal viruses such as the filamentous phages and plasmaviruses like those that infect *Mycoplasma* spp (Clokie *et al.,* 2011). The life cycle of *Mycoplasma* bacteriophages involves slow continuous shedding of phage copies by budding from the host bacteria (*Mycoplasma* spp) for a long time without lysing the host (Weinbauer, 2004).

2.4 Phage abundance and diversity

Phage abundance within the biosphere varies biogeographically. There is more phage in the world ocean than on land. The ocean waters in total are thought to have about 4.0×10^{30} phage copies (Suttle, 2007) while a gram of soil sample on land has about $10^{8\text{-}10}$ phage copies (Wilhelm & Suttle, 1999; Williamson, 2011). However, phage copies on land vary from place to place. Where a gram of marine sediments has about 10^{10} phage copies as compared to one gram fresh water lake sediment which has an estimate of 10^{9} phages (Danovaro *et al*, 2002). In addition, fresh water lakes have about 10^{9} phage particles per milliliter than sea water of the same volume which has 10^{7} phage particles (Breitbart, 2012). Phage are also present in marine snow (algal flocs), in which there about 10^{10} phage copies (Peduzzi *et al.,* 1993). Furthermore, phages are abundant in sea ice; which has an estimate of $10^{6\text{-}8}$ phage

particles (Weinbauer, 2004) and in air. The concentration of these viruses in air does vary from one place to another. In some cheese processing plants about 10^8 PFU/m^3 of phage particles have been isolated from the air within these factories (Daniel Verreault *et al.,* 2010).

Phages are also form part of animals and plants microbial systems where they ensure balance among the bacterial flora. Amongst animals, ruminating bovines have phage count of about 10^7 per gram of their feces while human's feces has 10^9 phage copies per gram (Niu *et al.,* 2009; Rohwer, 2003). Opportunistic infections are often as a result of broad spectrum antibiotics that eradicate useful bacteria that colonize the gut (Buffie *et al.,* 2012). Phages are also abundant in certain food eaten by humans and especially dairy products such as yogurt, cheese and raw milk. Cheese has been found to possess a phage content of about 10^9 PFU/gm that is clearly higher than that of yogurt which has 10^3 PFU/mL and raw milk has 10^4 PFU/mL (Madera *et al.,* 2004).

2.5 Staphylococcal Phages

The order *Caudovirales* has three families under which staphylococcal phages are classified. These includes *Herelleviridae*, *Podoviridae* and *Siphoviridae* families (International Committee on Taxonomy of Viruses, 2020). *Herelleviridae* family is a group of phages usually possessing a large capsid head with a diameter of about 85 - 100 nm to which is attached a complex uncontractile tail of 130 - 185 nm in length (Barylski *et al.,* 2020). Tails of phages from this family have a baseplate at the tip and a collar joining it to the head (Nováček *et al.,* 2016). *Herelleviridae* phages have a genome size greater than 106 kilobase pairs (kbp) (NCBI, 2020). They infect gram-positive and gram-negative bacteria (O'Flaherty *et al.,* 2004).The family is currently grouped into five subfamilies which include *Bastillevirinae*, *Brockvirinae, Jasinkavirinae, Spounavirinae* and *Twortvirinae* (International Committee on Taxonomy of Viruses, 2020). Only the *Twortvirinae* subfamily that hosts staphylococcal phages within the *Herelleviridae* family. *Twortvirina*e has seven

genera and five of them are consists of only staphylococcal phage and they are *Baoshnavirus, Kayvirus, Sciuriunavirus, Sepunavirus, Silviavirus* and *Twortvirus* genus. These genera were created based on the *Staphylococcus* spp a virus destroys, the number of tRNA encoded by a virus genome, the genome size and its terminal repeats range. Previous studies show that members of genus *Sciuriunavirus* and *Sepunavirus* are more virulence on the strains of *S. sciuri* and *S. epidermidis* respectively. Genome analysis of phages from *Sciuriunavirus, Sepunavirus* and *Twortvirus* genera shows that they do not encode for tRNAs. Phages within *Baoshanvirus, Silviavirus* and *Kayvirus* genera each encodes for tRNAs. However, it is only Kayviruses which have four tRNAs while the other genera each has one tRNA (International Committee on Taxonomy of Viruses, 2020).

Podoviridae phages have smaller icosahedral or prolate heads with a diameter of about 50 – 60 nm, and a short, stubby, non-contractile tail or no tail at all (Hrebík *et al.,* 2019; Khan Mirzaei *et al.,* 2014). The *Podoviridae* family consists of three sub-families that include *Picovirinae, Rakietenvirinae, Sepvirinae,* and a number of genera. All staphylococcal podoviruses are members of the *Rakietenvirinae* subfamily within the *Andhravirus* and *Rosenblumvirus* genera.hosts. These phages have distinct small genomes of about 16 - 18.5 kbp void of tRNAs (Cater *et al.,* 2017; Culbertson *et al.,* 2019). *Herelleviridae* and *Podoviridae* viruses are obligate lytic phages that destroy bacteria.

Siphoviridae family, a member of the order *Caudovirales* contains the largest numbers of the tailed-phages. These are some of the populous staphylococcal phages and consequently are the major drivers *Staphylococcus* bacteria diversity (Deghorain & Van Melderen, 2012). The phages have either prolate or icosahedral capsids attached to long non-contractile tails void of sheaths. Their genome size is about 39-43 kb (Moller *et al.,* 2019; Xia & Wolz, 2014). These viruses are temperate phages capable of either co-existing with host bacteria or destroying them. They turn lytic when there is change in environmental conditions like exposure to ultra-violet radiation and antibiotics, pH and nutrient changes (Howard-Varona *et al.,* 2017). In

lysogenic cycle, they exist as prophages within the host bacteria genomes. The genomes of siphoviruses harbour lysogenic modules which consists of integrase and regulatory genes, and CI and Cro genes (Xia & Wolz, 2014). Siphoviruses are associated with *Staphylococcus* bacteria pathogenicity and ability to endure harsh environmental conditions. Pathogenic association of siphoviruses with *Staphylococcus* bacteria turns them unsuitable for phage therapy against staphylococcal infection.

2.6 Genome structures of S. aureus phages

These viruses have a genome size ranging from 16 kilobase pair (kbp) to about 157 kbp. Podoviruses are the only lytic of *S. aureus* phages with the smallest linear-double stranded DNA genomes. The genome of staphylococcal podoviruses varies in size and can be from 16-18.5 kbp (https://blast.ncbi.nlm.nih.gov/Blast.cgi). They have fewer open reading frames (ORFs) of about 20-22 ORFs, obligate lytic life cycle and GC content (27-29%) (Oliveira *et al.,* 2019). These phages have conserved genome organization with two transcriptional units meeting near the centre. Locations of the DNA packaging and DNA polymerase genes of these phages are close at the start of the left genome terminus while the structural protein genes are at their right parts (Cater *et al.,* 2017). In addition, these phages have the least counts of hypothetical proteins because of their tiny genomes.

The *Twortvirinae* sub-family is the only group within the *Herelleviridae* family that house staphylococcal phages. *Twortvirinae* has seven genera but only one group that is exceptional. Genus *Harbinvirus* is the odd group with *Twortvirinae* subfamily that contains non-staphylococcal phages and especially Lactobacillus phages. Six other groups are genera *Baoshanvirus*, *Twortvirus*, *Sciuriunavirus*, *Sepunavirus*, *Silviavirus* and *Kayvirus*, and they specifically have staphylococcal phages. However, they all possess large linear double-stranded DNA genomes of about 125-170 kbp. The Herelleviruses have open reading frames (ORFs) of about 165-301, long terminal repeats (3-16 kbp), 29.97-30.60% GC content and 0-24 tRNAs

(Barylski *et al.,* 2020; Cui, Guo, *et al.,* 2017). *Baoshanvirus* are phages that destroy only *S.aureus* bacteria strains. They have a genome size of about 142.9-149.2 kbp, 201- 210 coding sequence (CDS) and 1 tRNA. Genus *Twortvirus* currently has Staphylococcus phage Twort species as the only members. They have 130 kbp genomes with 195 CDS void of tRNAs, and a GC- content of about 30.3 to 30.6% (Łobocka *et al.,* 2012). *Sciuriunavirus* are Staphylococcus phages targeting only *S. sciuri* and they possess genomes with about 139.6 kbp, encoding 202 genes and 0 tRNAs (https://blast.ncbi.nlm.nih.gov/Blast.cgi). While the *Sepunavirus* have genomes of approximately 139-142.6 kbp, encoding 200- 208 putative ORFs. Genes are tightly packed in the genome, occupying almost 90% of it and do not encode for tRNAs (Gutiérrez *et al.,* 2015; Melo *et al.,* 2014).

Unlike *Baoshanvirus,* phages of genus *Silviavirus* are smaller having genomes averaging 131.3-138.3 kbp, encoding 189 putative genes and with a single tRNA (Vandersteegen *et al.,* 2013). *Kayvirus* is dominant genus in the *Twortvirinae* subfamily and are distinguished by large genomes (140-151.6 kbp) encoding 200-254 putative ORFs. In addition, they have long terminal repeats (8-12 kbp), low and 3-5 tRNAs (Abatángelo *et al.,* 2017; Crane *et al.,* 2020; Gill, 2014; Haddad *et al.,* 2014; Philipson *et al.,* 2018).

Siphoviridae family has four genera exclusively made up of staphylococcal viruses and they are *Biseptimavirus, Fibralongavirus, Phietavirus, Sextaectvirus* and *Triavirus*. Siphoviruses genomes are often 39-47 kbp (Oliveira *et al.,* 2019; Yazdi *et al.,* 2019; Zeman *et al.,* 2019). However, their some siphoviruses either smaller or large genomes. Staphylococcus phage HOB 14.1 and Staphylococcus phage 6ec have genome size ≈ 18.66 kbp and 93.79 kbp respectively (Aswani *et al.,* 2014; Lassen *et al.,* 2017). Siphoviruses are void of tRNA genes like phages in *Podoviridae* and a few viruses in *Herelleviridae* families. These phages are distinct from others by having high GC contents (31.0-34.99%) (Coombs *et al.,* 2020; Daniel *et al.,* 2007). *Caudovirilae* viruses have a general genomic architecture that consists of DNA replication, DNA packaging, structural (head and tail proteins) and lysis functional

modules. However, siphoviruses are unique by possessing lysogen units (Xia & Wolz, 2014). Staphylococcal phages from the family *Herelleviridae* and *Podoviridae* are free of lysogeny genes but rich in hypothetical proteins (Cha *et al.,* 2019; Oduor *et al.,* 2019).

Lysogeny modules facilitate staphylococcal siphoviruses cohabitation with their host bacteria as prophages (Fernández *et al.,* 2018). The viruses are capable of endless replication with the host cells without producing virions. This association does only end when the environmental conditions get unfavourable for the relationship to continue (like exposure to antibiotics, pH and nutrients changes) (Howard-Varona *et al.,* 2017). In addition, the genes enhance bacterial virulence, enables the bacteria to jump into various host and survive harsh environmental conditions (Howard-Varona *et al.,* 2017; Kashif *et al.,* 2019; Xia & Wolz, 2014).

Siphoviruses such as phages ϕ11 and ϕ80α fortifies *S.aureus* survival in various environments by inducing biofilm formation (Fernández *et al.,* 2018). The phages facilitates bacterial aggregation on food industry surfaces (like preparation tables and milk tanks), implanted devices and on biological surfaces like human tissues (Khatoon *et al.,* 2018). Consequently making the bacteria highly infectious, more virulent, and insensitive to high temperatures and drugs (Y. Liu *et al.,* 2020; Moormeier & Bayles, 2017). The biofilm in turn enables environmental persistence and dissemination of the bacteria to humans and live stocks/pets (Bernier-Lachance *et al.,* 2020). ϕ11 and ϕ80α phages induces biofilm formation by upregulating production of polysaccharide intercellular adhesion (PIA). The polysaccharide is the main constituent of the extracellular matrix of *S.aureus* biofilms. In addition, the viruses downregulates several genes encoding dispersion factors such as proteases (*sspA, splF, splC, splB* and *splA*) or surfactants (*hld*) to keep bacteria aggregated (Fernández *et al.,* 2018).

Host jumping or adaptation to new environments is one characteristics staphylococcal bacteria have perfected over the years. The consequent has been the rapid emergence of livestock-acquired methicillin resistant *S. aureus* (LA-MRSA)

strains across the world (Anjum *et al.,* 2019). The host jumping capability has been associated with staphylococcal bacteria siphovirus infections. The phages possess genes like *fnbA* and *clfA* that generates adhesion proteins for attachment to host, for example humans when bacteria jumps from pets/live-stock animals (Fernández *et al.,* 2018; Laumay *et al.,* 2019).

As the bacteria shelter these viruses, they in turn provide protection to the host microbes against antibiotics and other invading phages (Haaber *et al.,* 2016). They express phage repressor protein that inhibit the transition from temperate to lytic and infection of the host bacteria with another competitive phage (Bondy-Denomy *et al.,* 2016; Davies *et al.,* 2016). Multi-drug resistance among staphylococcal bacteria has been the outcome of direct association with mobile genetic elements such as siphoviruses. In addition, these viruses have a major influence on the pathogenicity of the *S. aureus* strains (Moon *et al.,* 2016; Xia & Wolz, 2014). To enhance drug resistance the viruses carry genes encoding drug resistant or pathogenicity islands (such as staphylococcal cassette chromosome *mec* (SCCmec)) from one bacterium to another. The SCCmec in certain circumstances does harbour multiple drug resistant encoding genes, resulting to MDR-*S. aureus* strains (Monecke *et al.,* 2016). The virulence of these bacteria is also determined by these cassettes and subsequent diseases caused by the pathogens. Lethal genes transferred by the SCCmec include; Panton-Valentine leucocidin (*lukSF*), exfoliative toxin (ET), cell-wall anchored protein SasX and immune evasion cluster (IEC) genes (Xia & Wolz, 2014).

Skin and soft tissue infections namely furuncles, abscesses and otitis. In addition, serious diseases such as necrotising pneumonia and osteomyelitis are all associated with *lukSF* gene rich *S. aureus* (Hoppe *et al.,* 2019). There are four serotypes of exfoliative toxin (*et*) genes (*eta, etb, etc and etd*) but only *eta* and *etb* genes are known to induce staphylococcal infections in humans. These toxins are the tools used by *S. aureus* to initiate and sustain scalded skin syndrome in infants, and young children (Mohseni *et al.,* 2018). The toxin transferred from one staphylococcal strain to another by Sa1int phages. Staphylococcal bacteria carrying SasX genes are

associated with nasal colonization and subsequent fatal invasive infections. Also, it assists the microbe to evade activities of the human innate immunity. SasX are disseminated by phiSPß phages (Nakaminami *et al.,* 2017). Another gene that aids in human immunity evasion is the IEC gene that encodes for staphyokinase (*sak*), chemotaxis inhibitory protein (*chp*), staphylococcal complement inhibitory protein (*scn*)/Staphylococcal Complement Inhibitor (SCIN), staphylococcal enterotoxins (SEs) (Fisher *et al.,* 2018; Pietrocola *et al.,* 2017; Xia & Wolz, 2014).

SAK/*sak* degrades bactericidal properties of human antibacterial peptides α-defensins and LL-$_{37.}$ Moreover, it degrades the human immunoglobulin G (IgG) and human C_3b. In addition, the bacteria do inhibit complement proteins activities by using SCIN proteins to deactivate C_3bBb convertase of the alternative pathway. To ensure their safety *Staphylococcus* bacteria use *chp* to inhibit neutrophil cells' chemotaxis by blocking the functions of their receptors (C5a and formylated peptides). Consequently enabling the bacteria to escape the host innate immune system (Pietrocola *et al.,* 2017). The bacteria express their virulence in the host using the enterotoxins. Pneumonia, toxic shock syndrome, and food poisoning are the human diseases associated with the staphylococcal enterotoxins (Fisher *et al.,* 2018). The IEC genes are ferried from one bacteria to another mainly by the ΦSa3int prophages. This process is referred to as transduction and is a form of horizontal gene transfer (Haaber *et al.,* 2017; Sieber *et al.,* 2020). However, the host bacteria can pass these virulent genes to their progenies through vertical gene transfer.

2.7 Emergence of Multi-drug resistant S. aureus (MDRSA)

These bacteria are highly contagious and pathogenic in humans and animals. Transmission of the *S. aureus* is either from humans, animals or from human to animals (Ballhausen *et al.,* 2017). Therefore, *S. aureus* infections pose a serious threat to global food security and public health (Minarini *et al.,* 2020; World Health Organization, 2017a). Since they are easily contracted within hospital set-ups and in the community (Gnanamani *et al.,* 2017). The *E. coli, Salmonella* spp, and *Shigella*

spp are all acquired via oral-fecal route by ingesting contaminated water or food (Hodges & Gill, 2010; Majowicz *et al.*, 2010). Meanwhile *S. aureus* infections are transmitted through contact with infected animals (Hau *et al.*, 2018) and inhalation of dust loaded with the bacteria (Kozajda *et al.*, 2019). *S. aureus* is further contracted by getting in contact with contaminated inanimate objects such as animal/human beddings or ingesting food harboring the bacteria (Venkatesh, 2018). The pathogen is known to cause serious skin and soft infections in both humans and livestock (Abrahamian *et al.*, 2019; Krukowski *et al.*, 2020). In human *S. aureus* is an etiological agent of various diseases such as pneumonia, bacteremia/sepsis, bacterial hepatitis, osteomyelitis and meningitis (Al-Obaidi & Desa, 2018; L. S. Miller *et al.*, 2019; Sharifipour *et al.*, 2020; Vlaeminck *et al.*, 2020). The mortality rate of *S. aureus* infections due to drug resistant strains has been estimated to be between 10% and 30% (Tom *et al.*, 2014; Yilmaz *et al.*, 2016).

However, there are only a few safe and effective new antibiotics against the MDRSA. In addition, only a handful pharmaceutical firm are currently engaged in the search for new types of antibiotics with novel antibacterial mechanisms (Plackett, 2020). Currently all antibiotics are based on the old antibacterial molecules with identical mode of actions against pathogenic bacteria. These antibiotics include protein, DNA/RNA and cell wall synthesis inhibitors (O'Rourke *et al.*, 2020). The similarity in mode of action restricts the antibiotics to these few bacterial targets and in case a bacterium modifies them, it becomes resistant to all drugs within a specific class. This property has been exploited by most strains of bacteria like *S. aureus* to acquire resistance to β- lactam drugs such as penicillins, cephalosporins, carbapenems and monobactams (Foster, 2017). These facts affirm the urgency for novel antibacterial agents with different modes of action against multidrug resistant bacteria like MDRSA. Phages have shown high efficacy against all strains of bacteria including the multidrug resistant isolates (Fish *et al.*, 2016; Kutateladze *et al.*, 2016). This is possible due to the viruses' ability to co-evolve with the bacteria in the environment. Though, there are bacterial strains that develop resistance against phages (Seed, 2015). This hostile status often select for the most virulent phages to

tackle phage-resistant bacteria to ensure their continuity (Maxwell, 2016; Stern & Sorek, 2011).

The selected phages often have modified biosynthetic activities to enable them bypass bacteria's defense mechanisms such as adaptive and innate immunity (Samson *et al.,* 2013). The bacteria can defend against phages by changing or eliminating the phage receptor to prevent phage adsorption, by blocking the genetic material of the invader phage, or by restriction endonucleases that digest the phage DNA (Shabbir *et al.,* 2016). They are the innate immunity mechanisms of the bacteria as they are functionally similar to animal immunity. The adaptive immunity of bacteria, in turn, involves the use of clustered regularly interspaced short palindromic repeats (CRISPR); acquired from the past successfully defeated invading phages (Bonsma-Fisher *et al.,* 2018). The defense and counter-defense mechanisms are protein-driven which shows the need for phages' regular update of their genomes to synthesis appropriate proteins for invading perceived host bacteria. These proteins often evolve from "hypothetical genes" within the phage genomes. The phenomenon has been observed in lytic phages effective against generally *Lactococcus lactis* bacteria resistant to numerous phages within its environment (McGrath *et al.,* 1999).

In phage therapy whenever a phage resistant bacterium emerges another phage strain effective against it is often isolated from the environment (Mattila *et al.,* 2015). This calls for continuous upgrade of phage bio-banks and this approach has been practiced for years in former USSR and Russia to enhance effective phage therapy (Weber-Dąbrowska *et al.,* 2016).

2.8 Phage therapy against staphylococcal infections

In recent years, phage therapy against staphylococcal infections has gain momentum across the continents. Numerous safety and efficacy animal experimental studies on staphylococcal infection treatment with phages have preceded these applications. Experimental studies with animal models proved that phage therapy is not only safe,

but also effective. In addition, certain staphylococcal phages were observed to be strain specific (Pincus *et al.,* 2015). Results of studies focused on acute and chronic lethal *S. aureus* infections demonstrated that Staphylococcus phages were effective against the pathogen (Kishor *et al.,* 2016; Oduor *et al.,* 2016; Takemura-Uchiyama *et al.,* 2014). In certain instances single doses were sufficient to resolve the infections (Kifelew *et al.,* 2020; Ngassam-Tchamba *et al.,* 2020; Oduor *et al.,* 2016). These findings have cast out fears and doubts laid on the application of phages in veterinary and human medicine.

Numerous compassionate phage treatment done in Western Europe and North America were successful. Staphylococcus phages have been used to resolve MRSA corneal infections, diabetic foot ulcer, endocarditis and septic shock (Fadlallah *et al.,* 2015; Fish *et al.,* 2016; Petrovic Fabijan *et al.,* 2020). In either of the case reports, there were no report of adverse reactions such as diarrhoea, tachycardia, fever, hepatic/renal dysfunction, and inflammation.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

Sites of investigation included Nairobi (Kenya), Helsinki (Finland) and Shkodra (Albania). The study involved many sites to enhance the probability of fast isolation of strict lytic *Staphylococcus* phages. These phages are often difficult to isolate from the environment.

3.1.1 Nairobi- Kenya

Nairobi County (**Appendix I**), the most populous county in the Kenya with an estimated population of about 4.397 million(Kenya National Bureau of Statistics, 2019). The county has an area of 696.1sq Km and it has seventeen sub-counties namely; Westlands, Dagoretti North, Dagoretti South, Langata, Kibra, Roysambu, Kasarani, Ruaraka, Embakasi South, Embakasi North, Embakasi Central, Embakasi East, Embakasi West, Makadara, Starehe, Mathare and Kamukunji (https://nairobi.go.ke/devolution-public-service-administration/). Majority of its inhabitants dwell in slums such as Mathare, Kibera and Mukuru Kwa Jenga, which constitutes 60% of the city's settlement. Slum dwellers have poor access to basic human needs such as water, basic sanitation and health care services (Wamukoya *et al.,* 2020). This make them prone to numerous bacterial infections and in most cases these bacteria are drug resistant strains (Maina *et al.,* 2013; Njuguna *et al.,* 2013). The slums' drainage systems and sewage treatment plants in the county were selected for the study as they provided rich sources of multidrug resistant bacteria.

3.1.2 Shkodra - Albania

In Albania the study was conducted at Shkodra County, the third most populous county in the country with an estimated population of about 88 500 (World Population Review, 2019). Despite having good housing facilities, there are about 40% informal settlement in the City of Shkodra (Morelli *et al.,* 2019). The county has sewage and wastewater treatment plants. However, occasionally there are leakage from these plants into nearby rivers (Rivers Kir, Drin and Buna) and lake

Shkodër resulting to their pollution (**Appendix I**) (Pandi Skaka, 2019). The encriched organic nutrient conditions make the rivers rich in bacterial flora that is ambient for bacteriophage colonization.

3.1.3 Helsinki - Finland

Helsinki is the Finnish capital city situated on a peninsula at the Gulf of Finland. The town is within Uusimaa region and is one of the most populous city in Finland. The city's total surface area is 715.48 sq Km with an estimated population of above 1.4 million (HelsinkiRegion, 2020). The city comprises of eight major districts namely: Southern, Northern, Eastern, Western, Central, Northeastern, Southeastern and Östersundom (Tikkanen & Selander, 2011). The Uusimaa region has good social amenities that includes; proper waste management system, healthcare and residential apartments. The city has a state of art sewage and wastewater treatment plant at Viikki known as Viikinmäki wastewater treatment plant (study site map **Appendix I**). The plant serves Helsinki city and other sections of neighbouring cities like Vantaa, Kerava, Tuusula, Sipoo and Järvenpää (HSY, 2020). The wastewater plant was selected for the study since it was the only suitable place for sampling raw sewage and wastewater.

3.2 Sampling

3.2.1 Sampling design

The selected study sites were sampled using convenient sampling technique. Cluster sampling technique was used to pick the sampling sites within the county. The names of particular areas of the sites to be studied were selected using simple random sampling methods.

3.2.2 Sample size determination

Sampling size was determined using the formula used by (Kothari, 2004).

$$\mathbf{n} = \{\mathbf{Z^2}\,[\mathbf{pq}]\}/\boldsymbol{\ell}^2$$

Where **n** = desired number of water samples to be collected.

(Population is infinite)

Z = standard deviation is usually 1.96 which corresponds to 95% confidence interval.

ρ = the proportion of informal settlement in Shkodra is about 40% and Nairobi the estimate is 60% (Morelli *et al.,* 2019; Wamukoya *et al.,* 2020).

Sample size for Shkodra:

$\mathbf{q = 1 - \rho \rightarrow (}1 - 0.4\mathbf{) =} 0.5$ $\boldsymbol{\ell} = 0.05$ (5% absolute precision)

$\mathbf{n}_{\text{Shkodra}} = \mathbf{\{}1.96^2\,[0.6 \text{ X } 0.4]\mathbf{\}} / 0.05^2$

$\mathbf{n}_{\mathbf{Shkodra}} = \mathbf{368.7936} \geq \mathbf{368.0}$ samples

Sample size for Nairobi:

$\mathbf{q = 1 - \rho \rightarrow (}1 - 0.6\mathbf{) =} 0.5$ $\boldsymbol{\ell} = 0.05$ (5% absolute precision)

$\mathbf{n}_{\mathbf{Nairobi}} = \mathbf{\{}1.96^2\,[0.6 \text{ X } 0.4]\mathbf{\}} / 0.05^2$

$\mathbf{n}_{\mathbf{Nairobi}} = \mathbf{368.7936} \geq \mathbf{368.0}$ samples

The sample sizes were apportioned according to the **ρ**-value of each site. However, in Helsinki the sample size was five (**n = 5**) since the metropolitan does not have a significant **ρ**-value. The city is void of informal settlement. In addition, this was an exploratory study and any sample size above five was significant.

3.2.3 Sample collection and sampling techniques

Environmental waste and river water samples were collected in sterile dark containers from the drainage systems, rivers sewage and wastewater treatment plants of the selected cities. In Nairobi samples came from the drainage systems, river and

sewage treatment plant (Dandora Sewage and wastewater treatment plant). While in Helsinki, the sources were wastewater from a sewage and wastewater treatment plant (Viikinmäki wastewater treatment plant) and compost soil. However, in Shkodra the samples were withdrawn from river(s) Kir in the east, Drin in the south and Buna in west of the city. Water samples were chosen for the study since polluted water are known to be rich bacterial flora (Blaak *et al.,* 2015; Okemo *et al.,* 2013).

Purposive and convenience sampling methods were applied during sample collection. The techniques were chosen to enhance thorough analysis of the samples resulting to precise reproducible outcomes (Ames *et al.,* 2019; Etikan *et al.,* 2015).

3.3 Materials

Bacterial strains: They are as listed in **Appendix II**. The isolates were from American type cell culture (ATCC), Hospital District of Helsinki and Uusimaa Laboratories (HUSLAB-Helsinki-Finland) and pig isolates (Vantaa pig farms-Finland). *S. xylosus* DD 34 that was used as a host strain when isolating the phages is a natural sausage fermenter isolated from dried sausage (Møller *et al.,* 1998). Other strains used were wild *S.aureus* prevously isolated and analysed in Nairobi (Oduor *et al.,* 2016).

Media, reagents and other consumables. The media included mainly; nutrient broth, luria broth (constituents are 10 g of tryptone [MC005, Neogen-USA], 5 g of yeast extract [NCM0218A, Neogen-USA], 10 g of NaCl, and 1 liter of distilled water; adjust the pH to 7.0 with 1 N NaOH)(**LB**) and tryptic soy broth (**TSB**) [MC005, Neogen-USA] and Agar (MC006, Neogen-USA). The reagents were sodium chloride salt, calcium chloride salt, magnesium sulphate, agarose powder, sodium citrate, glycerol, hydrochloric acid, uranyl acetate (U.A), sodium hydroxide, double distilled water, sodium dodecyle sulphate (**SDS**), 30% acrylamide/Bis (#1610154, Bio-Rad), 2× Laemmli buffer (#1610737, Bio-Rad), ammonium chloride, MIDORI^{Green} dye (#MG04 – NIPPON Genetics Europe, Germany), sucrose, InstantBlue™ dye (#ISB1L, Sigma-Aldrich), TEMED, Page Ruler™-Plus preset (#26619, ThermoScientific), Gene Ruler (#SM0313, ThermoScientific), proteinase K

(#AM2546, ThermoScientific), DNase I (#EN0521, ThermoScientific), RNase A (#EN0531, ThermoScientific), restriction enzymes (EcoRV, EcoRI & HindIII) [EcoRI- #FD0274, ThermoScientific; EcoRV- #FD0304, ThermoScientific; HindIII- #ER0505, ThermoScientific], Gel loading dye purple (**6X**) [#B7024S- New England Biolabs], 1M Tris-Cl (pH 7.5), phenol, TM buffer, chloroform, ethanol, sodium acetate, TAE buffer, SM buffer, ammonium persulfate (APS), citrate-phosphate buffer. Other consumables were screw capped conical centrifuge tubes (15 and 20 mL), snap and screw capped eppendorfs (0.5mL and 1.5 mL), weighing boats, gloves, applicator sticks, inoculating loops, petri-dishes, concentrators (Vivaspin tubes,- (Sartorius, 2019)), tips (sterile-filtered and non-filtered), loading dye.

3.3.1 Bacterial cultures

Propagated the *S. aureus* strains (**Appendix II**) on LB agar (LA) plates. The bacteria were incubated at +37 ºC overnight, then a colony from the streaks was sub-cultured and grown as previously described (Oduor *et al.*, 2016). Thereafter, bacterial mass was collected with a sterile plastic loop from the plates and suspended into 20% glycerol nutrient broth from which 200 µl aliquots were distributed into sterile tubes for longer storage at -70ºC.

3.3.2 Phage isolation

Phages from Nairobi were isolated from the sewage and wastewater samples as described elsewhere (Oduor, *et al.*, 2016). Briefly, centrifuged sewage/wastewater sample at 1500 ×*g* to sediment the debris and stored the supernatants at +4 ºC. A colony of the host bacterium *S. aureus* (wild strain) was transferred from an overnight plate to 1.5 mL of LB and allowed to grow at +37 ºC until OD_{600} of 1.0 – 1.5. Added an aliquot (1.0 mL) of the culture into 20 ml of LB supplemented with 5mM $CaCl_2$ and 20 ml of sewage supernatant. Incubated the culture at +37 ºC overnight while shaking at 120 rpm. Added chloroform (200 µL per 3.0 mL of the culture) to the overnight enrichment culture. Thereafter, incubated at room temperature (RT) for 20 min while rocking gently on a rocker machine. After the chloroform-treatment the enrichment culture was centrifuged at 4500 ×*g* for 20 min

to sediment the dead bacteria and other debris. The supernatant was filter sterilized using 0.45 µm syringe-filters and stored at +4 ºC and later shipped to Helsinki for further analysis.

Phages from Helsinki were isolated from sewage water and compost soil samples as previously described. However, the host strain used were a mixture of four MSSA clinical strains (#5523 & #5857) listed in **Appendix II** instead of wild host *S. aureus.*

Phages from Shkodra were isolated from the sewage and wastewater samples was carried out as described elsewhere (Oduor, *et al.,* 2016 & Kadija et. al., Unpublished). Briefly, centrifuged sewage/wastewater samples at 1500 ×*g* to sediment the debris and the supernatants stored at +4 ºC. A colony of the host bacterium *S. xylosus* DD-34 was transferred from an overnight plate to 1.5 mL of LB and allowed to grow at +37 ºC until OD_{600} of 1.0 – 1.5. A 1 ml aliquot of the culture was mixed with 20 ml of 10× nutrient broth supplemented with 20mM $CaCl_2$ and 20 ml of sewage supernatant. Incubated the culture overnight at +37 ºC while shaking at 120 rpm. Added chloroform at 200 µL per 3.0 mL of the overnight enrichment culture, and incubated at room temperature (RT) for 20 min while rocking gently on a rocker machine. After the chloroform-treatment the enrichment culture was centrifuged at 4500 ×*g* for 20 min to sediment the dead bacteria and other debris. The supernatant was filter sterilized using 0.45 µm syringe-filters and stored at +4 ºC and later shipped to Helsinki for further analysis.

3.3.3. Soft agar spot assay

Detected the presence of phages in the filtrates was using spot assay. A bacterial overlay was prepared by mixing 0.2 mL of host bacteria (OD_{600} 1.0-1.5) to 3.0 mL molten 0.3% soft agar maintained at 55 ºC. This mixture was immediately poured on pre-warmed LA plates and allowed to solidify. The phage suspensions were ten-fold serially diluted with sterile PBS, 5 µL drops of different dilutions pipetted on the solidified soft-agar, and allowed to adsorb for 30 minutes. Thereafter, incubated the plates at +37 ºC overnight and observed for lysis zones under the drops.

3.3.4. Plaque purification of phages

Positive lysates were ten-fold serially diluted up to 10^{-10}, and 100 µL aliquots of 10^{-5} -10^{-10} dilutions were mixed with 0.2 mL of host-bacteria and added into 3.0 mL molten soft agar and poured on pre-warmed LA plates. Once cooled the plates were incubated overnight at +37 ºC and the plaques observed the following day and counted to determine the plaque forming units (pfu). Picked single separated plaques with clear plaque-morphology, from the plates using a Pasteur-pipette. Transferred the agar plugs into 0.5 mL SM buffer for the phages to diffuse out of the agar, overnight at +4 ºC. The phage titre was determined by spot assay and the plaque purification repeated 3-4 times to make certain that a phage prepared of a single phage was reached. Thereafter, phage stocks with high titres were prepared.

3.3.5. Preparation of phage stocks using semi-confluent double-layer plates

Several LA plates were pre-warmed, fresh host bacteria culture prepared to an OD_{600} of 0.5 – 1.5 and 0.3% soft agar melted and cooled to +50 ºC. Appropriately diluted phage lysates to achieve plates with semi-confluent plaques. Then added 0.04 mL of the diluted phage to 0.2 mL of host bacteria. Afterwards dispensed it into a tube with 3.0 mL molten soft agar. The tubes were then rapidly but gently vortexed and the soft agar poured on dry LA plates. Incubated the plates at +37 ºC for 16 hrs, after cooling them for 30 min on the table. The plates with semi-confluent plaques were flooded with 3 mL of SM-buffer and rocked gently for 2.0 hrs. The soft agar and the remaining fluid was transferred into a sterile 15 ml centrifuge tube, 0.2 mL of chloroform was added for every 3.0 mL, and the tube rocked at RT for 20 min. Thereafter, centrifuged the tubes at 4500 ×*g* for 15 min., and the supernatants filtered through 0.45 and 0.22 µm syringe filters. The phage samples were further purified to remove chloroform traces as described by Invisorb® Spin Virus DNA mini kit (Stratec SE, 2019). Finally, added 0.6 mL of 40% sucrose for every 3.0 mL of the filtered lysate.

3.3.6. Phage titration

The phage lysates were ten-fold serially diluted with SM-buffer up to 10^{-10} dilution. One hundred µL aliquots of the 10^{-6} – 10^{-10} dilutions were analyzed using the soft-agar overlay method described in **3.3.5**. The plaques on plates were counted and counts between 30 and 300 pfu were used to determine the titre. The formula below was used for calculation:

$$\text{Phage titer (}\mathbf{pfu/mL}\text{)} = \{\text{pfu}\} / \{0.1\ \text{mL} \times \text{lysate dilution factor}\}$$

The lysates with titers above 10^{10} pfu/mL were stored at +4 ºC for further analysis.

3.4 Characterization of phages

3.4.1 Morphological analysis

15 mL of an overnight lysates with titers above 10^{7} pfu/mL were prepared as indicated above (**3.3.5**) and concentrated to 0.5 mL. Performed lysate concentration using 6.0 mL Vivaspin® concentrators with 100 000 molecular weight (M.W) cut-off, at +4 ºC, 4500×*g*. Thereafter, washed phage concentrates three to four times with 2.0 mL SM-buffer. Afterwards, pelleted the phages at +4 ºC by centrifugation at 16100×*g* for 90 min. Then, re-suspended them into 450µL 0.1 M ammonium acetate.

A 3.0 µL droplet of the phage sample was loaded onto a copper-carbon grid (diameter, 3 mm; 300 meshes) and allowed to adsorb for 60 seconds. Dried the grids with blotting papers, and stained them with 3.0 µL 2% uranyl acetate (pH 7.4). Thereafter, dried them for 15 to 30 seconds. The observation, micrography and dimension estimation of the phages was done with JEOL JEM-1400 TEM (Jeol Ltd., Tokyo, Japan) fitted with a bottom mounted Gatan Orius SC 1000B camera (Gatan Inc., USA). The TEM ran at 80Kv with a magnification power of 8,000-150,000. The work was performed at the Electron Microscopy Unit (Institute of Biotechnology, University of Helsinki-Finland). The phage particle dimensions of five to ten virions

were determined and the measurements used to calculate the averages and standard errors (Oduor *et al.,* 2020).

3.4.2 Genome analysis

3.4.2.1 DNA isolation

Phenol-chloroform extraction method was used for DNA isolation and it was performed as described elsewhere (Green & Sambrook, 2017). Briefly, fresh 10.0 mL lysates with phage titer of at least 10^9 pfu/mL were prepared as described above (**3.3.5**), then concentrated to 0.4 mL as mentioned earlier (2.4.1) and transferred into 1.5 mL microtubes. 1.3 µL DNase I (IU/µL) and 4.0 µL RNase A (1.0 mg/mL) were added to the tubes containing the phages and incubated at +37 ºC for 30 min to digest bacterial DNA and RNA. Thereafter, 16.0 µL 0.5M EDTA, 1.2 µL Protenase K (20.0 mg/mL) and 20.0 µL 10% SDS were added to the above mixture and incubated at +56 ºC for 60 min to degrade the phage capsids. After cooling the samples to RT 1 VOL phenol (pH 8.0) (volume equivalent to the sample) was added and gently rocked/mixed for 15 mins using a rocker machine. This was followed by centrifugation at 16100 × *g* at RT for 5 min to sediment bacterial debris and other dirt. The clear aqueous upper phase was transferred into a new microtube for each sample. 1 VOL of phenol-chloroform (1:1) was added to the harvested aqueous samples and mixed as previously described for 15 min followed by 5 min centrifugation at 16100 × *g* at RT. The aqueous upper phase was again collected and transferred to new microtubes. This procedure was repeated until a clear upper phase aqueous supernatant was emerged.

Finally, the aqueous sample was extracted with 1 VOL chloroform followed by DNA precipitation that was achieved by adding 0.1 VOL 3M NaOAc (pH 7.0) and 2 VOL absolute EtOH to the samples. The precipitated DNA formed a thread that was transferred into new 1.5 mL microtube containing 1.0 mL 70% EtOH and centrifuged at RT, 16,100 × *g* for 20 min. The supernatant was discarded, the DNA pellet air-dried for 5 min, dissolved in 0.1 mL TE buffer (10.0 mM Tris-HCL, 1.0 mM EDTA, pH 8.0) and incubated at + 4 °C overnight. The DNA quantity and

quality was determined by NanoDrop1000 Spectrophotometer (Thermo Scientific, 2019) and finally the quantification was also carried out using Qubit® 2.0 fluorometer as described by the instrument's manual (Qubit ThermoFisher, 2010).

3.4.2.2 DNA analysis by Agarose gel electrophoresis

The genomic DNA of the phages were characterized by restriction enzyme digestions. Briefly, phage 1.0 µL of DNA (approximately 300-600 ng/ µL), 10 × digestion buffer (1.0 µL), restriction enzyme (EcoRI, EcoRV or HindIII) (0.5 µL), and nuclease free water (7.5 µL) were mixed in a microtube for each enzyme and incubated at +37°C for 1.0 hr. Addition of 2.0 µL of Gel loading dye purple (**6X**) stopped the digestion. The samples and appropriate controls together with Gene Ruler were then loaded into the wells of a stain free 1% agarose gel impregnated with Midori green dye. Then separated DNA fragments at 65 kV, 200 mA for 150 mins. Thereafter, the stained gel for 20 min with Midori green dye and rinsed for 30 min with double distilled water. Thereafter, visualized using the Bio-Rad XR+ gel documentation system (Bio-Rad, 2020).

3.4.2.3 Next generation sequencing

Good quality genomic DNA of the phages was shipped for next generation sequencing (NGS) to Eurofins Genomics company (https://eurofinsgenomics.eu/) (Eurofins, 2019). The sequence reads received from NGS analysis were *de novo* assembled by A5-miseq pipeline [https://docs.csc.fi/apps/] (Coil *et al.,* 2015). The physical ends of the linear phage genomes were determined using the PhageTerm tool [https://galaxy.pasteur.fr] (Garneau *et al.,* 2017). Using this information, and read-coverage visualization using the Integrated genome viewer (IGV) [download: http://software.broadinstitute.org/software/igv/] (Robinson *et al.,* 2017) the phage genome sequences were re-arranged to reflect the physical form of the DNA packaged into phage particles. The genomes were submitted to rapid annotations-using-subsystem-technology (RAST)-server (Aziz *et al.,* 2008) to achieve preliminary prediction of the phage genes. The RAST annotations were manually verified using the Artemis tool (Carver *et al.,* 2008). The predicted functions of the

gene products were later confirmed and revised using the BLASTP, smart BLAST, PSI-BLAST (acceptable results threshold; query-cover: 90-100%, E-value: 1e-3 and percentage identity: 90-100%) (BLAST, 2019), InterProScan [https://www.ebi.ac.uk/interpro/search/sequence/] (Jones *et al.,* 2014; Quevillon *et al.,* 2005), HHpred (cutoffs used for considering meaningful results were, probability:90-100% and E-value:1e-3) and HMMERscan (reporting threshold at E-value:1e-3) [https://toolkit.tuebingen.mpg.de/tools/hhpred] (Zimmermann *et al.,* 2018) search tools. The tRNA genes were predicted with the aid of ARAGORN [http://www.ansikte.se/ARAGORN/] (Laslett & Canback, 2004) and tRNAscan-SE [http://lowelab.ucsc.edu/tRNAscan-SE/] (Lowe & Chan, 2016). Promoter genes were predicted using PePPER [http://genome2d.molgenrug.nl/]. While ARNold [http://rssf.i2bc.paris-saclay.fr/toolbox/arnold/] was used to predict for terminator genes (Gautheret & Lambert, 2001). Thereafter, promoter motif consensus of the phages were probed by MEME/MAST [https://meme-suite.org/meme/] (Bailey & Elkan, 1994). The genomes were screened for antibiotic resistance, virulence factor and toxin encoding genes using the antibiotic resistance database (ARDB) [http://arpcard.mcmaster.ca] (B. Liu & Pop, 2009) and ResFinder 3.2 systems [https://www.genomicepidemiology.org/] (Zankari *et al.,* 2012).

3.4.2.4 Phylogenetic analysis

The close relatives of the phages were identified using BLASTN search of the GenBank (NCBI GenBank, 2019). The similarity and alignment of the genomes was established using the EMBOSS stretcher tool [https://www.ebi.ac.uk/services/all] (Madeira *et al.,* 2019). The genomes and the reference phage genomes were also aligned and visualized using progressiveMAUVE [download: http://darlinglab.org/mauve/mauve.html] (Darling *et al.,* 2010). The VICTOR [https://ggdc.dsmz.de/home.php] (Meier-Kolthoff & Goeker, 2017) and Phylogeny fr-"One Click " Mode [http://phylogeny.lirmm.fr/phylo_cgi/index.cgi] (Dereeper *et al.,* 2008) tools were used to construct the phylogenetic trees of the phage genomes and of the selected phage proteins.

3.4.3 Proteomics of the phages

The phages were concentrated by centrifugation for 30 min at 4 °C and 4500 ×*g* using 100 kDa molecular weight cut-off Vivaspin concentrator® 20 (Sartorius, 2019). The phages were further purified by through glycerol step gradient (5% and 40% glycerol concentration v/v in TM-buffer) as described (Sambrook & Russell, 2006). The resulting phage titres were each > 6×10^{10} pfu/mL. Thereafter, diluted the phage stocks appropriately to get a final concentration of 6×10^{8} pfu/mL. Then 20 µL of the diluted phage was mixed with 20 µL of 2× Laemmli buffer (loading buffer) and heated at 100 °C for 5 min. Ten µL of the heated ice-cooled sample were loaded to the wells of 10% SDS-PAGE with 5% stacking gel, and the electrophoresis was run at 80 V for 2 hr 50 min. The gel was stained for 3 hrs with InstantBlue dye. Thereafter, rinsed with milli-Q water (double distilled sterile water) and imaged with Bio-Rad gel-imaging system.

Analysed the proteomes of the purified phage particles using the liquid chromatography tandem mass spectrometry (LC-MS/MS) method. Prior to trypsin digestion of the proteins to peptides, tris (2-carboxyethyl) phosphine (TCEP) reduced the phage proteins and afterwards alkylated with iodoacetamide. Tryptic peptide digests were purified by C18 reversed-phase chromatography columns (Varjosalo *et al.*, 2013) and the mass spectrometry (MS) analysis was performed on an Orbitrap Elite Electron-Transfer Dissociation (ETD) mass spectrometer (Thermo Scientific, Waltham, MA, USA), using Xcalibur version 2.2, coupled to a Thermo Scientific nLC1000 nanoflow High Pressure Liquid Chromatography (HPLC) system. Peak extraction and subsequent protein identification were achieved using Proteome Discoverer 1.4 software (Thermo Scientific). Calibrated peak files were searched against all the predicted amino acid sequences of the Stab20, Stab21, Stab22 and Stab23, and *Staphylococcus aureus* subsp. aureus ST398 proteins (ASM188707v1, NCBI) by the SEQUEST search engine. Error tolerances on the precursor and fragment ions were ±15 ppm and ±0.8 Da, respectively. For peptide identification, a stringent cut-off (0.05 false discovery rate or 5%) was used. Performed LC-MS/MS

experiments at the Proteomics Unit, Institute of Biotechnology-University of Helsinki.

3.4.4 Nucleotide sequence accession numbers

The annotated sequences of the Stab20, Stab21, Stab22 and Stab23 phages are deposited at the nucleotide sequence databases under the accession numbers LR215718, LR215719, LR215720, and LR215721, respectively.

3.5 Methods to assay physico-chemical properties of phages

All the physico-chemical property experiments were done in triplicates to ensure reproducibility and statistical significance.

3.5.1 Thermal stability

Fresh phage lysates were prepared from the stocks and their pfu/mL count determined as indicated previously described (2.3.6). Working stocks with 10^9 pfu/mL were prepared for each phage. Aliquots of the phages were incubated at +35°C, +37°C, +40°C, +45°C, +50°C, +55°C and +60°C for 30 min. The phages incubated at +37°C were used as control samples. Cooled the samples to RT and afterwards viable phage particles were enumerated by the double-layer assay. The pfu values were counted after 18 hr incubation at +37°C using the colony/plaque counter machine (Stuart Scientific SC5). The pfu of the control plates (+37°C) were set to 100% and the values of the other samples were normalized to it to establish the effect of various temperatures on the phages.

3.5.2 Ultra-violet (UV) stability

Working stocks with 10^9 pfu/mL were prepared as described above and 200 µL aliquots dispensed into microtiter plate wells for irradiation. The energy applied on the phages were 0, 25, 50, 75, 100, 125 and 150 µJ/cm^2 for 30 min. In this experiment, 0 µJ/cm^2 was used as the positive control and PBS as the negative control. The phage numbers were enumerated as described above (2.5.1). The pfu of

the non-irradiated samples was set to 100% and the values of the other samples were normalized to it.

3.5.3 pH stability

Buffers with the pH-values of pH 1.4, 3.4, 5.4, 7.4, 9.4, 11.4 and 12.9 were prepared as follows. The low pH (1.4 to 5.4) buffers were prepared using citric acid monohydrate and adjusted with 1.0 M sodium hydroxide. The higher pH (9.4 to 12.9) buffers were made using sodium bicarbonate and adjusted with 1.0 M HCL. PBS was used as a neutral medium for pH 7.4, and was used as the control diluent for this experiment. Thereafter, 100 µL of the phage lysate was added to 900 µL of either PBS, acidic or alkaline medium. The mixtures were incubated at +37°C for 1 hr shaking at 120 rpm, serially diluted to 10^{-5} and pfu was enumerated as described above (2.5.1.). The pfu of the pH 7.4 samples was set to 100% and the values of the other samples were normalized to it.

3.5.4 Chloroform stability

Since chloroform is not water-soluble, one part of the phage working stocks (10^9 pfu/mL) was mixed with nine parts of chloroform and the mixture was vortexed and incubated at +37°C for 1 hr shaking at 120 rpm. For the positive control the phage stock was mixed with nine parts of PBS. Then the samples were serially diluted to 10^{-3} and 5 µL drops were spotted on double-agar plates with host bacteria. The plates were incubated at +37°C overnight and observed for lysis zones under the drops.

3.5.5 Ethanol stability

Ethanol solutions of 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70% and 80%. (vol/vol %) were prepared. Phage stocks were diluted as described in (2.5.3) and 1 part of the diluted stock was mixed with 9 parts of the different ethanol solutions to give 10^{-1} diluent factor. The mixtures were vortexed and incubated at +37˚C, 120 rpm for an hour. The samples were diluted to 10^{-3} for each sample and 5 µL drops were spotted double-agar plates with host bacteria, and allowed to adsorb for 30 min. The plates were incubated at +37°C overnight and observed for lysis zones under the drops. Phage mixed with PBS acted as the positive control, and

sterile PBS, as the negative control. Experimental outcomes were recorded as either positive or negative.

3.6 Biological properties of the phages

The biological properties of the phages were characterized with adsorption rate and growth curve assays. Growth characteristic (life cycle) experiments begun by first determining the phages' adsorption rates and afterwards the burst size using the one step growth curve (O.S.G.C) experiment.

3.6.1 Adsorption curve

The experiment was set up by using a fresh host bacterial culture at an OD_{600} of 0.5 – 1.0, and a serially ten-fold diluted phage sample of predetermined titre. Briefly, 500 µL of host bacteria (*S. xylosus*) was sub-cultured in 5 mL fresh sterile LB and incubated at +37 °C to an OD_{600} of 0.5 to 1.0. Then the culture was pelleted through centrifugation at 4500 ×*g*, the supernatant was discarded, and the pellet resuspended in 0.9 mL fresh LB. Thereafter, added 100 µL of 10^{-5} dilution of phage lysate to an experimental tube (A) and to a control tube (B). The control tube only contained 0.9 mL of fresh LB medium. Incubated **A** and **B** at +37°C, 120 rpm for 15 min and sampling of 50 µl aliquots at 5 min intervals from each tube. Dispensed the aliquot into pre-chilled microtubes. The samples were briefly vortexed then centrifuged at 16,100 × *g* at +4 °C for 10 min. 50 µL of the supernatant was added to 200 µL host bacteria in 3 mL molten soft agar media tubes previously maintained at 50°C. The mixtures were briefly vortexed, dispensed on pre-warmed LB agar plates and allowed to set (solidified) at RT (also called double plaque layer assay). Thereafter, the plates were incubated at +37 °C overnight and the experiment was run in triplicates. Plaques were counted from the plates and recorded at their respective time points (from 0 min to 15 min) on excel sheets. PFU from control tubes (tube B) were used as time point 0 min reference points. The values were normalized by having the average PFU of tube B representing 100% and calculating the ratio pfu count at various time points in reference to pfu counts of tube B. The outcomes of these ratios

were multiplied with 100% and the data presented in curves and adsorption rate (***k***) calculated using the formulae below. Where ***B*** represented the bacterial titre**,** ***t*** time after infection**,** P_0 initial number of phages/ plaque counts and ***P*** number of phage unadsorbed (post adsorption plaque count) (Vandersteegen *et al.*, 2013).

$$k = \{[(2.3/Bt)]\ [\log (P_0 /P)]\}$$

3.6.2 One step growth curve (O.S.G.C)

Experiments were set up by first establishing the phages' multiplicity of infection (M.O.I). Briefly, 50 µL of an overnight culture (OD_{600} of 1.0 - 1.5) of the host bacteria (*S. xylosus*) was diluted to 5.0 mL of fresh LB and grown for 60 to 120 min to reach a desired OD_{600} of about 0.8. The culture was serially ten-fold diluted to 10^{-6} in PBS (pH 7.4), and then 100 µL aliquots of the dilutions were spread on LA plates. The plates were incubated at +37 °C overnight and the colonies counted the following day to determine the bacterial numbers as colony forming units (CFU) per mL The remaining culture was pelleted at 5000 rpm for 10 min and resuspended in 0.9 mL LB. 0.1 mL of appropriately ten-fold serial diluted phage lysate was added to 0.9 mL of host bacteria in tube A. Similarly, another 0.1 mL of lysate was dispensed to 0.9 mL LB without bacteria (blank control) in tube B. Tubes A and B were incubated at +37 °C for 10 min. The cultures were then centrifuged at +4 °C, 16,100 × *g* for 10 min. Supernatant in tube A was harvested into a sterile tube Y, and 1.0 mL of fresh LB was added to the pellet (now tube Z1). Aliquots of 50 µL were collected from tubes B and Y, and added to the host bacteria in soft agar tubes. After gentle mixing, the mixture was poured on warm LA plate, and allowed to set before incubating at +37 °C overnight. The plaques were counted from each plate the following day. The difference between the plaque counts in tubes B and Y represented the number of adsorbed phage particles. Number of adsorbed phage particles (pfu/mL) was divided by the CFU/mL and the obtained value defined the

M.O.I (PFU/CFU). One step growth experiment continued with tube Z1 and two more tubes (Z2 and Z3). Z2 and Z3 tubes had 0.9 mL LB and were incubated concurrently with Z1 which had bacteria pellet infected with phage. Incubation took place at +37 °C, 120 rpm for 60 min. There was sampling at every 5 min interval. However, at 5 min after the incubation begun two samples were picked; first 100 µL sampling that was dispensed to tube Z2 and second 50 µL which was picked for double plaque layer assay. After 10 min another pair of sampling was done but from different tubes. A 50 µL aliquot of Z1 was taken for double plaque layer assay and 100 µL pipetted from Z2 to Z3. Sampling continued from Z1 until 30 min time point, at this time point onwards sampling took place from Z1 and Z2; all for double layer assay. Z2 provided double layer assay samples from time point 30 min to 40 min. However, at 40 min there was a pair of sampling; with one from tube Z2 and another from Z3. Thereafter, between 45 min and 60 min sampling was carried out only from tube Z3. The double layered plates cooled at RT for 30 min before being incubated at +37 °C overnight. The plaques were counted from each plate time point 5, 10, 15, 20… 60 min and tabulated as per corresponding time point. For each phage (Stab20 - 23) the experiments were repeated five to ten times on different days. The data was analyzed by Prism GraphPad.

3.7 Host range analysis

3.7.1 Spot assay

Overnight cultures were prepared for test bacteria. A colony from each test strain was inoculated into 1.5 mL LB and the cultures were incubated at +37˚C, 120 rpm to an OD_{600} of 1.0 – 1.5 for about 100 min. The LA plates were warmed-up in 37 ˚C incubator, and 0.3% soft agar molten and cooled to +50˚C. 100 µL of the bacterial culture was mixed into 3.0 mL molten agar, vortexed mildly and poured on LA plates. The top agar was allowed to solidify for 30 min and 5.0 µL drops of each phage was pipetted on it. After the spots had dried, the plates were incubated overnight at +37˚C and observed the next day for lysis zones under the drops.

3.7.2 Relative efficiency of plating (R.E.O.P)

The strains with positive spot assay were subjected to R.E.O.P experiments using the double layer assay. The indicator and test bacteria were grown to an OD_{600} of 1.0 – 1.5, and 200 µL aliquots were added to 3.0 mL of 0.3% molten soft agar maintained at +50˚C. To each tube, 50 µL of Stab20, Stab21, Stab22 and Stab23 lysate was added. The tubes were vortexed and poured on pre-warmed and dry LA plates. Next day, the plaques were counted and the R.E.O.P established for each phage and strain was determined by dividing the plaque counts from test strain with those from indicator bacteria.

R.E.O.P = Test strain plaque count ÷ Indicator bacteria plaque count.

The plaque counts and R.E.O.P results were tabled in excel sheets.

3.8 Quality assurance and ethical consideration

3.8.1 Quality assurance

The isolates used in the study such as the ATCC bacteria were certified isolates approved by both the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2017) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2017). While the Finnish isolates had been analysed and approved as either MRSA or MSSA by the HUSLAB. The phages' genomes were compared against well-annotated phage genomes deposited in the NCBI global GenBank. All sensitive and bio-hazardous experiments were done either in biosafety cabinet class II type B2 or in fume hoods.

3.8.2 Ethical consideration

The study involved neither the human research participants nor the use of laboratory animals. This was *in vitro* study involving only laboratory bacterial isolates and

phages isolated from the environment. In addition, there were no genetic manipulation of the viruses. The study was carried out at the Department of Bacteriology and Immunology, Medicum, and the Human Microbiome Research Program, the Research Programs Unit, Faculty of Medicine, University of Helsinki. The facilities are authorized to carry out research with biosafety level BS2 pathogens. However, this work was approved by the KNH-UoN ethical review committee and it ERC number is **P262/05/2017**.

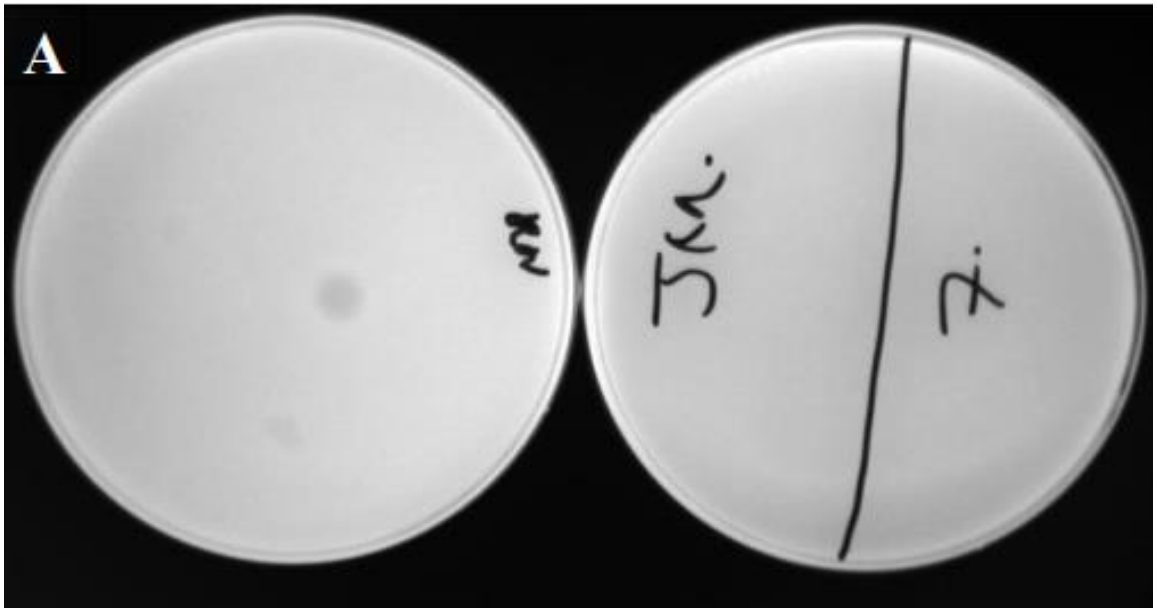
3.9 Statistical analysis

The experiments were performed at least in triplicates. The dimensions and plaque counts were presented as mean ± S.D. Physico-chemical (thermal, pH and U.V stability), adsorption and one-step growth curve experimental data were analysed by GraphPad Prism version 8.0 (GraphPad software, San Diego. CA) (Prism 8, 2019). The comparative analysis on the stability of Stab phages was carried out using the 2way ANOVA accompanied with Bonferroni post-test at 95% and 99% confidence intervals.

CHAPTER FOUR: RESULTS

4.1 Phage isolation and purification

Nine phages were isolated during the study. **RN**, **JM** and **7** (Nairobi-**[A]**), **fWa-Sau02** and **fHe-Sau2a** (Helsinki-**[B]**) and Stab20, Stab21, Stab22 and Stab23 (Shkodra-**[C]**). Phages from Nairobi had turned impotent after several attempts to recover them after long storage (**Figure 1A**). Isolates from Helsinki were active but only on a few *S. aureus* strains but, often gave low yields (phage particles per millilitre). Minute clear lysis zones (plaques) are present on fWa-Sau02 plates but blurred on fHe-Sau02a plate where they were very tiny (**Figure 1B**). The Shkodra originating Stab phages depicted large countable plaques on their host bacteria (*S. xylosus* DD34) lawn (**Figure 1C**). The Stab phages displayed clear lytic characteristic in all *Staphylococcus* sp lawns they were active against and thus they became the major phages of interest in this work.



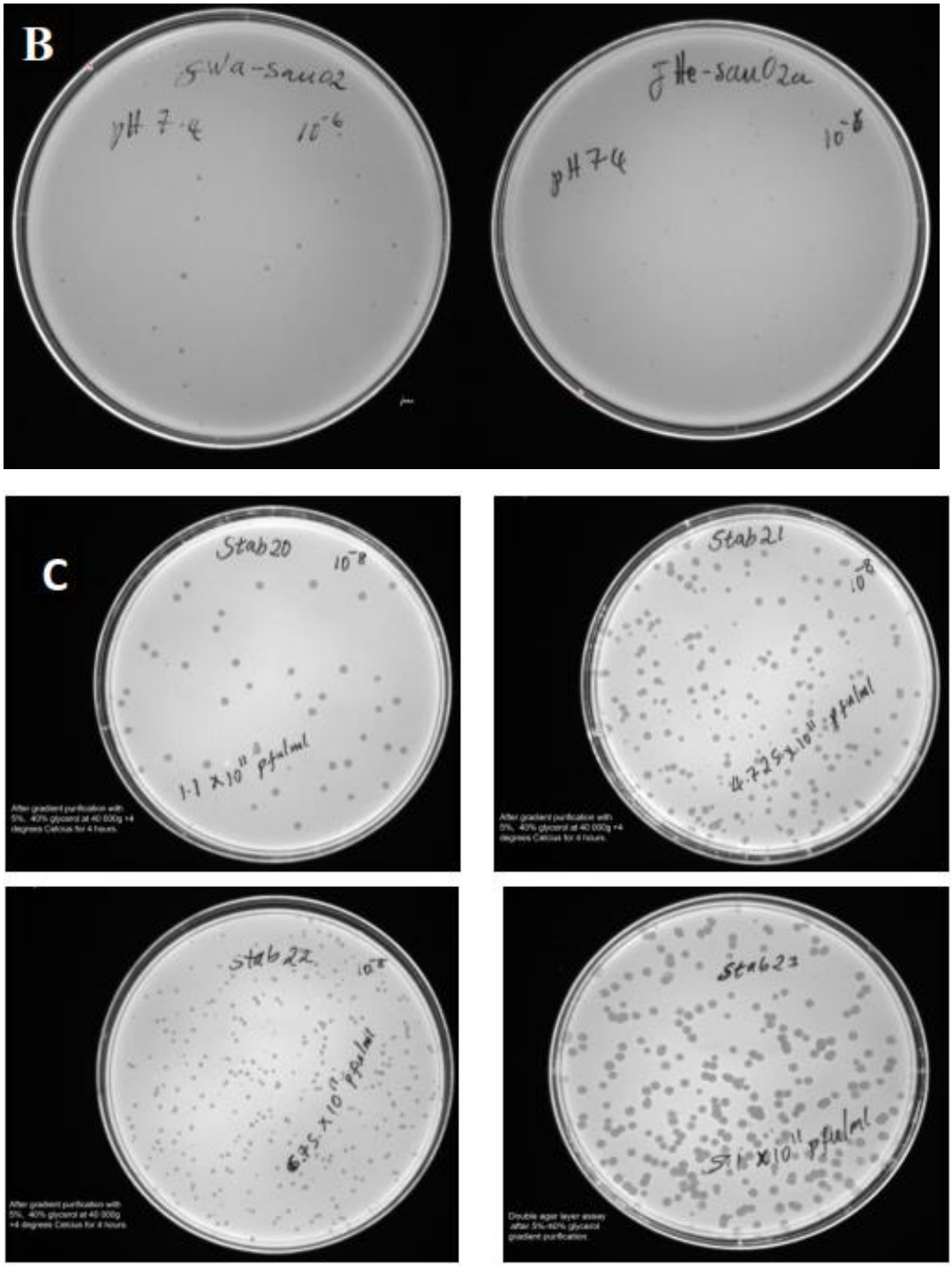
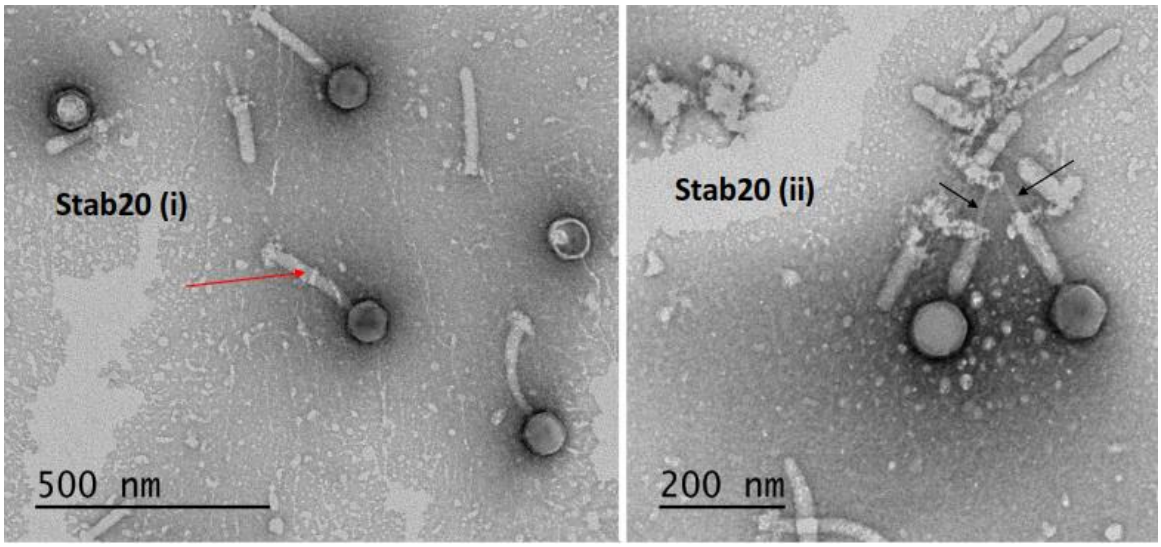


Figure 1: Plaque assay of the isolated phages from various study sites, which included Nairobi (A), Helsinki (B) and Shkodra (C). The clear lytic zones on the plates B and C are illustrations of plaques created phages after eating their hosts.

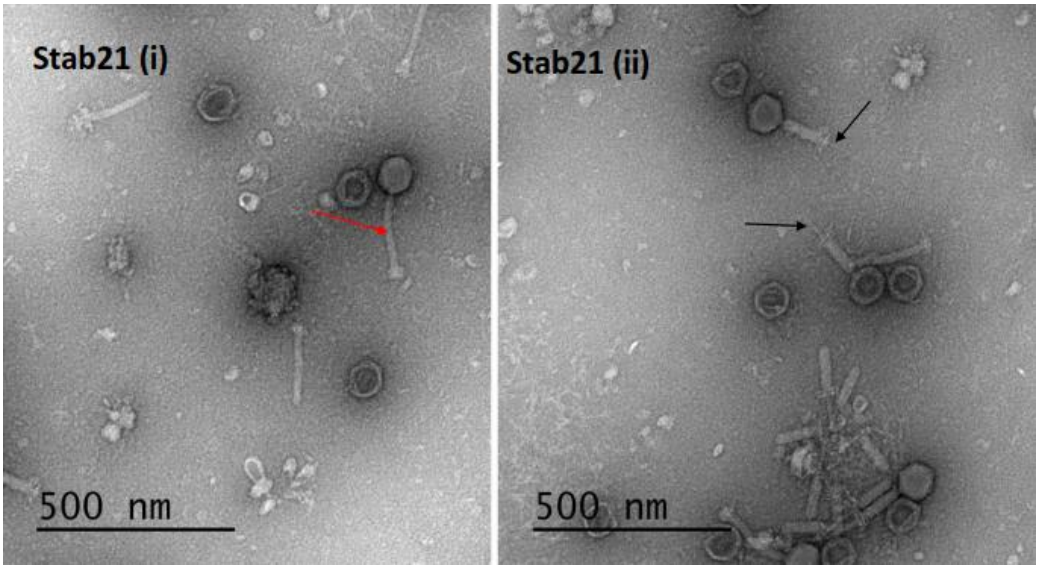
4.2 Characterization of phages

4.2.1 Morphological identification

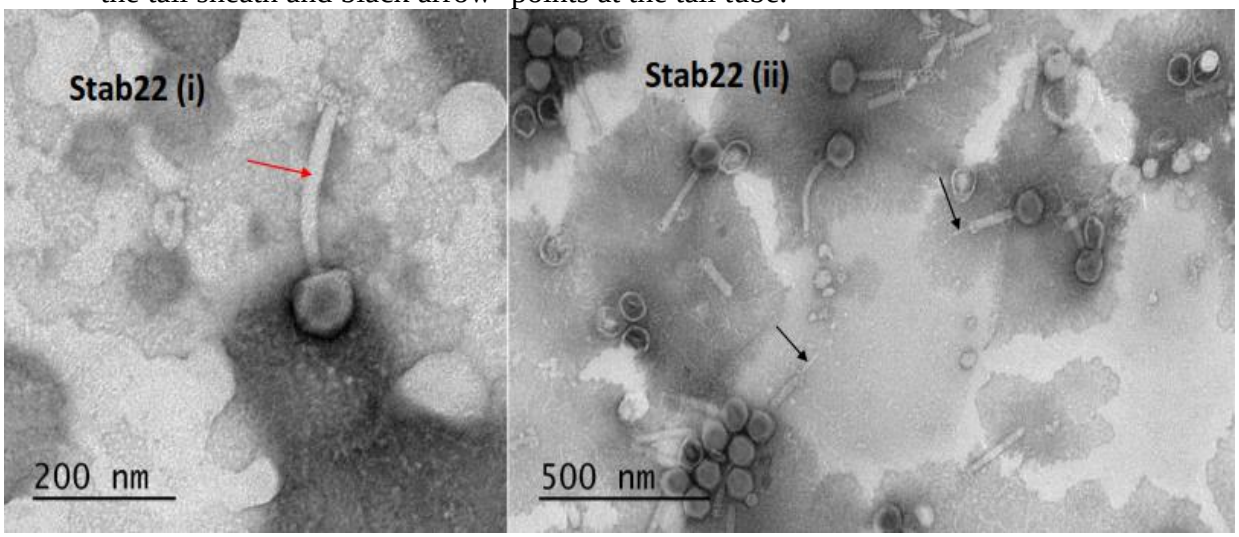
The TEM micrographs showed that Stab20, Stab21, Stab22 and Stab23 were complex viruses made up of icosahedral heads attached to long flexible contractile tails. Their tail tips had wide structures known as the baseplate from which numerous fibre-like structures extended (**Figure 2 a-d**). Second images (**ii**) depict the phages' contracted tails and tail-tubes.



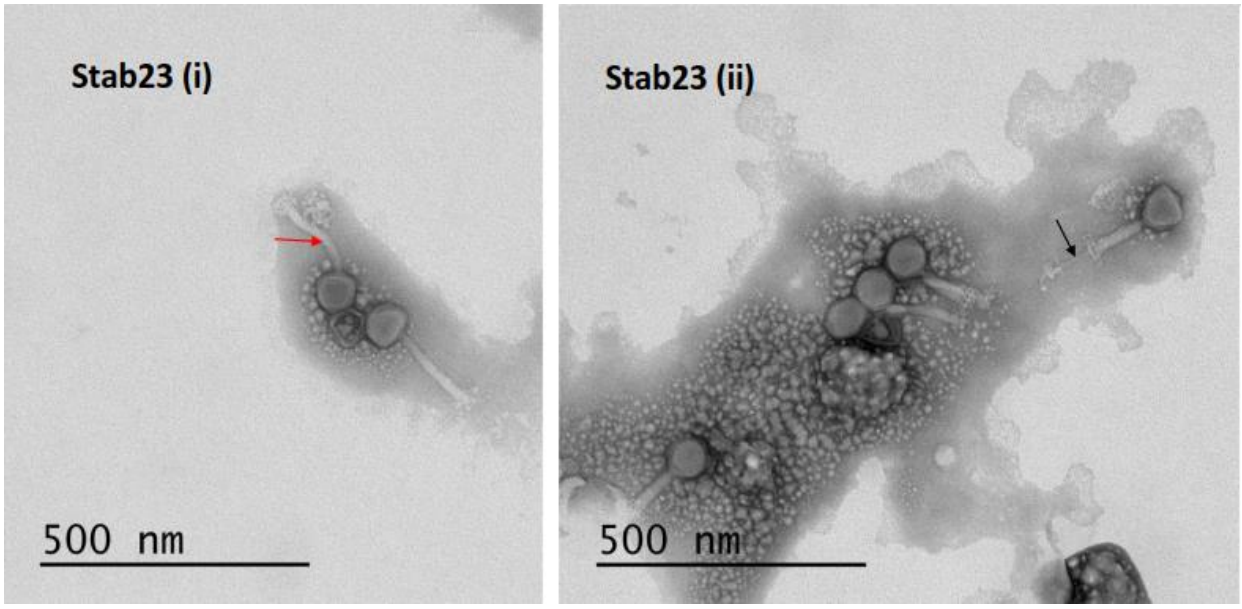
a. Stab20 phage status at normal stage (**i**) and during contraction (**ii**) the tail sheath becomes short and tail tube protrudes out of it. The red arrow- points the tail sheath and black arrow- points at the tail tube.



b. Stab21 phage status at normal stage (**i**) and during contraction (**ii**) the tail sheath becomes short and tail tube protrudes out of it. The red arrow- points the tail sheath and black arrow- points at the tail tube.



c. Stab22 phage status at normal stage (**i**) and during contraction (ii) the tail sheath becomes short and tail tube protrudes out of it. The red arrow- points the tail sheath and black arrow- points at the tail tube.



d. Stab23 phage status at normal stage (**i**) and during contraction (**ii**) the tail sheath becomes short and tail tube protrudes out of it. The red arrow- points the tail sheath and black arrow- points at the tail tube

Figure 2: Transmission electron microscopy images of phages Stab20 (a), Stab21 (b), Stab22 (c), and Stab23 (d). Uranyl acetate negative staining at original magnification of 25,000× illustrating phage particles with contracted and non-contracted tails.

These descriptions coupled with the measured virion dimensions (**Table 1**) associate the phages with the *Herelleviridae* family of the order *Caudovirales*.

Table 1: Phage particle dimensions were measured using the TEM-camera inbuilt software at a magnification of 15 000 ×. The results depict odd features of Stab20, which has the smallest capsid and tail, but possess a broader baseplate. Each data represent the mean ± standard deviation for five to ten independent measurements.

Phage	**The dimensions of the structural features**			
	Capsid head	**Tail length**	**Tail width**	**Baseplate width**
Stab20	83.96 ± 3.1 nm (n = 5)	163.2 ± 11.4 nm (n = 5)	21.1 ± 0.7 nm (n = 5)	48.14 ± 1.22 nm (n = 5)
Stab21	91.3 ± 0.25 nm (n = 8)	196.5 ± 3.1 nm (n = 8)	23.4 ± 0.6 nm (n = 5)	44.9 ± 1.5 nm (n = 7)

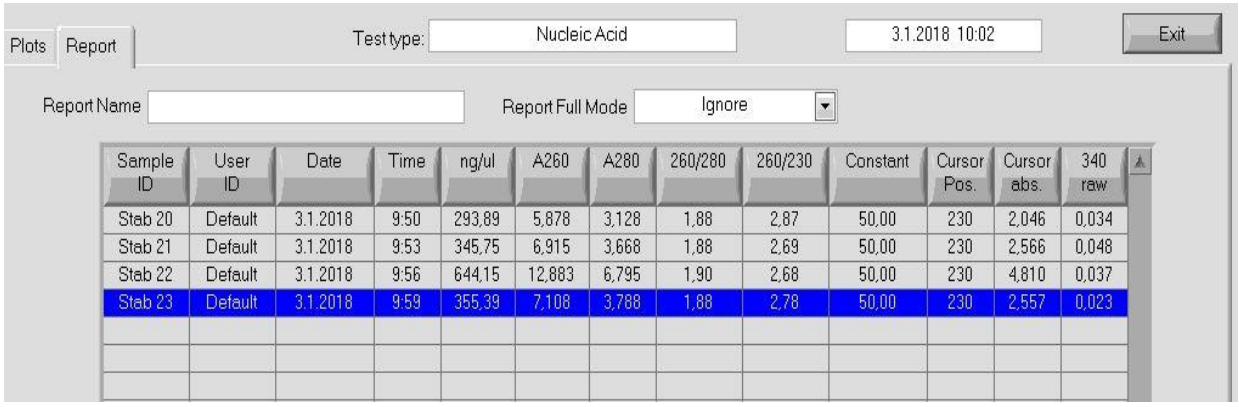
Stab22	94.3 ± 0.5 nm (n = 10)	201.6 ± 0.6 nm (n = 5)	21.3 ± 0.4 nm (n = 5)	41.84 ±.0.74 nm (n = 5)
Stab23	92.50 ± 2.6 nm (n = 10)	198.9 ± 2.9 nm (n = 9)	20.3 ± 0.3 nm (n = 9)	42.3 ± 0.8 nm (n = 5)

4.2.2 Genome analysis

4.2.2.1 DNA extraction and gel analysis

Preliminary quantification of the phages' DNA with NanoDrop indicated the success of the extraction after purification with ethanol to remove chloroform traces. Chloroform does influence UV absorbance that in turn inflates the NanoDrop results. In addition, DNA samples with chloroform-free for a successful restriction digestion to be realised since the compound denatures restriction enzymes. The concentrations were above the concentration required for sequencing which was 100.0 ng/µL (**Table 2**).

Table 2: NanoDrop quantification for Stab20, Stab21, Stab22 and Stab23 nucleic acid (DNA) samples. The table indicates extraction of sufficient DNA samples for sequencing since none was below the concentration threshold (100.0 ng/µL) as lowest was 293.89 ng/µL.



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
Stab 20	Default	3.1.2018	9:50	293,89	5,878	3,128	1,88	2,87	50,00	230	2,046	0,034
Stab 21	Default	3.1.2018	9:53	345,75	6,915	3,668	1,88	2,69	50,00	230	2,566	0,048
Stab 22	Default	3.1.2018	9:56	644,15	12,883	6,795	1,90	2,68	50,00	230	4,810	0,037
Stab 23	Default	3.1.2018	9:59	355,39	7,108	3,788	1,88	2,78	50,00	230	2,557	0,023

The DNA samples were then analysed using agarose gel electrophoresis that showed intact high-molecular weight bands. The analysis showed that the DNA samples were of good quality as only minimal smearing was detectable under the bands (**Figure 3**).

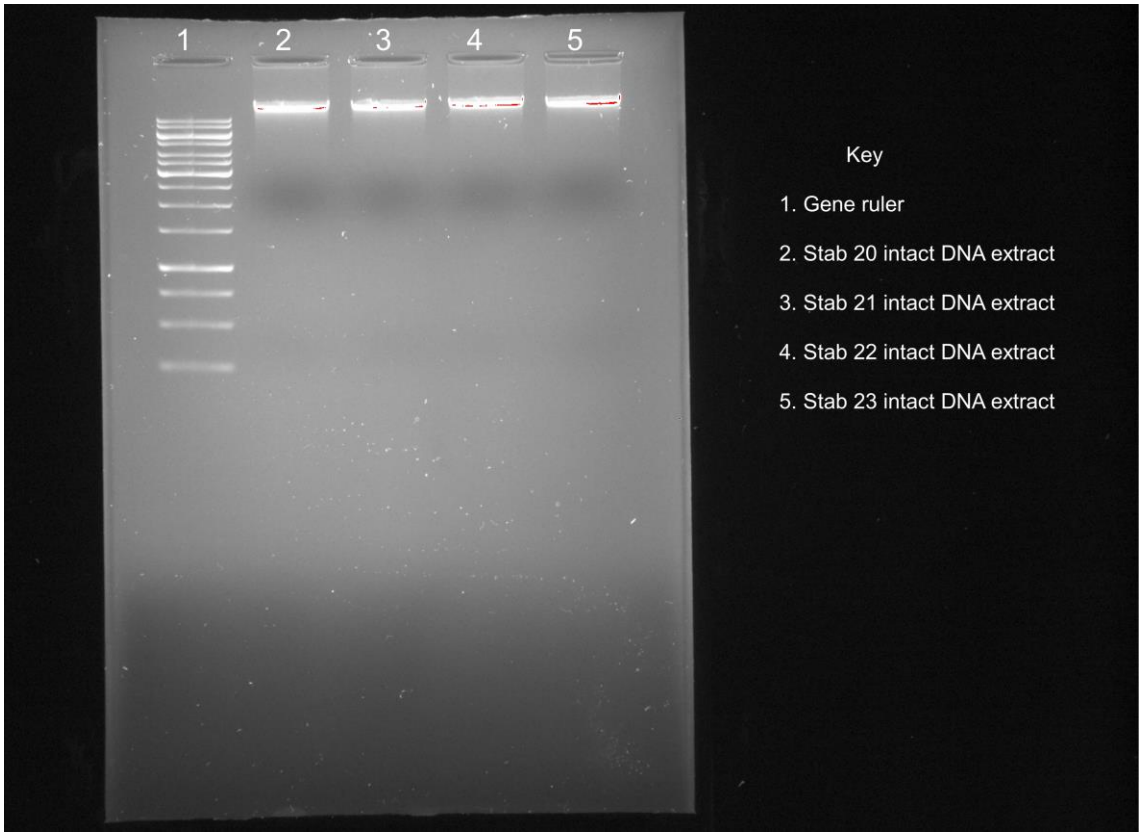


Figure 3: Gel exhibition of Stabs' DNA samples with minimal contamination. There is absence smears up the ladder after DNA bands.

Afterwards, phage DNA samples were analysed by restriction digestion. The enzymes used included EcoRI, EcoRV and HindIII. Restriction fragments of the digested DNA samples were analysed by agarose gel electrophoresis (**Figure 4**). The analysis revealed numerous restriction fragments indicating that the phages had large genomes. This indicates that these phages might be *Herelleviridae* viruses.

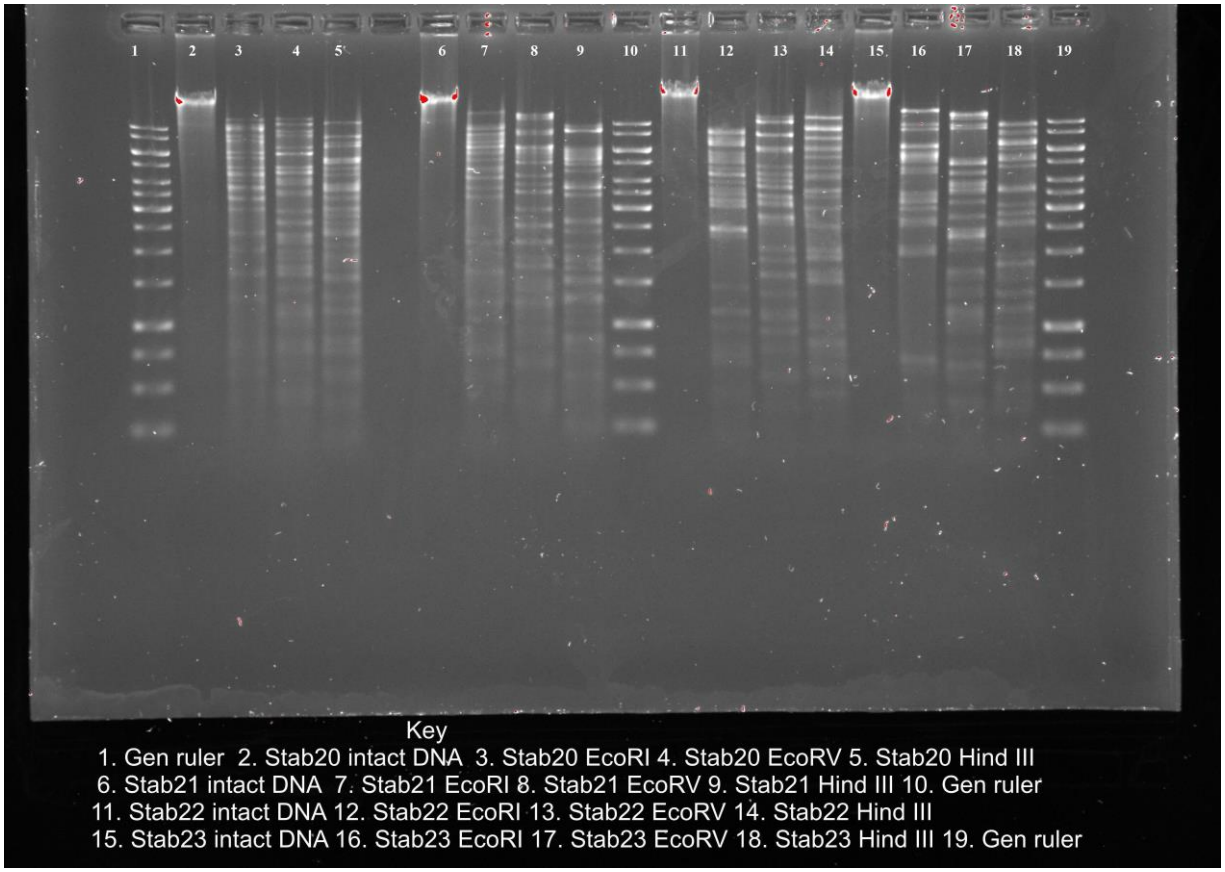


Figure 4: Restriction analysis of Stabs showing the size of phages' genomes. The presence of numerous bands shows that these phages have large genomes.

4.2.2.2 Sequencing and annotation

The NGS results of the phage genomes were received as paired-end 150-base pairs (bp)-long reads. The reads were assembled *de novo* using the A5-miseq pipeline and the physical ends and terminal repeats of the phage genomes were determined as described in section 3.4.2.3. General properties of the phage genomes are shown in Table 3. The Stab20, Stab21, Stab22, and Stab23 genomes were 153,338 bp, 153,797 bp, 155,962 bp and 154,499 bp in size, respectively. The PhageTerm analysis was ran on Galaxy/Pasteur platform and it revealed that Stabs had long direct terminal-repeats ranging from 10814 to 12225 bp.

The annotation of the phage genomes showed that Stab20, Stab21, Stab22 and Stab23 had 223, 217, 218 and 206 predicted genes, respectively (Table 3). *In silico* analysis of 100 bp sequences upstream and downstream of each phage's conservative regulatory region indicated that Stabs had 28- 48 host specific putative promoters and 33-37 terminators (**Appendix IV & V**). The phages' promoters probing with MEME and MAST generated uniform consensus motifs (**Figure 5**) that resembles phage ISP motifs (Vandersteegen *et al.,* 2011). Genomes analysis with ARTEMIS established that guanine-cytosine contents of the phages were between 30.2 and 30.9 %, slightly lower than that of the staphylococci in general (32.7 %). The genome analysis suggested that the phages are new members of the genus *Kayvirus* of the subfamily *Twortvirinae*. The genomes were compared using the progressiveMauve tool and the result is shown in Figure 5. The putative functions of the predicted gene products were annotated using BLASTP, HHMER, InterProScan, PSI/BLAST analysis, smart BLAST, PSI-BLAST and HHpred analyses. The tRNAscan-SE and ARAGORN showed that the phages possessed 1-4 tRNA genes. Pairwise sequence identity and BLASTn analyses identified clear differences between the Stab phages and the genus *Kayvirus* reference phage K (**Table 3**) (**Figure 6**).

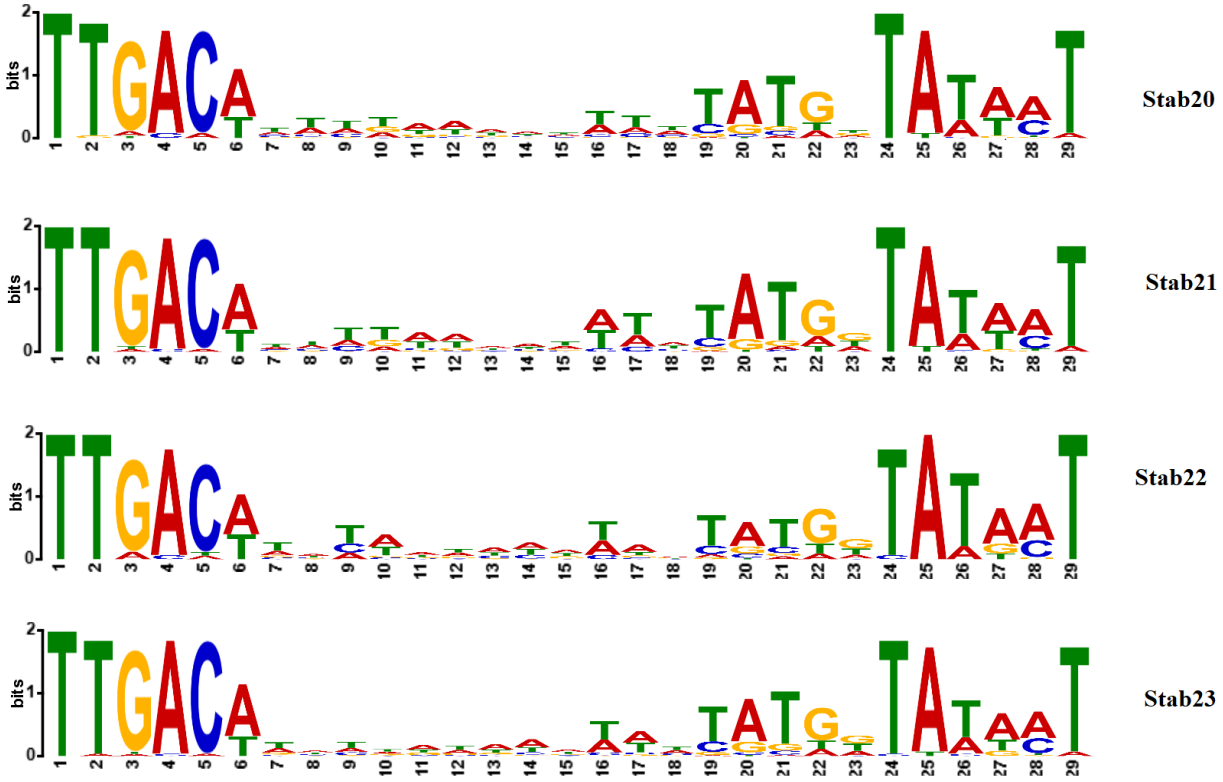


Figure 5: Consensus motifs of the Stab phages' putative promoter sequences. Sequence pair 1-6 and 24-29 respectively represents -35 box and -10 box. The boxes have similar sequences but the spacer sequence (7-23) for each phage is unique and they illustrate that these phages are distinct.

Table 3: Summary of the Stab phage genome properties. The table displays how varied these phages are from each other with regard to genome size and analysis of nucleotide identity (ANI) percentages. In addition, it shows Stabs' close association with phage K.

Staphylococcus phages					
	Stab20	**Stab21**	**Stab22**	**Stab23**	**Phage K***
Genome size (bp)	153338	153797	155962	154499	148317
Direct terminal-repeats size (bp)	10814	11149	12304	12225	8486
% GC content	30.21	30.32	30.61	30.88	30.4
Number of predicted genes	223	217	218	206	233

Number of tRNA genes	4	4	2	1	4
Stab20 identity (%)	**100**	84.4	49.7	49.6	81.6
Stab21 identity (%)	84.4	**100**	73.4	76.9	76.2
Stab22 identity (%)	49.7	73.4	**100**	77.5	72.2
Stab23 identity (%)	49.6	76.9	77.5	**100**	67.4
Phage K identity (%)	81.6	76.2	72.2	67.4	**100**

*****Phage K refers to Staphylococcus phage K, which is type member of the genus *Kayvirus* recognized by the International Committee on Taxonomy of Viruses (ICTV).

Putative functions were assigned to 75 predicted gene products of the phages including both non-structural and structural proteins (**Appendix III**). The structural proteins included capsid and scaffold protein, portal protein, prohead protease, membrane protein, tail tube, major tail sheath, tail morphogenetic, and tail tape measure proteins, baseplate proteins, adsorption-associated and carbohydrate-binding domain-containing tail proteins (**Appendix III**). The non-structural predicted gene products included DNA primase, DNA helicase, exonuclease, DNA polymerases (A/I and B/II), RNA polymerase sigma factor, integration host factor, thioredoxin, ribonucleotide reductase large and small subunits, resolvase, ribonucleotide reduction protein, replication protein, nucleoside 2-deoxy-ribosyltransferase, RNA ligase, ribonuclease H, and a tran-scriptional regulator (**Appendix III**). Majority of non-structural predicted proteins had a function in DNA/RNA synthesis and metabolism. The identified bacterial cell wall degrading enzymes and compounds associated with them included endolysin, holin and amidase. In addition, these viruses have abundant hypothetical proteins (**Appendix III**). Submitted the annotated Stab20, Stab21, Stab22 and Stab23 genomes to the European Nucleotide Archives and received the accession numbers **LR215718**, **LR215719**, **LR215720** and **LR215721,** respectively.

4.2.2.3 Phylogenetic analysis

The whole genome phylogenetic comparisons showed that the Stab phages are closely related to the *Staphylococcus* phage K and the other members of *Kayvirus* genus (**Figure 7A**). Phylogenetic trees constructed based on the predicted amino acid sequences of the tail sheath (**Figure 7B**) and primase (**Figure 7C**) proteins illustrated the closest association between Stab20 and Stab21, and another, between Stab22 and Stab23.

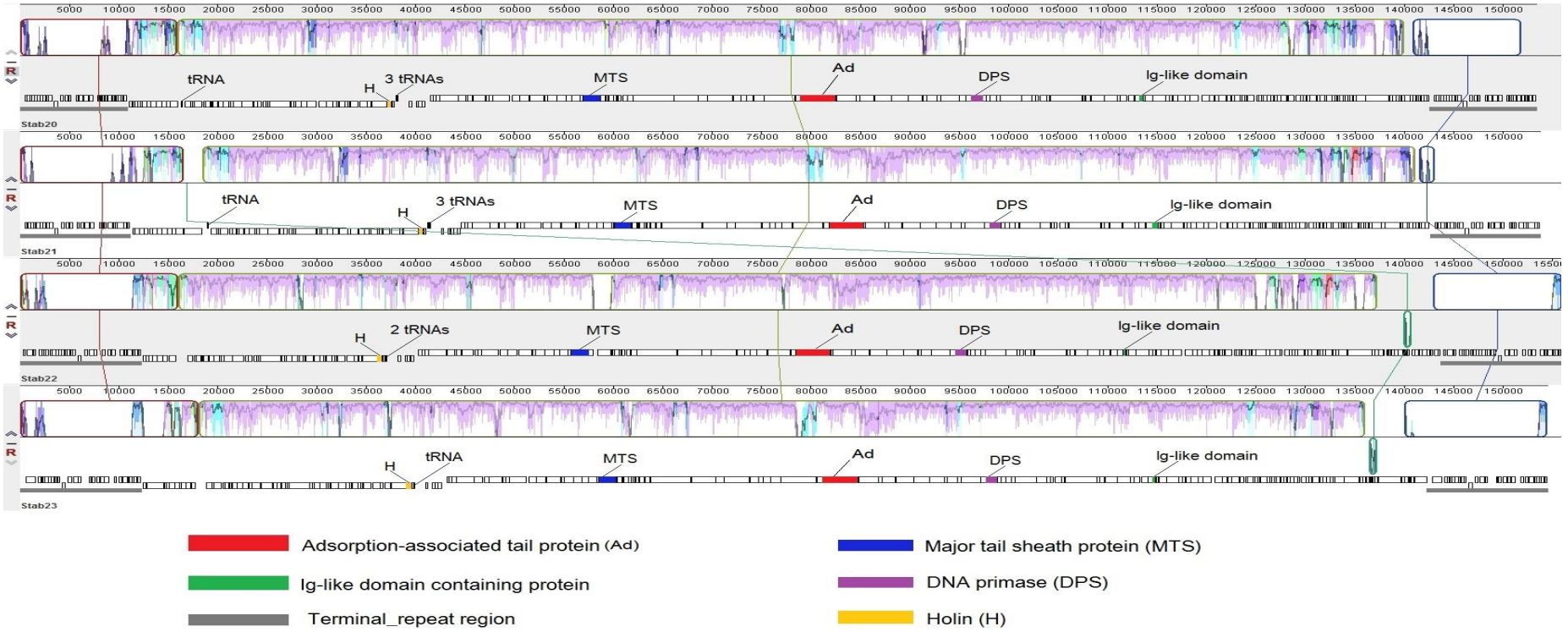
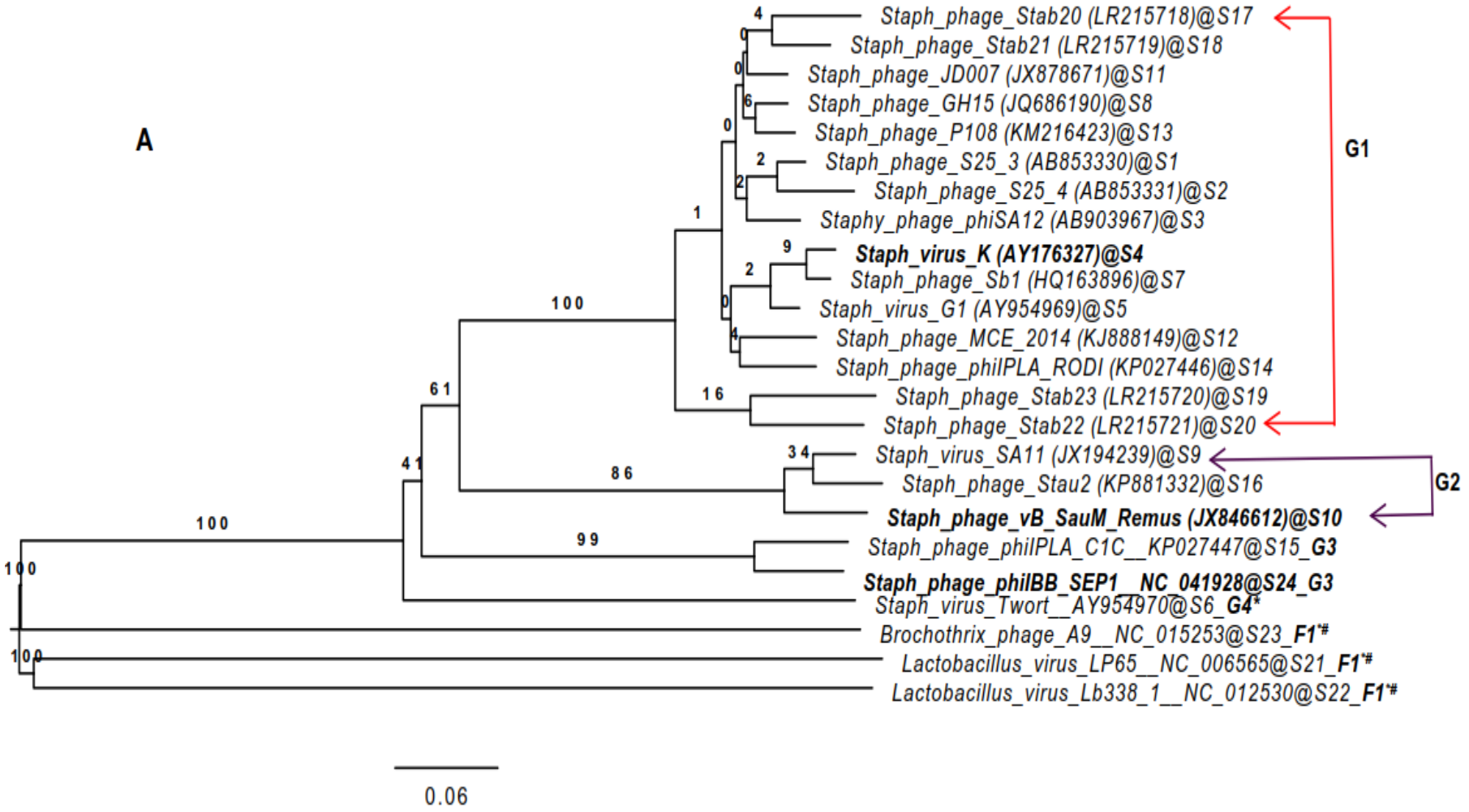


Figure 6: Mauve alignment of annotated complete genomes of Stab20, Stab21, Stab22 and Stab23 from top to bottom; showing the locations of tRNAs, and the genes for proteins such as major tail sheath protein (blue), holin (yellow), DNA primase (purple), Ig-like domain containing protein (green) and adsorption-associated tail protein (red) within the genomes. The thick grey bar represents terminal-repeat regions. The mauve plots show conserved genomic regions of the four phages. The similarity levels vary with the heights of the curves and the intensities within the blocks that are proportional to the average nucleotide identities. The white spaces inside or outside the blocks represent regions of difference between the genomes of these phages.


A
Staph_phage_Stab20 (LR215718)@S17
Staph_phage_Stab21 (LR215719)@S18
Staph_phage_JD007 (JX878671)@S11
Staph_phage_GH15 (JQ686190)@S8
Staph_phage_P108 (KM216423)@S13
Staph_phage_S25_3 (AB853330)@S1
Staph_phage_S25_4 (AB853331)@S2
Staphy_phage_phiSA12 (AB903967)@S3
Staph_virus_K (AY176327)@S4
Staph_phage_Sb1 (HQ163896)@S7
Staph_virus_G1 (AY954969)@S5
Staph_phage_MCE_2014 (KJ888149)@S12
Staph_phage_philPLA_RODI (KP027446)@S14
Staph_phage_Stab23 (LR215720)@S19
Staph_phage_Stab22 (LR215721)@S20
Staph_virus_SA11 (JX194239)@S9
Staph_phage_Stau2 (KP881332)@S16
Staph_phage_vB_SauM_Remus (JX846612)@S10
Staph_phage_philPLA_C1C__KP027447@S15_G3
Staph_phage_philBB_SEP1__NC_041928@S24_G3
Staph_virus_Twort__AY954970@S6_G4*
Brochothrix_phage_A9__NC_015253@S23_F1*#
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G2
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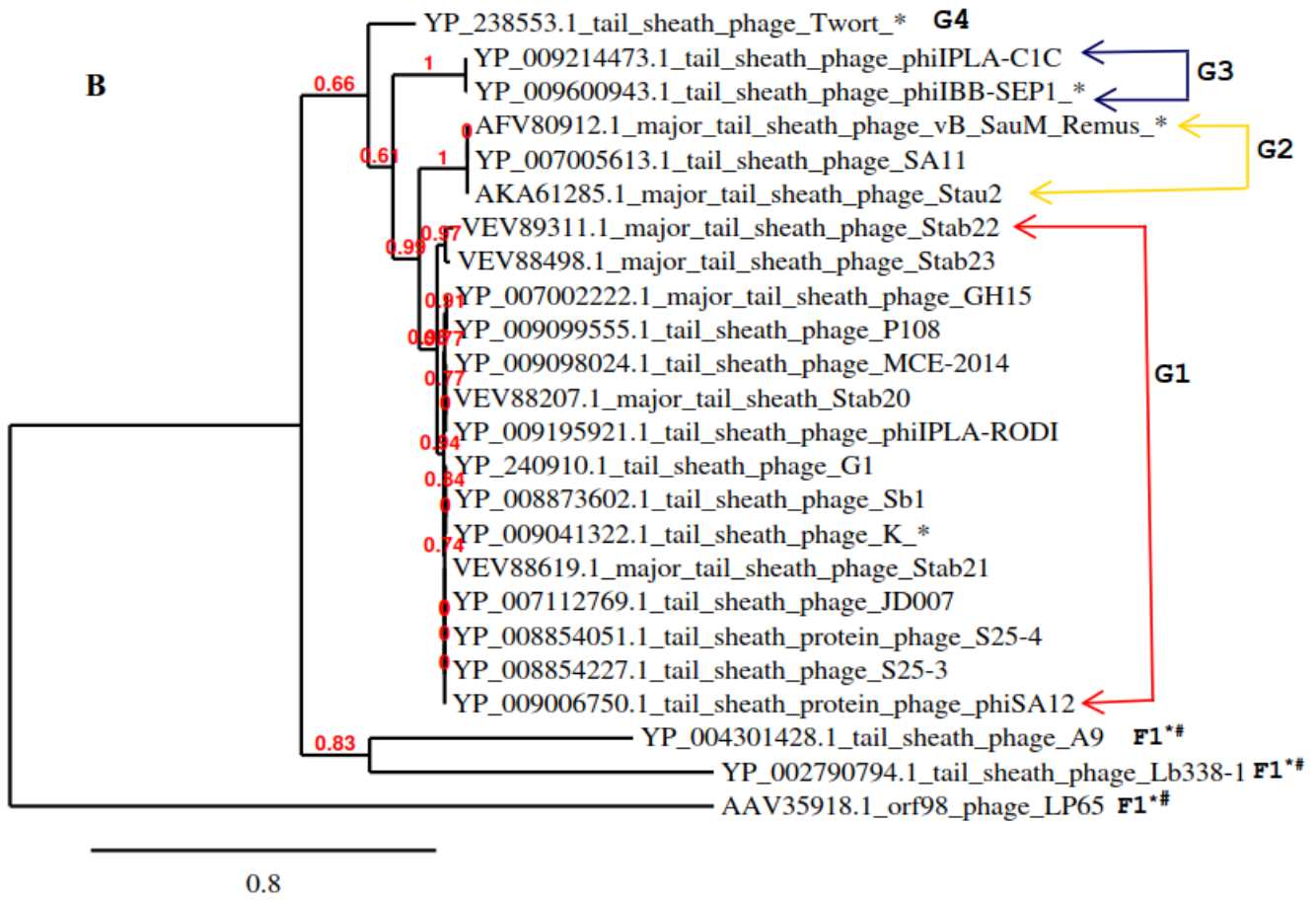
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YP_009214473.1_tail_sheath_phage_phiIPLA-C1C
YP_009600943.1_tail_sheath_phage_phiIBB-SEP1_*
G3
AFV80912.1_major_tail_sheath_phage_vB_SauM_Remus_*
YP_007005613.1_tail_sheath_phage_SA11
AKA61285.1_major_tail_sheath_phage_Stau2
G2
VEV89311.1_major_tail_sheath_phage_Stab22
VEV88498.1_major_tail_sheath_phage_Stab23
YP_007002222.1_major_tail_sheath_phage_GH15
YP_009099555.1_tail_sheath_phage_P108
YP_009098024.1_tail_sheath_phage_MCE-2014
VEV88207.1_major_tail_sheath_Stab20
YP_009195921.1_tail_sheath_phage_phiIPLA-RODI
YP_240910.1_tail_sheath_phage_G1
YP_008873602.1_tail_sheath_phage_Sb1
YP_009041322.1_tail_sheath_phage_K_*
VEV88619.1_major_tail_sheath_phage_Stab21
YP_007112769.1_tail_sheath_phage_JD007
YP_008854051.1_tail_sheath_protein_phage_S25-4
YP_008854227.1_tail_sheath_phage_S25-3
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G1
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F1*#
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F1*#
AAV35918.1_orf98_phage_LP65
F1*#
0.66
1
0.61
1
0.99
0.97
0.91
0.87
0.77
0.84
0.84
0.74
0.83
0.8

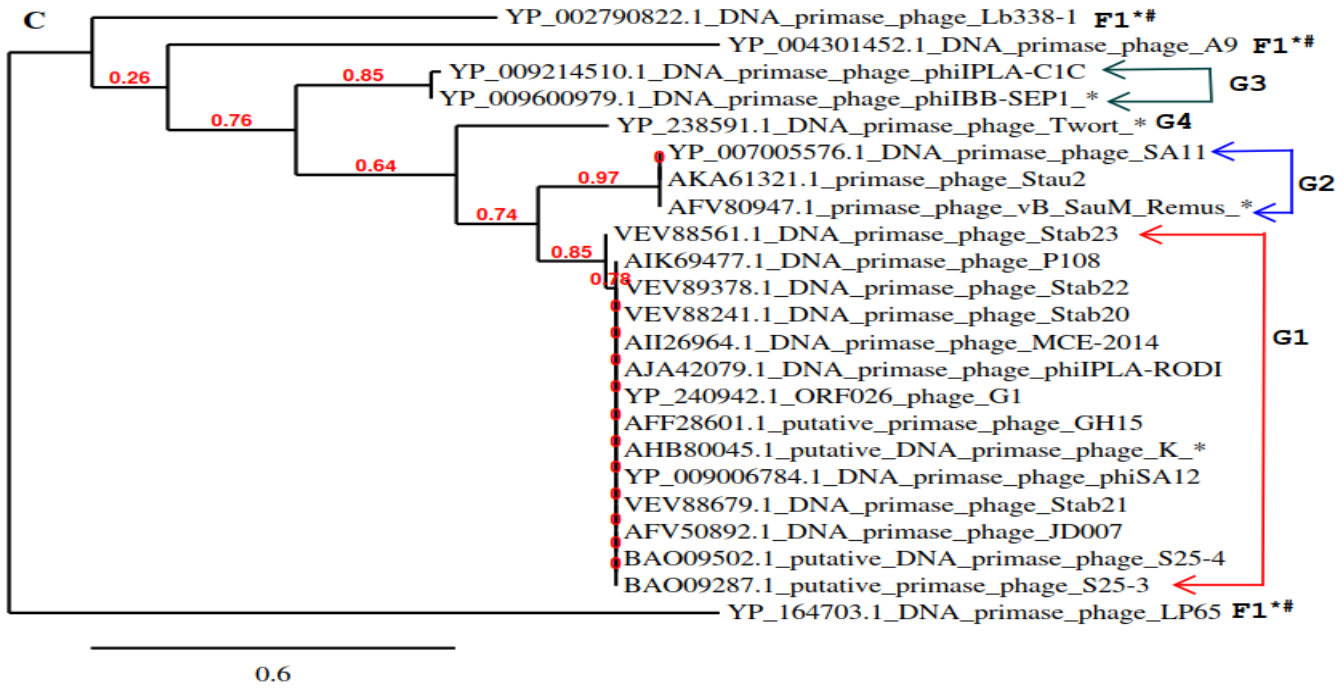


Figure 7: Taxonomic classification of the Stabs. These are phylogenetic trees of the *Twortvirinae* subfamily illustrating the Stab phages genus. Phylogenetic trees created with whole genomes (A), predicted tail sheath (B) and DNA primase amino acid sequences (C) of the Stab phages and representatives of closely related phages selected from the ICTV database. The branch length is proportional to the number of substitutions per site. The abbreviations G1 – genus *Kayvirus*; G2 - genus *Silviavirus*; G3 - genus *Sepunavirus*; G4 – genus *Twortvirus*; F1$^{*\#}$ – are phage species which are members of the family *Herelleviridae* with neither subfamily nor genus. Used the VICTOR tool to create the phylogenetic tree in panel A, and those in panels B and C were constructed using Phylogeny.fr. “One Click” tool.

4.2.3 Screening for lethal genes

No antibiotic resistance, virulence factor, toxin-encoding gene or chromosomal point mutation (such as 23S, dfrB, fusA, grlA, grlB, gyrA, ileS, pbp2, pbp4, rpoB and pbp4_promoter_size_304bp) was present in the Stab genomes (**Table 4**). Furthermore, comparative protein analysis with HHpred/ HMMER scan/ BLASTp/ InterProscan showed that the Stabs were integrase free.

Table 4: This list shows that the Stabs were free of antibiotic resistance genes and chromosomal point mutations associated with *Staphylococcus* spp drug resistance.

No	Antibiotic resistance encoding genes	Stab20	Stab21	Stab22	Stab23
1	Aminoglycoside	-ve*	-ve	-ve	-ve
2	Beta-lactam	-ve	-ve	-ve	-ve
3	Colistin	-ve	-ve	-ve	-ve
4	Trimethoprim	-ve	-ve	-ve	-ve
5	Nitroimidazole	-ve	-ve	-ve	-ve
6	Fosfomycin	-ve	-ve	-ve	-ve
7	Fluoroquinolone	-ve	-ve	-ve	-ve
8	Fusidic Acid	-ve	-ve	-ve	-ve
9	MLS - Macrolide	-ve	-ve	-ve	-ve
10	Rifampicin	-ve	-ve	-ve	-ve
11	Tetracycline	-ve	-ve	-ve	-ve
12	Sulphonamide	-ve	-ve	-ve	-ve
13	Glycopeptide	-ve	-ve	-ve	-ve
14	Oxazolidinone	-ve	-ve	-ve	-ve
15	Phenicol	-ve	-ve	-ve	-ve
16	Chromosomal point mutations (23S, dfrB, fusA, grlA, grlB, gyrA, ileS, pbp2, pbp4, rpoB and pbp4_promoter_size_304bp)	-ve	-ve	-ve	-ve

*Negative results

4.2.4 Proteomic

This work showed that these phages are related to each other and to the typed *Staphylococcus* virus K that represents the *Kayvirus* genus associated phages. Analysis of phages' structural protein via SDS-PAGE revealed the common physical features of the Stabs (**Figure 8**). Dense conspicuous uniform bands across the gel illustrate the shared proteins with similar migration distance such as those at 70 kDa and 55-50 kDa. In addition, they represent the phages' dominant structural proteins with numerous copies such as the capsid and tail-sheath. 50-55 kDa bands of the SDS-PAGE are the most conspicuous and a deeper look at them gives an impression of two or more overlapping bands. Therefore, they might refer to the *in silico* genome analysis predicted major capsid and scaffold proteins of Stab20, Stab21, Stab22 and Stab23 with calculated molecular mass of 50.4 kDa and 51.5 kDa that might have co-migrated down the gel. Further, up the gel bands within molecular mass 70 kDa are visible across all the phages. The bands are perceived to be the *in silico* predicted phage terminase large-subunit proteins with calculated molecular mass of 70.2 – 70.4 kDa. Other most conspicuous bands are present just above 130 kDa mark of the molecular ladder. They are speculated to be the Stabs' tail tape-measure proteins since the bands' molecular weight seems to correspond with the suggested *in silico* calculated molecular mass of the proteins (143.1-143.9 kDa) (**Appendix III**).

The LC-MS/MS work provided information on the phage structural and non-structural proteins predicted by genome sequencing (**Appendix VI**). The selection criteria for valid proteins were at least 5% sequence coverage and identification of ≥ 2 unique peptides. LC-MS/MS outcome depicted that structural proteins (capsid, portal protein, tail tape-measure, major tail-sheath, tail morphogenetic protein and adsorption-associated tail protein) of the Stab phages had minor molecular weight variations (**Table 5**). In addition, the results showed that certain sequence annotated as Stabs' hypothetical proteins make up the structural units of these phages. These hypothetical proteins include *g102* (Stab20), *g163* (Stab21), *g097* (Stab22) and *g160* (Stab23) (**Appendix VI**). Furthermore, the LC-MS/MS also confirmed the existence

of common non-structural proteins like DNA polymerase, Ribonucleotide reductase large-subunit, DNA repair recombinase, DNA helicase A/B, PhoH-related protein and AAA family ATPase (**Appendix VI**). However, host bacteria proteins were absent and these indicate that these non-structural proteins originated from phages.

Table 5: A list of Stabs' major structural proteins identified with LC-MS/MS. The proteins' molecular weights are less distinct.

Protein	**Phages' protein molecular weight (kDa)**			
	Stab20	**Stab21**	**Stab22**	**Stab23**
Tape-measure	143.79	143.9	143.66	143.71
Major capsid	51.24	51.21	51.3	51.26
Major tail-sheath	64.42	64.46	64.23	64.49
Tail tube	15.93	15.93	15.20	15.87
Adsorption-associated tail protein	129.26	129.18	129.84	129.76
Tail morphogenetic protein	20.96	20.92	21.23	21.02

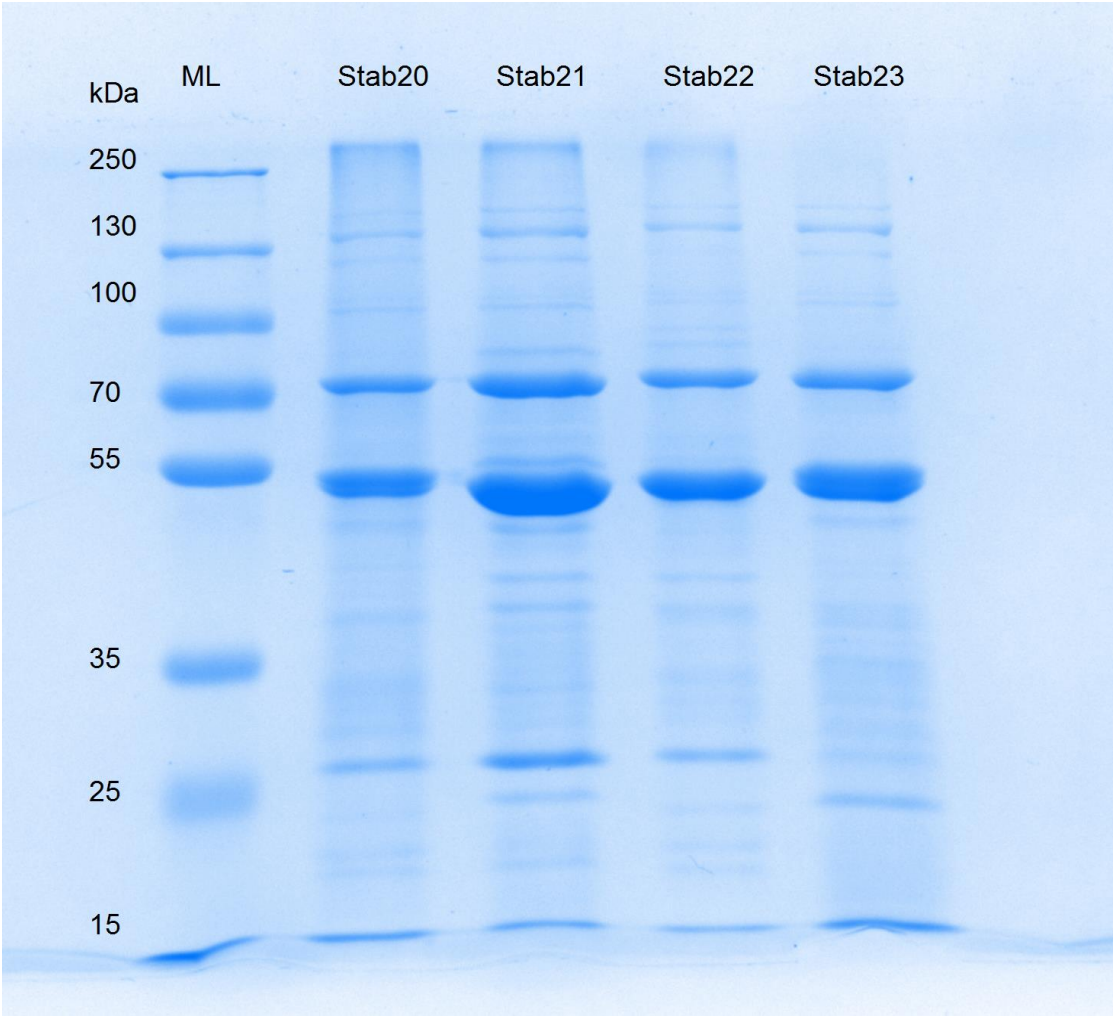
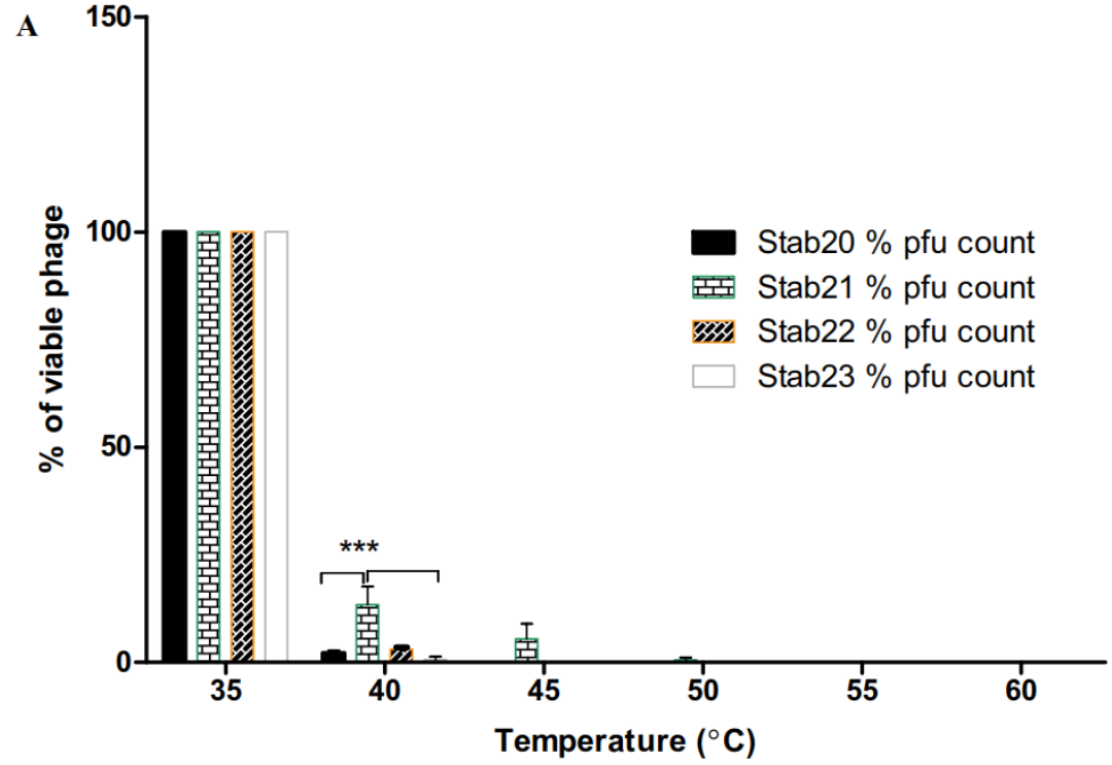


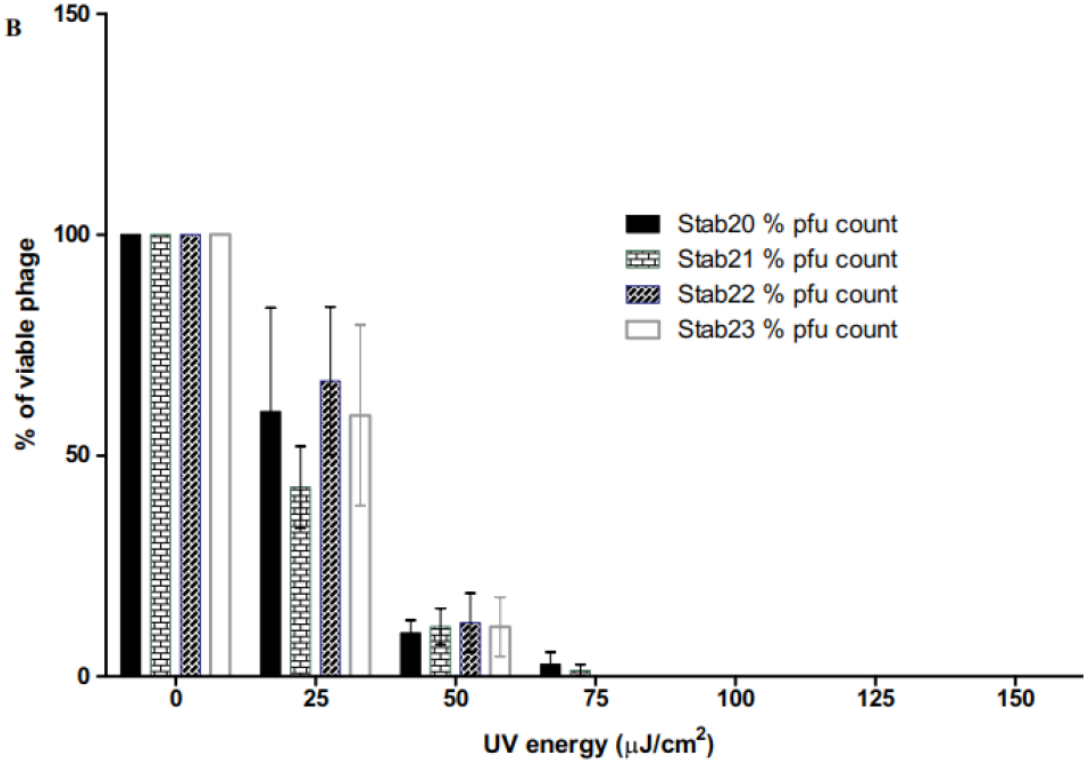
Figure 8: SDS-PAGE (10% acrylamide) of Stab20, Stab21, Stab22 and Stab23 showing major similarity and distinction among the phages' structural proteins. **ML**-molecular ladder (broad range molecular marker), **kDa**- kiloDalton**.**

4.3 Physico-chemical properties

There was little variation on the phages' stability when subjected to different environmental conditions such as ultra-violet (UV) irradiation, temperature, pH and exposure to organic solvents (ethanol and chloroform). All phages exhibited significant viral titre ($p < 0.0001$) when incubated at temperatures above 45 °C or exposed to 75 µJ/cm2 of UV-irradiation. Increased acidity or alkalinity had negative impact on these viruses' viability. Each was inactivated below pH 5.4 or above pH

9.4 (**Figure 9A-C**). Ethanol concentrations above 25% vol/vol were enough to inactivate all the four phages (**Figure 10A** and **B**). However, they exhibited resistance to chloroform (**Figure 11**).


A
150
100
50
0
% of viable phage
***
Stab20 % pfu count
Stab21 % pfu count
Stab22 % pfu count
Stab23 % pfu count
35
40
45
50
55
60
Temperature (°C)


B
150
100
50
0
% of viable phage
Stab20 % pfu count
Stab21 % pfu count
Stab22 % pfu count
Stab23 % pfu count
0
25
50
75
100
125
150
UV energy (μJ/cm²)

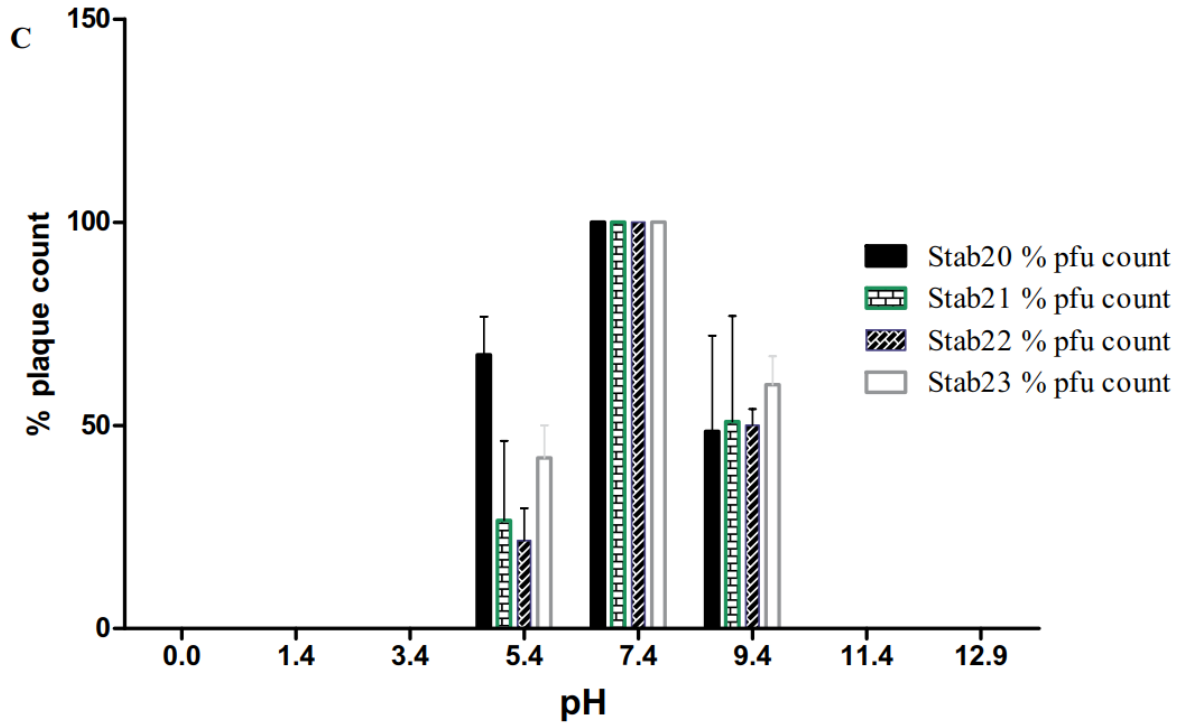
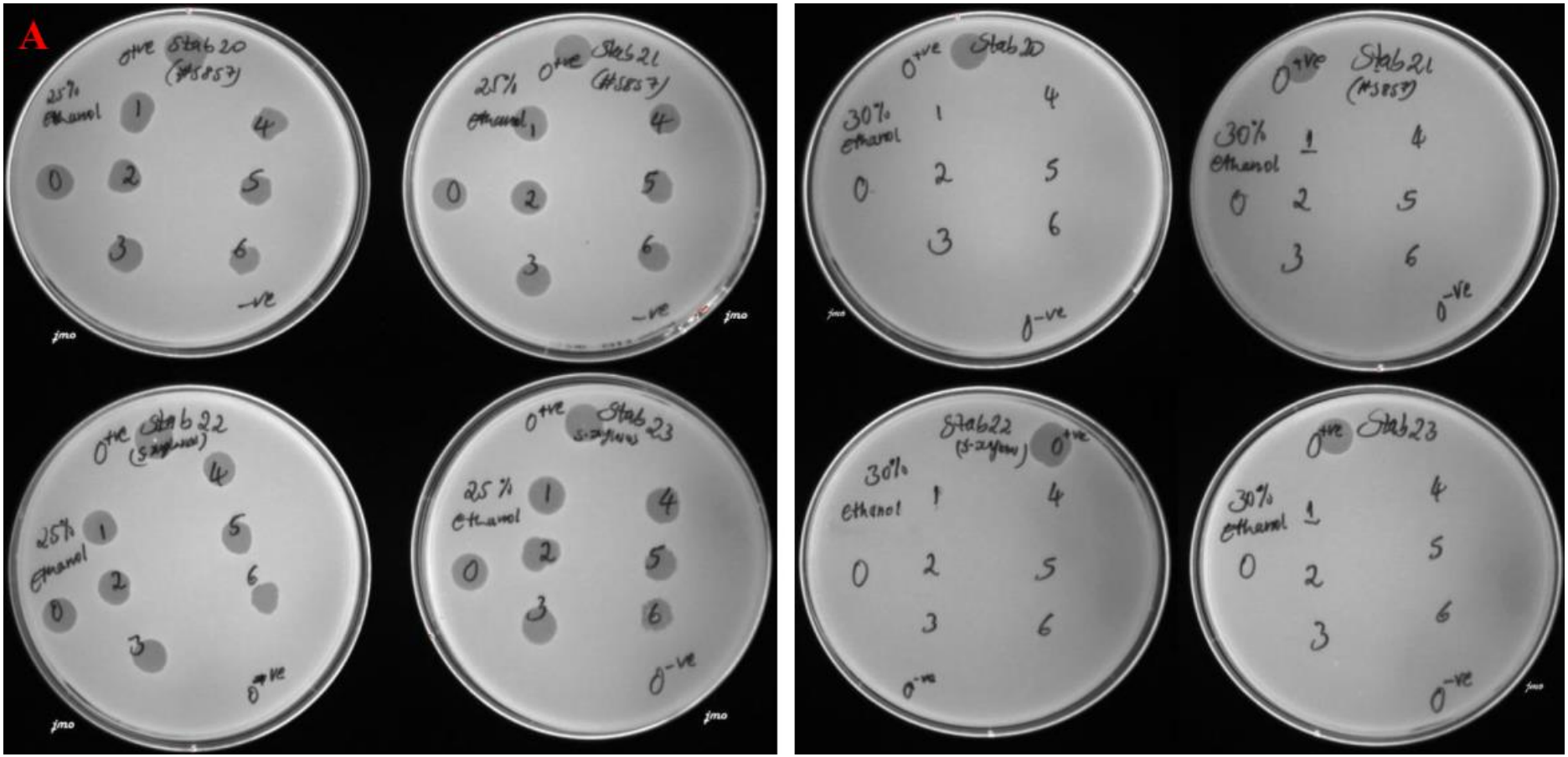
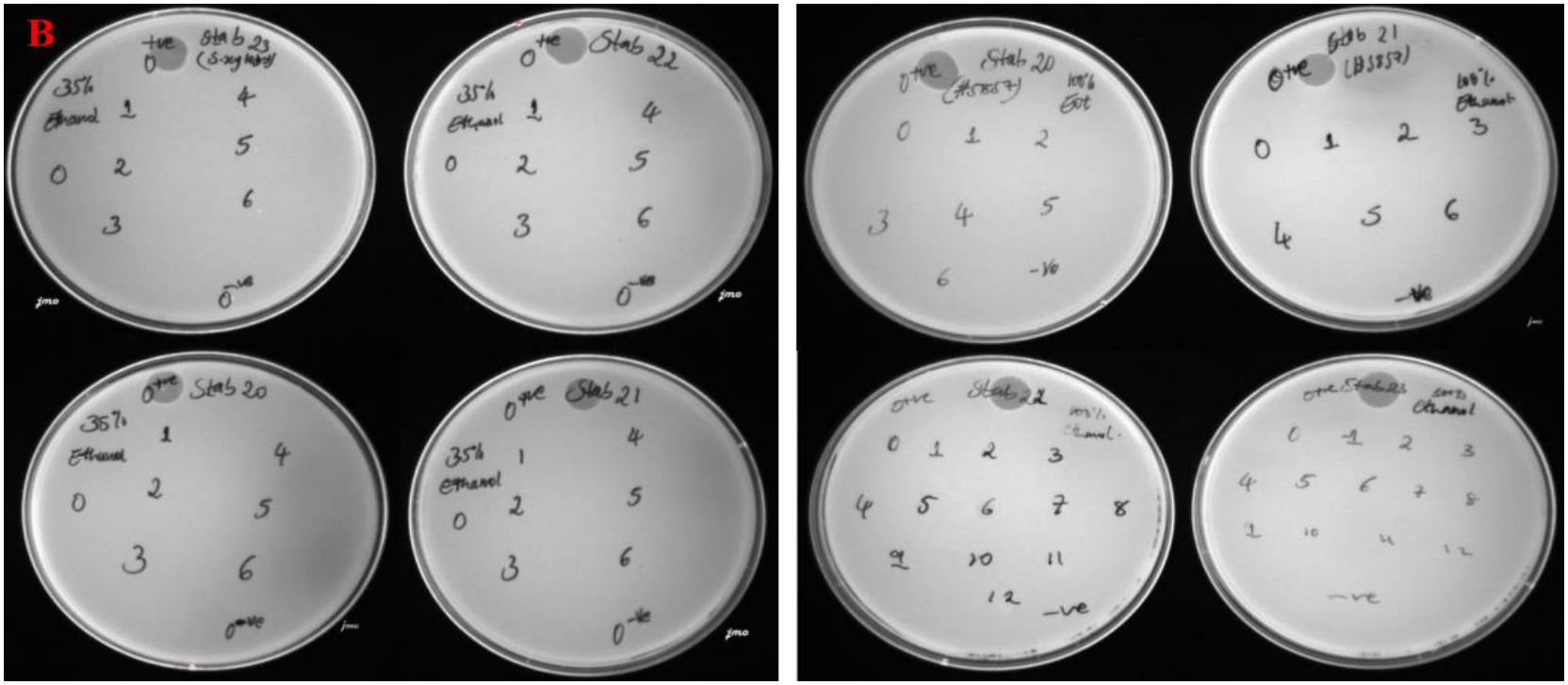


Figure 9: Stability of Stabs at various environmental conditions such as temperature (**A**), UV-irradiation **(B)** and pH **(C)**. The charts show the sensitivity of the phages to temperature, UV-irradiation and pH. Each data point shows the mean ± standard deviation for three independent experiments.



a. The phages were active at 25% vol/vol of ethanol/PBS but, inactive at 30% vol/vol of a similar medium.



b. The phages were inactive at either 35% or 100% vol/vol of ethanol/PBS.

Figure 10: Stabs' stability at ethanol concentration (vol/vol %). These viruses are denatured (killed) by ethanol concentration above 25%; (**A**) and (**B**).

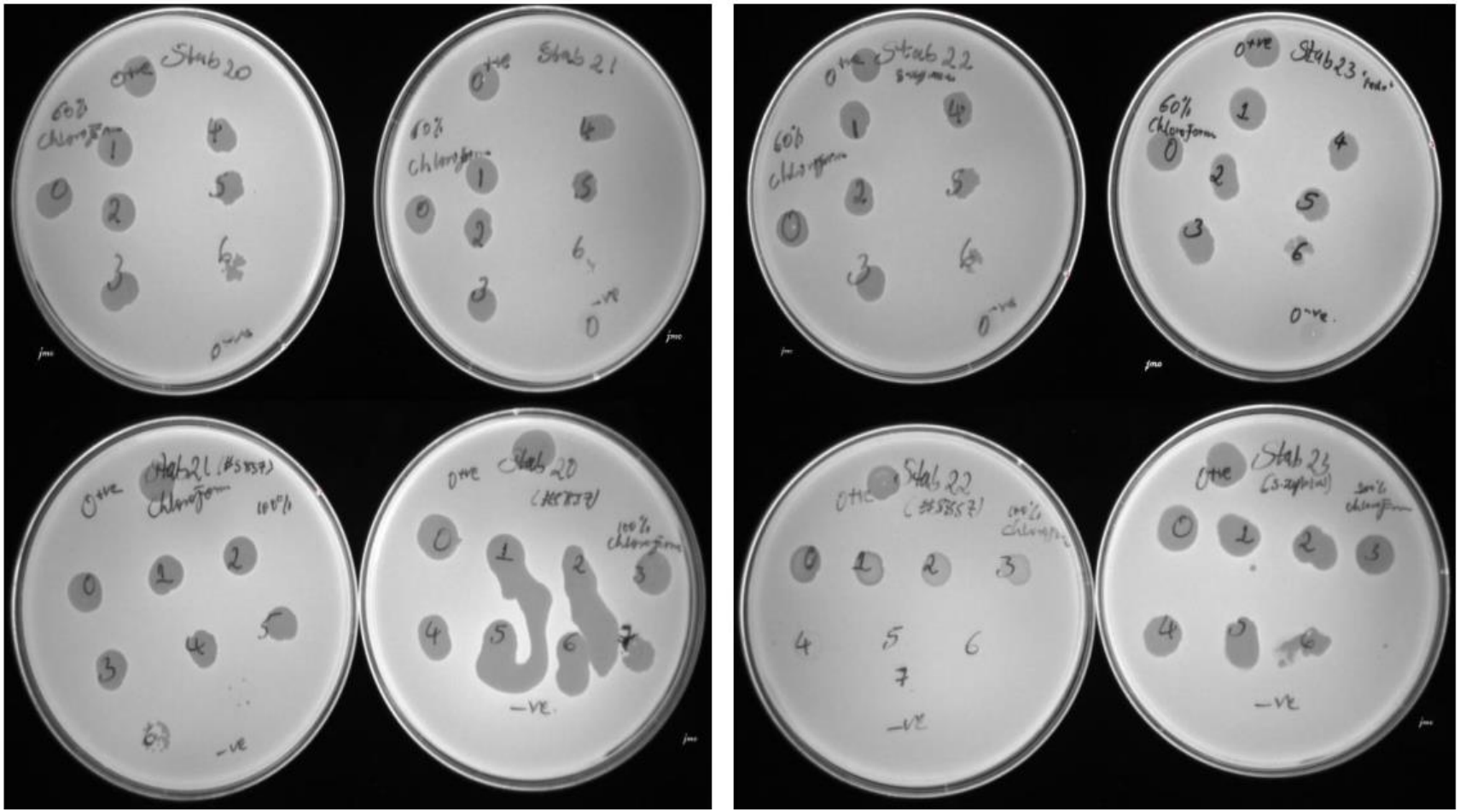


Figure 11: Stabs' stability at various chloroform concentration (vol/vol %). The phages exhibit resistance to chloroform.

4.4 Growth properties of the Stab phages

The Stabs exhibited distinct nature through their growth curves which shows varied adsorption rates and burst sizes. The adsorption curves represents the rate at which phages attach to their hosts, also known as adsorption kinetics (Storms & Sauvageau, 2015). Of the phages, Stab21 adsorbed rapidly, ca 90% in just 5 min while only 40, 60 and 70% for Stab20, Stab22 and Stab23 respectively in a similar moment (**Figure 12**). There were no observable significant variations between the phages' calculated adsorption rate constants for the 5 min time point. However, each phage displayed unique one step growth curve characterized by varied latent and lag phase per 30 min. The burst size varied between 42 and 130 (**Figure 13**).

4.5 Host range analysis of the phages

The tested strains that were positive with spot assay were further analysed with relative efficiency of plating (REOP) assay. In order to obtain countable plaques in all plates a dilution of 10^{-5} was used for the assay. This resulted to negative results with less virulent phage isolates. Dilutions between 10^{0} and 10^{-4} gave semi-confluent results with the control/host bacteria (*S. xylosus* DD-34) but few countable plaques with less sensitive strains. However, REOP can only be established with countable plaques. Subsequently <0.1 was considered the minimal REOP for strains that only had plaque counts at lower dilutions (10^{0} and 10^{-4}) (**Figure 14 - 18** & **Table 6**).

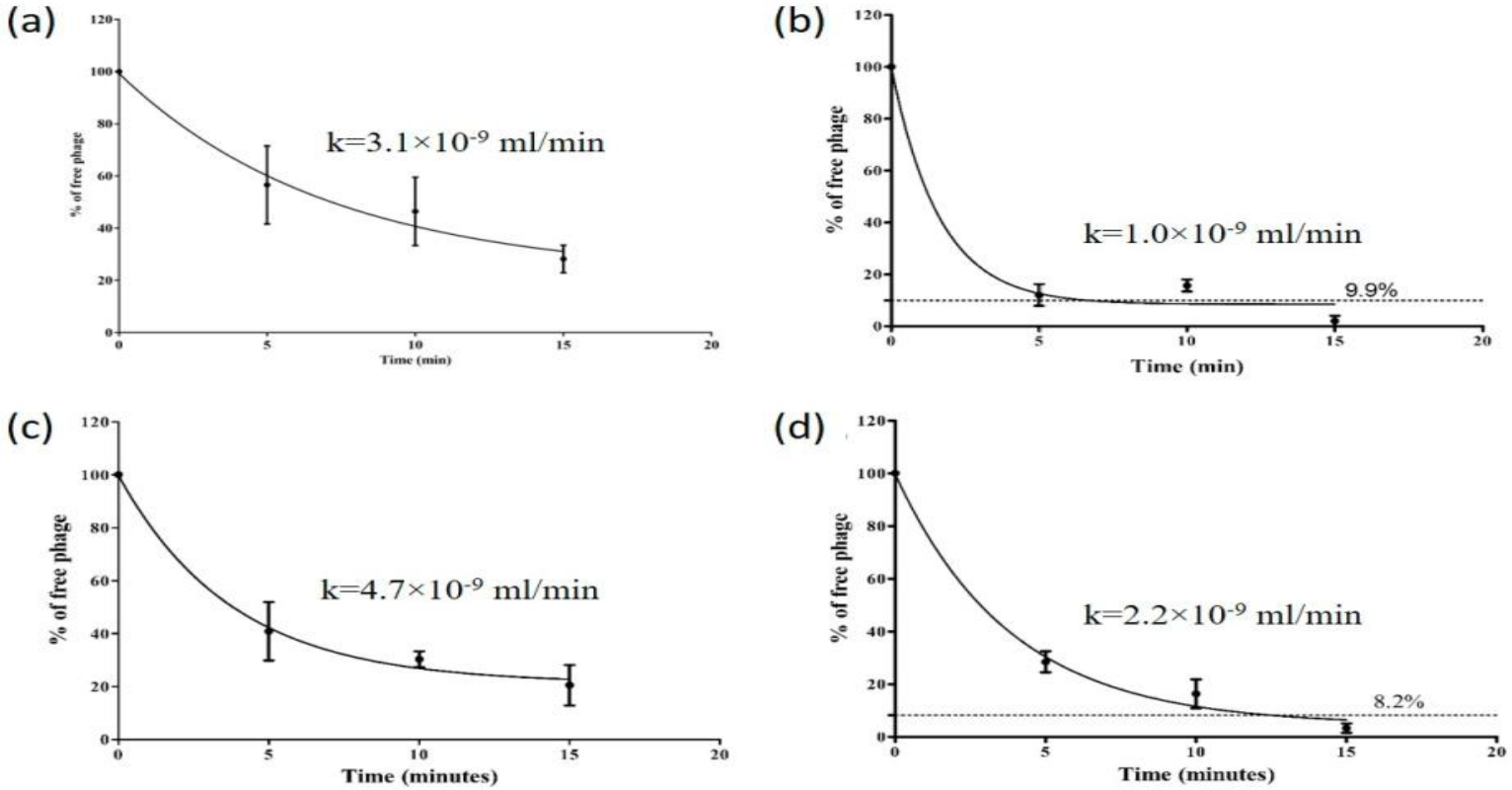


Figure 12: Adsorption curves and adsorption rate constants (**k**) of Stab20 (**a**), Stab21 (**b**), Stab22 (**c**) and Stab23 (**d**) displayed by phages when interacting with *S.xylosus* DD-34 as host bacteria at 37 ºC. The data is the average of three independent experiments carried out on separate days average bacterial titers were 7.2×10^8 CFU/mL (**a**), 3.93×10^7 CFU/mL (**b**), 3.83×10^7 CFU/mL (**c**), 1.2×10^8 CFU/mL (**d**). The point bars represents mean ± standard deviation.

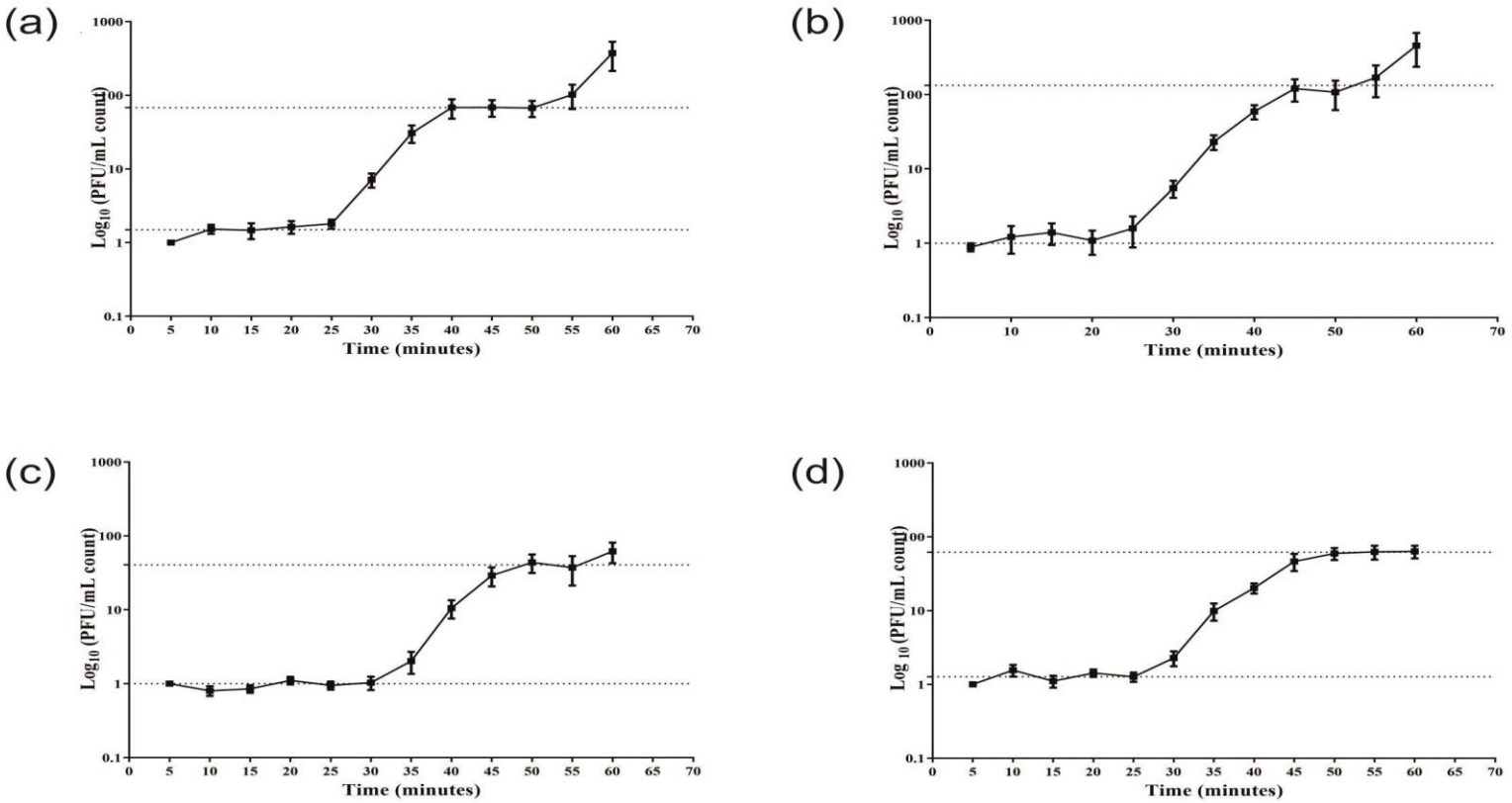


Figure 13: One step growth curves Stab20 (**a**), Stab21 (**b**), Stab22 (**c**) and Stab23 (**d**) in S.xylosus DD-34 when incubated at 37 ºC. The average burst size were 66, 130, 42 and 62 for Stab20, Stab21, Stab22 and Stab23, respectively. Each point data represent the mean ± standard deviation for eight independent experiments.

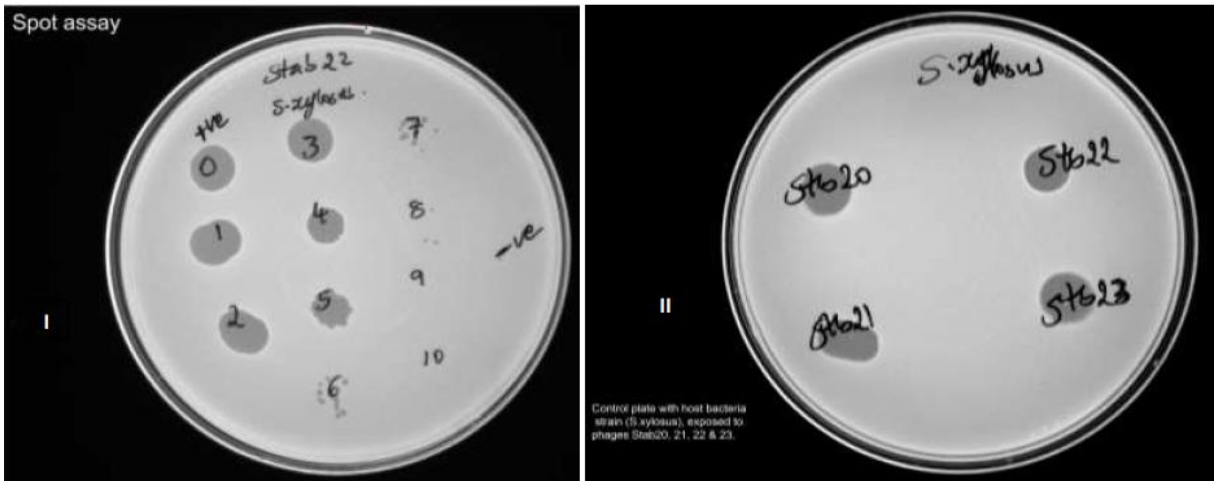


Figure 14: Efficiency of plating (EOP) (**I**) and Spot assay (**II**) of the Stabs on *S.xylosus* (indicator/host bacteria).

Figure 15: Spot assay tests of the Stabs on various *Staphylococcus* species.

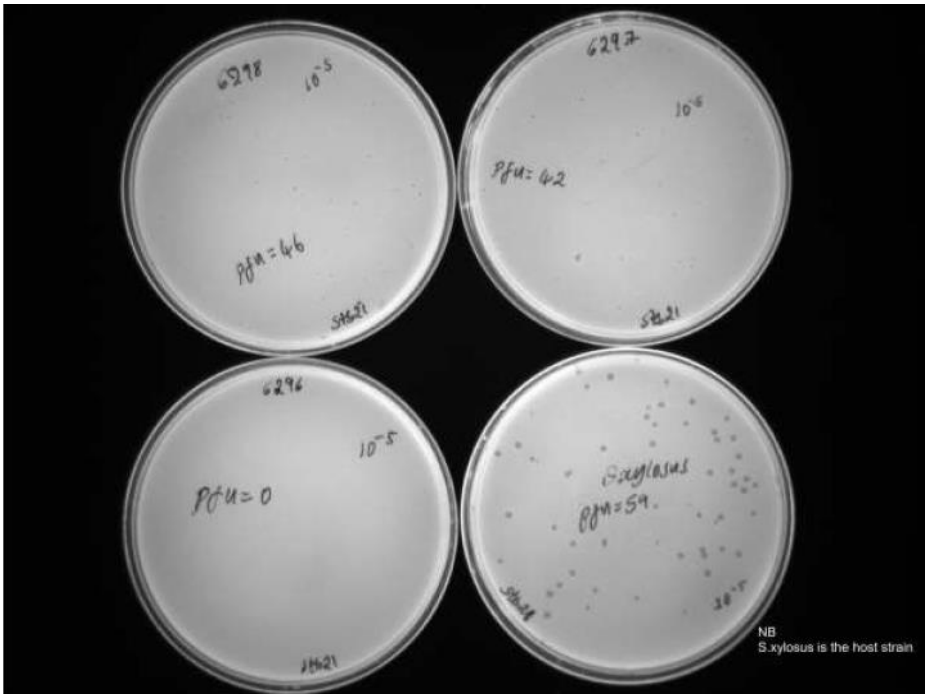


Figure 16: The relative E.O.P of Stab21 against *S.aureus* test strains 6298, 6297, 6296.

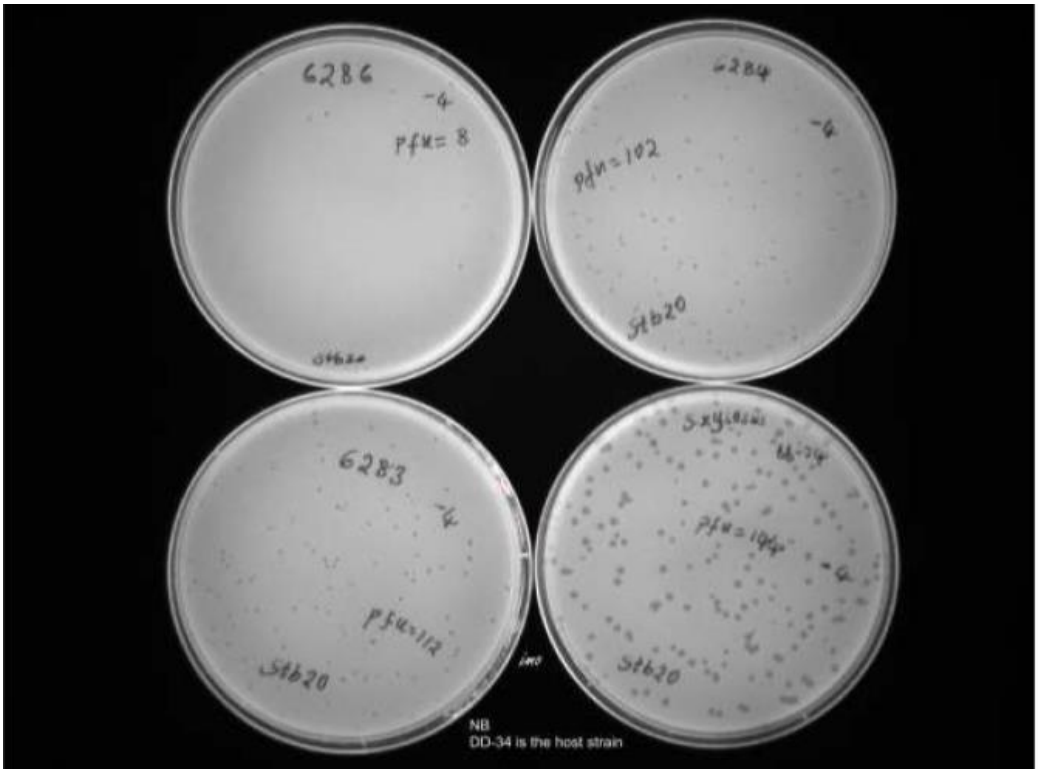


Figure 17: The relative E.O.P of Stab20 on various *Staphylococcus* spp (6283, 6284 & 6286).

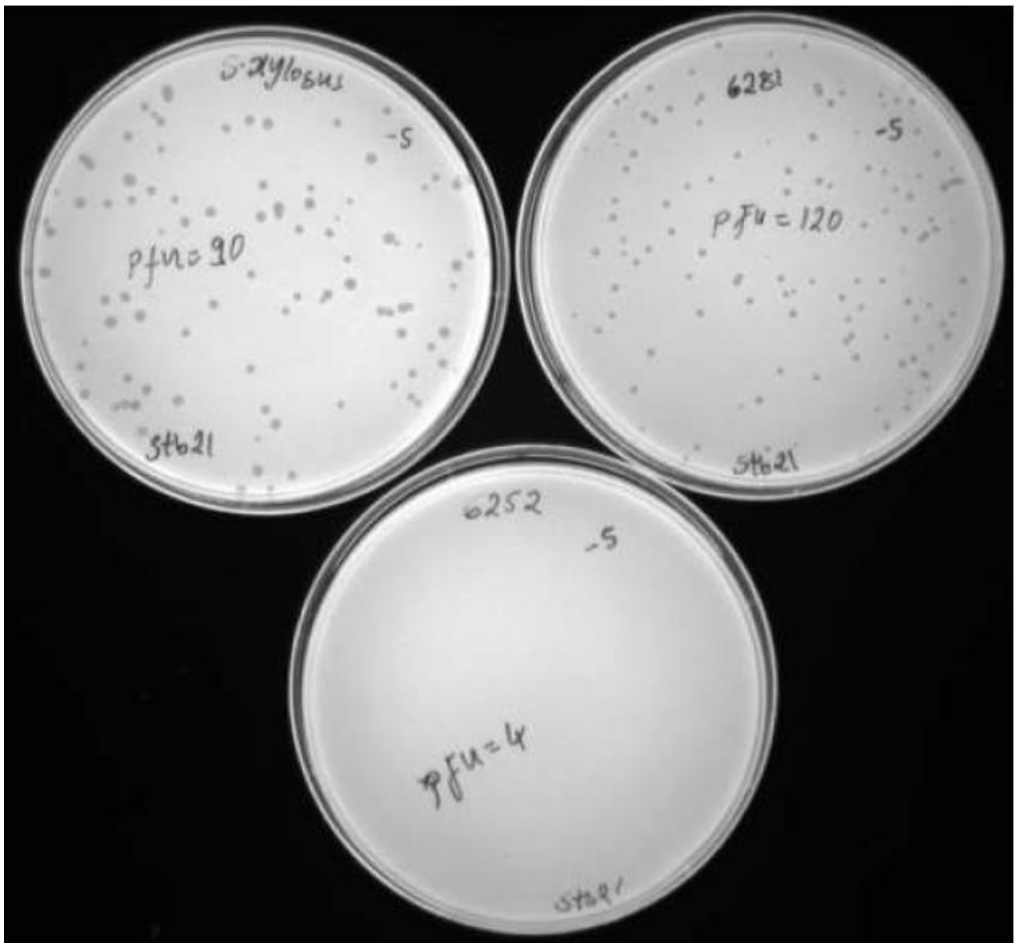


Figure 18: The relative E.O.P of Stab21 on various *Staphylococcus* spp (6281 & 6252).

Table 6: This list shows that Stabs produced lysis zones (positive) in some *Staphylococcus* spp but could not form plaques on their lawns (negative).

No.	Strains	Origin	ID	Spot assay					Average REOP			
				Stab20	Stab21	Stab22	Stab23		Stab20	Stab21	Stab22	Stab23
1	*S. aureus*	Human	5511	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
2	*S. aureus*	Human	5515	Pos	Pos	Neg	Neg		2.0	0.1	0.0	0.0
3	*S. aureus*	Human	5523	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
4	*S. aureus*	Human	5526	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
5	*S. aureus*	Human	5527	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
6	*S. aureus*	Human	5528	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
7	*S. aureus*	Human	5530	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
8	*S. aureus*	Human	5531	Pos	Pos	Neg	Neg		2.4	0.5	0.0	0.0
9	*S. aureus*	Human	5535	Neg	Pos	Neg	Neg		0.0	0.2	0.0	0.0
10	*S. aureus*	Human	5676	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
11	*S. aureus*	Human	5677	Pos	Neg	Neg	Neg		2.2	0.0	0.0	0.0
12	*S. aureus*	Human	5678	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
13	*S. aureus*	Human	5679	Pos	Pos	Neg	Neg		1.6	0.2	0.0	0.0
14	*S. aureus*	Human	5680	Pos	Neg	Neg	Neg		1.8	0.0	0.0	0.0
15	*S. aureus*	Human	5681	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
16	*S. aureus*	Human	5682	Pos	Pos	Neg	Neg		0.5	0.1	0.0	0.0
17	*S. aureus*	Human	5683	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
18	*S. aureus*	Human	5684	Pos	Pos	Neg	Neg		0.9	0.8	0.0	0.0
19	*S. aureus*	Human	5685	Pos	Pos	Neg	Neg		0.4	0.3	0.0	0.0
20	*S. aureus*	Human	5686	Pos	Pos	Neg	Neg		2.4	0.4	0.0	0.0
21	*S. aureus*	Human	5689	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
22	*S. aureus*	Human	5690	Pos	Pos	Neg	Neg		1.0	0.3	0.0	0.0
23	*S. aureus*	Human	5691	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
24	*S. aureus*	Human	5692	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
25	*S. aureus*	Human	5693	Pos	Pos	Neg	Neg		1.2	0.5	0.0	0.0

26	*S. aureus*	Human	5694	Pos	Pos	Neg	Neg		1.3	0.7	0.0	0.0
27	*S. aureus*	Human	5695	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
28	*S. aureus*	Human	5696	Pos	Pos	Neg	Neg		1.3	0.1	0.0	0.0
29	*S. aureus*	Human	5697	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
30	*S. aureus*	Human	5698	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
31	*S. aureus*	Human	5699	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
32	*S. aureus*	Human	5700	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
33	*S. aureus*	Human	5701	Pos	Pos	Neg	Neg		1.3	0.1	0.0	0.0
34	*S. aureus*	Human	5702	Pos	Pos	Neg	Neg		0.8	0.1	0.0	0.0
35	*S. aureus*	Human	5703	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
36	*S. aureus*	Human	5704	Pos	Pos	Neg	Neg		0.3	0.1	0.0	0.0
37	*S. aureus*	Human	5705	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
38	*S. aureus*	Human	5849	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
39	*S. aureus*	Human	5851	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
40	*S. aureus*	Human	5852	Pos	Pos	Neg	Neg		1.5	0.1	0.0	0.0
41	*S. aureus*	Human	5853	Pos	Pos	Neg	Pos		0.2	0.2	0.0	<0.1
42	*S. aureus*	Human	5854	Pos	Pos	Neg	Neg		0.6	0.4	0.0	0.0
43	*S. aureus*	Human	5855	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
44	*S. aureus*	Human	5856	Pos	Pos	Neg	Neg		1.7	1.1	0.0	0.0
45	*S. aureus*	Human	5857	Pos	Pos	Neg	Pos		0.7	0.3	0.0	0.0
46	*S. aureus*	Human	5858	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
47	*S. aureus*	Human	5859	Pos	Pos	Neg	Neg		2.2	2.1	0.0	0.0
48	*S. aureus*	Human	5860	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
49	*S. aureus*	Human	5861	Pos	Neg	Neg	Neg		0.6	0.0	0.0	0.0
50	*S. aureus*	Human	6209	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
51	*S. aureus*	Human	6210	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
52	*S. aureus*	Human	6211	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
53	*S. intermedius*	Human	6212	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
54	*S. intermedius*	Human	6213	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
55	*S. intermedius*	Human	6219	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
56	*S.epidermidis*	Human	6220	Pos	Pos	Neg	Neg		<0.1	<0.1	0.0	0.0

57	*S. epidermidis*	Human	6221	Neg	Pos	Neg	Neg		0.0	<0.1	0.0	0.0
58	*S. epidermidis*	Human	6222	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
59	*S. epidermidis*	Human	6223	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
60	*S. haemolyticus*	Human	6224	Pos	Neg	Neg	Neg		<0.1	0.0	0.0	0.0
61	*S. haemolyticus*	Human	6225	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
62	*S. haemolyticus*	Human	6226	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
63	*S. haemolyticus*	Human	6227	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
64	*S. haemolyticus*	Human	6228	Pos	Pos	Neg	Neg		<0.1	0.1	0.0	0.0
65	*S. saprophyticus*	Human	6229	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
66	*S. saprophyticus*	Human	6230	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
67	*S. saprophyticus*	Human	6231	Pos	Pos	Pos	Pos		1.8	1.3	<0.1	<0.1
68	*S. saprophyticus*	Human	6232	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
69	*S. saprophyticus*	Human	6233	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
70	*S. aureus* (MRSA)	Pig	6248	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
71	*S. aureus* (MSSA)	Pig	6249	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
72	*S. aureus* (MRSA)	Pig	6250	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
73	*S. aureus* (MRSA)	Pig	6251	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
74	*S. aureus* (MSSA)	Pig	6252	Pos	Pos	Neg	Neg		<0.1	0.1	0.0	0.0
75	*S. aureus* (MRSA)	Pig	6253	Pos	Pos	Neg	Neg		0.7	0.4	0.0	0.0
76	*S. aureus* (MRSA)	Pig	6254	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
77	*S. aureus* (MRSA)	Pig	6258	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
78	*S. aureus* (MRSA)	Pig	6259	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
79	*S. aureus* (MRSA)	Pig	6259	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
80	*S. aureus* (MRSA)	Pig	6260	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
81	*S. aureus* (MRSA)	Pig	6261	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
82	*S. aureus* (MRSA)	Pig	6262	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
83	*S. aureus* (MRSA)	Pig	6263	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
84	*S. aureus* (MRSA)	Pig	6264	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
85	*S. aureus* (MRSA)	Pig	6265	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
86	*S. aureus* (MRSA)	Pig	6266	Neg	Neg	Neg	Neg		0.0	0.0	0.0	0.0
87	*S. aureus* (MRSA)	Pig	6273	Pos	Pos	Neg	Neg		0.1	0.3	0.0	0.0

88	*S. aureus* (MRSA)	Pig	6274	Pos	Pos	Neg	Neg		0.9	0.4	0.0	0.0
89	*S. aureus* (MSSA)	Pig	6278	Neg	Pos	Neg	Neg		0.0	0.1	0.0	0.0
90	*S. aureus* (MRSA)	Pig	6280	Pos	Pos	Neg	Neg		0.4	0.3	0.0	0.0
91	*S. aureus* (MRSA)	Pig	6281	Pos	Pos	Neg	Neg		0.7	1.2	0.0	0.0
92	*S. aureus* (MRSA)	Pig	6283	Pos	Pos	Neg	Neg		0.8	1.0	0.0	0.0
93	*S. aureus* (MRSA)	Pig	6284	Pos	Pos	Neg	Neg		0.9	1.1	0.0	0.0
94	*S. aureus* (MRSA)	Pig	6286	Pos	Pos	Neg	Neg		0.1	0.4	0.0	0.0
95	*S. aureus* (MRSA)	Pig	6287	Neg	Pos	Neg	Neg		0.0	0.2	0.0	0.0
96	*S. aureus* (MRSA)	Pig	6288	Pos	Pos	Neg	Neg		<0.1	0.1	0.0	0.0
97	*S. aureus* (MRSA)	Pig	6295	Pos	Pos	Neg	Neg		0.4	0.5	0.0	0.0
98	*S. aureus* (MRSA)	Pig	6296	Pos	Pos	Neg	Neg		0.5	0.3	0.0	0.0
99	*S. aureus* (MRSA)	Pig	6297	Pos	Pos	Neg	Neg		0.4	0.5	0.0	0.0
100	*S. aureus* (MRSA)	Pig	6298	Pos	Pos	Neg	Neg		0.5	0.8	0.0	0.0
101	*S. xylosus*	**Sausage**	**DD-34**	Pos	Pos	Pos	Pos		1.0	1.0	1.0	1.0

REOP: relative efficiency of plating.

CHAPTER FIVE: DISCUSSION, CONCLUSION, LIMITATION, RECOMMENDATION AND SUGGESTION FOR FURTHER STUDIES

5.1 Discussion

The arm race between phage and bacteria is an ongoing issue that is never going to end any time soon. Regular update of the global phage bio-bank with novel and safe phage isolate is imperative (Oduor *et al.,* 2020; Yerushalmy *et al.,* 2020). In this study, four novel phages have been isolated and characterized with the objective of exploring their therapeutic or bio-remedial significance.

5.1.1 Indicator/host bacteria

Staphylococcus xylosus is a coagulase negative Staphylococcus that forms part of the mammalian skin bacterial flora. Some strains of the bacterium such as *S. xylosus* DD-34 are used in food processing (Kaur *et al.,* 2016; Leroy *et al.,* 2017). On rare occasions *S. xylosus* has been found to be pathogenic in human and livestock (Akhaddar *et al.,* 2010; Bochniarz *et al.,* 2014). Furthermore, some strains of *S. xylosus* harbor genes encoding antibiotic resistance or virulence factors. In addition, their genomes does possess mobile genetic elements like plasmids, prophages, phages and transposons that facilitates dispersal of lethal genes among *Staphylococcus* bacteria (Firth *et al.,* 2018; Kaur *et al.,* 2016). These factors necessitated the screening for presence of unwanted/lethal encoding genes within the genomes of Stab phages. However, the **DD-34** isolate used in this study was a food-quality *S. xylosus* strain free of genes encoding antibiotic resistance or virulence factors. Propagation of phages for therapeutic or bio-control purposes in food-grade bacteria is much safer than with clinical staphylococcus strains that often harbor lethal prophages and antibiotic resistant genes (Cervera-Alamar *et al.,* 2018; Haddad *et al.,* 2014). The **DD-34** strain bacteria are used in meat processing industry (Møller *et al.,* 1998).

5.1.2 Characterization of Stab phages

The International Committee on Taxonomy of Viruses (ICTV) classifies the viruses based on morphological properties and the Baltimore system. Viruses appear in

various forms such as cylindrical/rod-like, icosahedral and complex. Staphylococcus phages are complex in structure since they consist of cylindrical and icosahedral features.

The morphological analysis of the Stab20, Stab21, Stab22 and Stab23 phages showed that they are myoviruses (**Figure 2**). These phages had long contractile tails ending with complex appendage (baseplate), full tail fibers and large icosahedral symmetrical heads. These features are similar to those observed in other previously isolated myovirus phages such as phages K, vB_SauM_Remus, JD007, and Sb_1 (Cui, Feng, *et al.*, 2017; Gutiérrez *et al.*, 2015; Kvachadze *et al.*, 2011; Łobocka *et al.*, 2012; Vandersteegen *et al.*, 2013). These findings show that the Stabs are closely associated with members of the subfamily *Twortvirinae* of *Kayvirus* genus. The dimensions of isolated viruses were 83.9-94.3 nm, 163.1-201.6 nm, 20.3-23.3 nm and 41.8-48.1 nm for head, tail, tail width and baseplate width, respectively (**Table 1**). These measures fell within the values of other *Kayvirus* genus phages (Ajuebor *et al.*, 2018; Cui *et al.*, 2017; Rees & Fry, 1981).

Stabs had 153.3kbp to 155.9 kbp genomes, larger than most viruses in their subfamily (*Twortvirinae*) (https://blast.ncbi.nlm.nih.gov/Blast.cgi; https://talk.ictvonline.org/). This huge genome size variation indicates that the Stabs might be members of a new genus within the *Twortvirinae* subfamily. However, analysis of nucleotide identity of these phages showed that they are associated with major typed viruses within the group of *Twortvirinae*. The results further affirmed their association with the genus *Kayvirus* phages such as Staphylococcus phage K (**Table 3**).

Stab20, Stab21, Stab22 and Stab23 genomes consist of 58%, 71%, 61% and 63% hypothetical proteins respectively. Most of these hypothetical proteins are homologous with those found in *Twortvirinae* phages (Barylski *et al.*, 2020; Imam *et al.*, 2019). Proteomic analysis of the Stabs designated some hypothetical proteins as structural proteins but the functions of a number of them could not be ravelled (**Appendix III**). Phylogenetic findings indicate that they are all members of this

genus in the *Twortvirinae* subfamily (**Figure 6**). In addition, it further supports similarity between Stab20 and Stab21, and Stab22 and Stab23 that had been observed by EMBOSS analysis **(Table 3)**. These observations illustrate the uniformity amongst the predicted phage-encoded proteins (**Table 5)**. In addition, SDS-PAGE analysis further affirms the close phylogenetic association of the Stabs (**Figure 8**). However, comprehensive protein analysis shows that these viruses are of different species as none of them is 95% identical to one another (**Table 3**) (Barylski *et al.,* 2020). Morphological and genomic analyses clearly illustrate that these phages are new members of the *Twortvirinae* subfamily. The ICTV groups all *Staphylococcus / Lactobacillus* infecting phages with genomes 135-150 kbp as *Twortvirinae* but the Stabs have larger genomes (https://talk.ictvonline.org/taxonomy/). This implies that there is need to adjust the classification criteria *Staphylococcus* myoviruses to accommodate bigger staphylococci phages. Alternatively, the Stabs and other phages with such genomes (150 kbp>) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) ought to be placed under a new genus but within *Twortvirnae* subfamily.

5.1.3 Screening for lethal genes

The Stabs were free of all lethal unwanted genes encoding for toxins, antibiotic resistance and integrase, and chromosomal point mutations (**Table 4**). Absence of integrase encoding genes indicates that these phages cannot engage in a lysogenic lifestyle with the target bacteria as observed with siphoviruses (J. Wang *et al.,* 2019). Therefore, if used for therapeutic or as sanitizers these phages would not pass lethal genes to the target bacterial pathogens. The absence of the antibiotic resistance-conferring genes such as *dfrB, fusA, grlA, grlB, gyrA, ileS,* and *rpoB* carrying point mutations or the *pbp4* promoter of 304 bp in size (**Table 4**), asserts safety of the Stabs. Furthermore, it infers the inability of these phages to instigate bacterial resistance to antibiotic classes such as beta-lactam, rifampin, ciprofloxacin, mupirocin, linezolid and trimethoprim-sulfamethoxazole drugs (Chatterjee *et al.,* 2017; Harris *et al.,* 2018; Iguchi *et al.,* 2016; Lai *et al.,* 2018). In addition, the results deduce the inability of the Stabs to physically release unwanted lethal genes into the

environment for uptake by other bacteria. These findings concur with the results of previous studies which describes the safety and therapeutic efficacy of phages related with the Stabs such as ISP and Sb_1 (Kvachadze *et al.,* 2011; Vandersteegen *et al.,* 2011).

5.1.4 Stability status of the Stabs

Phage stability determines the efficacy and application of the microbe either as therapeutic or bio-control agent. In addition, it is a pertinent factor to during packaging, shipment of phage cocktails and storage. The Stabs were stable for 1 hr at 35˚C with over 90% viable phage particles. Incubation at temperatures over 40℃ for 1 hr resulted in denaturing the phages with more than 85% decrease in viability (**Figure 9A**). These results concur with numerous previous studies which shows that *Staphylococcus* phages of either therapeutic or biocontrol significance are known to have an optimum viable temperature of 37 ± 2℃ (Cui *et al.,* 2017; Vandersteegen *et al.,* 2013). However, certain studies indicates that thermal stability of the phages is directly associated with the host bacteria temperature tolerance levels. Previous findings have shown that phages isolated from regions with high temperatures or mammals with high body temperatures like birds have high thermal stability. The reverse of this observation is notable with phages from cold regions (Borriss *et al.,* 2003; Cui *et al.,* 2017; Prestel *et al.,* 2013). Besides temperature, storage media also determines the shelf life of phages. Stabs depicted long shelf life in SM-buffer at +4 ˚C with a viability loss of 20% in 12 months. However, their viability in normal saline media at a similar temperature dropped to about 50%. This property does influence the application and storage of phage.

Ultra-violet (UV) energy is destructive to almost all biological life forms but with lethal dose variations. The toxic radiation energy that kills the host is often sufficient to destroy the predator virus as observed with this work. Radiation energy of UV at 25µJ/cm^2 or more resulted to over 50% reduction of viable pfu/mL for each phage ($p < 0.0001$) (**Figure 9B**). This illustrates how sensitive the Stab phages are to UV energy; a feature that has been observed with other myoviruses (Ramirez *et al.,*

2018). Unlike the podoviruses that are known to be stable even at high UV irradiation (Wang *et al.,* 2016). This indicates that Stabs cocktail for topical applications require precise formulation with ingredients that protects them from UV-irradiation for them to be effective.

Acidity or alkalinity of the medium does influence the potency of a phage. Phages isolated in this study were stable at a broad pH range of 5.4 - 9.4 at 37 ˚C. However, their performance was much better at pH 9.4 than at pH 5.4. Stab20 was at pH 5.4 statistically ($p < 0.01$) more stable than Stab21, Stab22 and Stab23. Nonetheless, these phages tolerate alkaline conditions at pH 9.4 but with 40% viability reduction (**Figure 9C**). The results show that oral administration of Stabs to patients without modification to protect them from the acidic environment of the stomach is impossible. However, they can be issued to patients as rectal and urethral suppositories since their pH stability coincides with these organs' pH 6.0 – 8.0 (Bono & Reygaert, 2019; Turner *et al.,* 2012). In addition, they can be administered nasally as aerosols to patients since the nasal pH 5.5- 6.5 favour their existence (Baroody, 2011). These findings corroborate other pH endurance observations made on other members of *Kayvirus* genus isolated from a similar environment but elsewhere. Phage JD007 and phiIPLA-RODI have shown tolerance to broad range of pH 5-11 but at room temperature (Cui *et al.,* 2017; Gutiérrez *et al.,* 2015). These results signify the influence of the phage source to its pH endurance range and the microbe's origin as noted in other phages like of the acidophiles and halophiles (Akhwale *et al.,* 2019; Yu *et al.,* 2006).

Organic solvents are destructive to many viruses but not all. Viruses with high lipid capsulation or envelope are more susceptible to denaturation by organic solvents than those with no or low lipid content (naked virus) (Rheinbaben *et al.,* 2007). This characteristics is observables among phages which are prokaryotic viruses but with variations from one to another (Jurczak-Kurek *et al.,* 2016). Observation made on the Stab phages deduced that they were highly sensitive to ethanol concentration (volume-by-volume percentage – Vol/Vol %) in SM-buffer or PBS above 25%

Vol/Vol (**Figure 10**). However, they were tolerant to 0-100% Vol/Vol chloroform concentration on similar buffers (**Figure 11**). These outcomes are in line with other findings which showed myoviruses to be less sensitive to chloroform but highly susceptible to ethanol (Jurczak-Kurek *et al.,* 2016).

5.1.5 Growth properties of the novel phages

In this work, the adsorption rates and burst-sizes of the phages (**Figure 12** and **Figure 13**) inversely correlated for certain phages. Direct association of the two growth factors was shown for Stab20 (3.1×10^{-9}mL/min; 66 pfu), Stab21 (1.0×10^{-10}mL/min; 130 pfu) and Stab23 (2.2×10^{-9}mL/min; 62). Stab22 (4.7×10^{-9}mL/min; 42) had a high adsorption rate but with less progeny output. Adsorption rates for these phages were greater compared to those of Staphylococcus phage K and DRA88 but less to those of phages phi812 and SK311. (Alves *et al.,* 2014; Pantůcek *et al.,* 1998). In addition, adsorption curves of these phages illustrate that at no time interval was the culture medium free of phages. This suggests that the lowest free-phage count was an equilibrium point at which the rates of adsorption and replication of the phages were equivalent. This duration is what defines the latent phase of one-step growth curve of phages. 25 min, 20 min, 30 min and 25 min were the latent periods for Stab20, Stab21, Stab22 and Stab23 respectively. Eclipse period occurs when there is active production of phages per actively infected bacteria. Replication rate is what determines the phage's eclipse period and subsequently its burst-size. Moreover, emergence of mutant phage-resistant bacterial strains is associated with low phage outburst pace. Since the bacterium has more chances of evolving against the predator and consequently colonization of the medium with mutant strains. However, the appearance of lag phase is often due to establishment of old bacteria population in the medium which does not support rapid phage replication (Bull & Gill, 2014). The curves also show the susceptibility of a target bacterium varies from one phage to another. Furthermore, it is worth noting that adsorption is just a physical property of a phage. Therefore, adsorption rate is not directly associated with a phage's burst size since phage can adsorb to dead bacteria (Krueger, 1931). Stab22 (4.7×10^{-9}mL/min; 42) had a high adsorption rate but with less progeny output

which suggests that it might be viable therapeutically, only if used in large numbers. Therapeutic or bio-control potential of a phage is determined by its growth properties which includes adsorption rate and burst-size. Since phages with fast adsorption rates and large progeny, outputs are suitable for bio-remediation. Nonetheless fast adsorption and low burst-size may be important specifically in passive bioremediation where there are low bacterial concentrations (Bull & Gill, 2014). However, phage's exhibition of these properties may vary from one bacteria to another as observed with Staphylococcus phage philPLA-RODI (González-Menéndez *et al.,* 2018).

5.1.6 Host range analysis

Determination of the antibiogram plays a similar role in the use of antibiotic therapy as the determination of the phagogram in the use of phage therapy. In this work, host range analysis infers to robust phagogram since it entailed both spot and relative efficiency of plating (REOP) assays. A confluent bacterial lawn or a significantly low plaque count on the target-strain accompanied with low REOP (<0.1) as compared to host bacteria inferred to a negative result. Medium or large REOP (>0.1) backed with large zone of lysis inhibition marked a positive outcome. Greater REOPs (>2) indicated higher efficacy of phage against a target strain (**Figure 18**). Spot assay is the preferred technique for host range analysis in most labs since it is less demanding in terms of time and cost. However, this is not the efficient way of determining the virus virulence against bacteria because it can mislead. Occurrence of inhibition zones might be due to phage's lysis from without due to residual endolysin or bacteriocin in the lysate. Furthermore, they could be due to excessive adsorption of bacteria with high phage titre resulting to irreversible extensive damage to the bacteria (Abedon, 2011). False positives in the analysis is eliminated through spotting of serially diluted stock lysate on bacterial lawn (Kutter, 2009). In this study spot analysis samples were lysate diluent of 10^{-5} with a definite predetermined plaque counts of about 50 – 200 pfu/mL to eliminating the effects of residual bacteriocin and endolysin. However, spot assay does not exhibit the exact fitness of phages against their target bacteria. Reason being that the virus particles might only

be concentrated at the specific point of application during the assay. In addition, the phage might encounter a resistance from the target bacteria in form of abortive infection which inhibits its replication (Hyman & Abedon, 2010).

Positive results exhibited by this study's modified spot assay were further analysed with efficiency of plating (E.O.P) using double agar layer method. The outcome plaque counts varied from one-target bacteria to another compared with the host's plaques. In certain instance, the plaques were higher for the tested strains than for the original host bacteria and vice versa (**Figure 18**). There were no plaques in some bacteria strains which had previously tested positive with spot assay analysis such as strain ID 6220 and 6221 (**Table 6**). In addition, the phages exhibited different plaque sizes from one strain of test bacteria to another. Most susceptible bacteria did present large plaques (≈1.5 – 2.0 mm) while least sensitive strains either had pinpoint plaques (<1 mm) or none (**Figure 16- 18**). The counts ratios provided relative E.O.P (R.E.O.P) values that showed the fitness of each phage against specific bacteria. *Staphylococcus* species/strains susceptible to Stabs produced large R.E.O.P and the lowest or none for non-susceptible strains (**Table 6**).

Abortive infections and emergence of mutant phage-resistant bacteria are detectable through efficiency of plating by spot and double agar layer assays. However, bacteriophage-insensitive mutants (BIMs) assay is the accurate means of determining emergence of phage-resistant strains among susceptible bacteria. Double agar assay is the most preferable means for determining the fitness of a phage against target-bacteria. High REOP should be the determinant factor when selecting a phage for bioremediation purposes. Classical Spot or classical efficiency of plating method (modified spot assay) is full of setbacks and might be influenced by residual bacteriocin or endolysin in the lysate. Failures of these techniques have been noted in some recent phage therapy works where bacteria have turned resistant against candidate phages during treatment (Krylov *et al.,* 2016; Schooley *et al.,* 2017). Despite the shortcomings spot and E.O.P, the assays are suitable in establishing the polyvalent nature of a phage. These assays illustrate Stab20 as a polyvalent phage

with large REOP across various targeted strains (Table 6) inferring to it potential as candidate for bioremediation purposes. However, Stab22 and Stab23 are monovalent phages due to their narrow host range. The phages grow well in *S. xylosus* but poorly or do not produce plaques in other species/strains of *Staphylococcus*.

5.2 Conclusion

In this study I have isolated the phages, and characterized them morphologically and genomicaly to elucidate their taxon. Structural analysis by TEM unveiled their morphology that consisted of icosahedral head attached to a tail with baseplate at the other end. The outcome of phylogenetic analysis showed that they are novel phages of the *Kayvirus* genus, *Twortvirinae* subfamily and *Herelleviridae* family of the order *Caudoviralea.* Genomic analysis depicted that they were free of lysogen, bacterial-virulence/chromosomal point mutations and antibiotic resistance encoding genes. In addition, the Stabs' genomes possess several hypothetical proteins but their functions could not be determined experimentally.

The findings further showed that the phages now known as Stabs (Stab20, Stab21, Stab22 and Stab23) are sensitive to U.V irradiation and temperatures over 40 ºC. Thermal and UV findings exhibits these phages' potential as topical anti-staphylococcal agents against SSTIs caused by staphylococcus bacteria. However, they are stable at pH range of 5.4 to 9.4, in chloroform but very sensitive to ethanol concentration above 25% vol/vol. In addition, they have varied growth properties as displayed by their adsorption and one step growth curves. The curves present Stab21 as a more virulent phage compared to other Stabs. Nonetheless, the growth activity of a phage directly correlated with the host bacteria. The two-fold higher plaque formation depicted by Stab20 in some *Staphylococcus* strains tested in the study compared to that in the original host clearly shows that phage's replication varies from one bacterium to another.

Exhibition of broad host range by Stab20 and Stab21 proves that these viruses have bio-remedial potential application. However, the poor lytic activities presented by Stab22 and Stab23 did not mean that they are of no significance. The specificity of Stab22 and Stab23 is of great importance especially in the events of tackling *Staphylococcus* sp specifically sensitive to them. In addition, they are of immense potential as candidates for phage therapy cocktails or bio-control products. The Stabs

are in the Skurnik lab phage bio-bank for possible use in case of emergency phage therapy where conventional antibiotics have failed.

5.3 Limitation of the study

Shortage of funds and time made it impossible to deduce functions of Stabs' numerous hypothetical proteins and *in vitro* ability of Stabs to act as "superspreaders". Host range analysis exhibits the Stabs potential as therapeutic and bio-control agents against *Staphylococcus aureus* and *Staphylococcus* sp. However, *S. aureus* clusters were not identified to establish whether they represented dominant circulating pathogens within either Finland, Europe or globally.

Genomic analysis of the phages suggested that they are safe but for clarity, there is need to assess it *in vivo* with an appropriate animal model.

5.4 Recommendations

There is need for more funding on phage biology/application research to unlock the therapeutic and bioremediation potential of these microbes.

5.5 Suggestion for further studies

There is need to study the significance of hypothetical proteins to phages' biological activities. The approach might involve studying virulence of phages with knocked-out hypothetic proteins (genetically engineered phages with the proteins removed). Alternative could be the harvesting and purification of these phage proteins. Thereafter, test their toxicity against battery of bacteria as described by Ushanandini and her group (Mohanraj *et al.,* 2019). Understanding of these proteins might be the key to development of better and effective novel antibacterial drugs. Finally, there is need to establish whether Staphylococcus phages can act as "superspreaders".

CHAPTER SIX: REFERENCES

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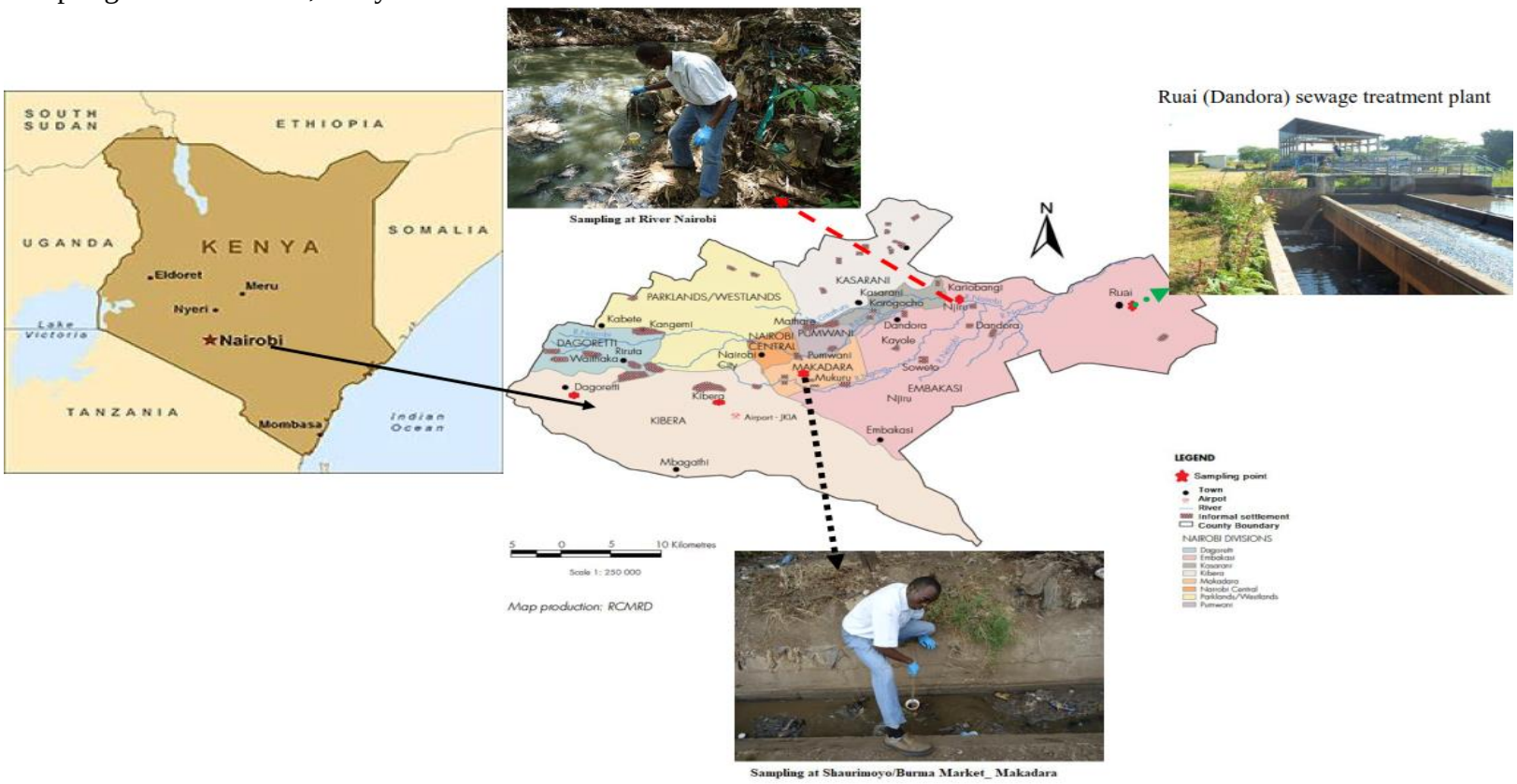
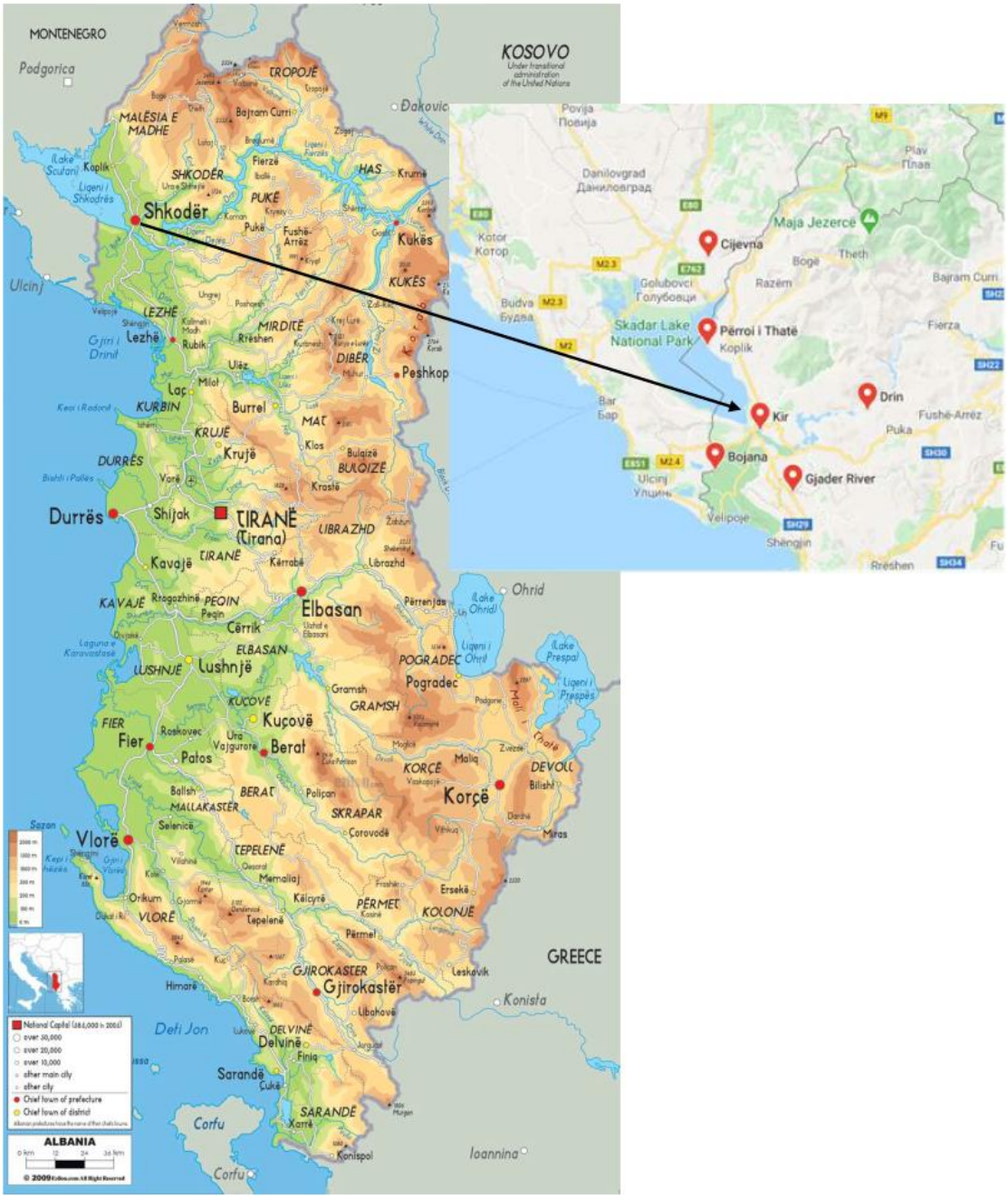
APPENDICES

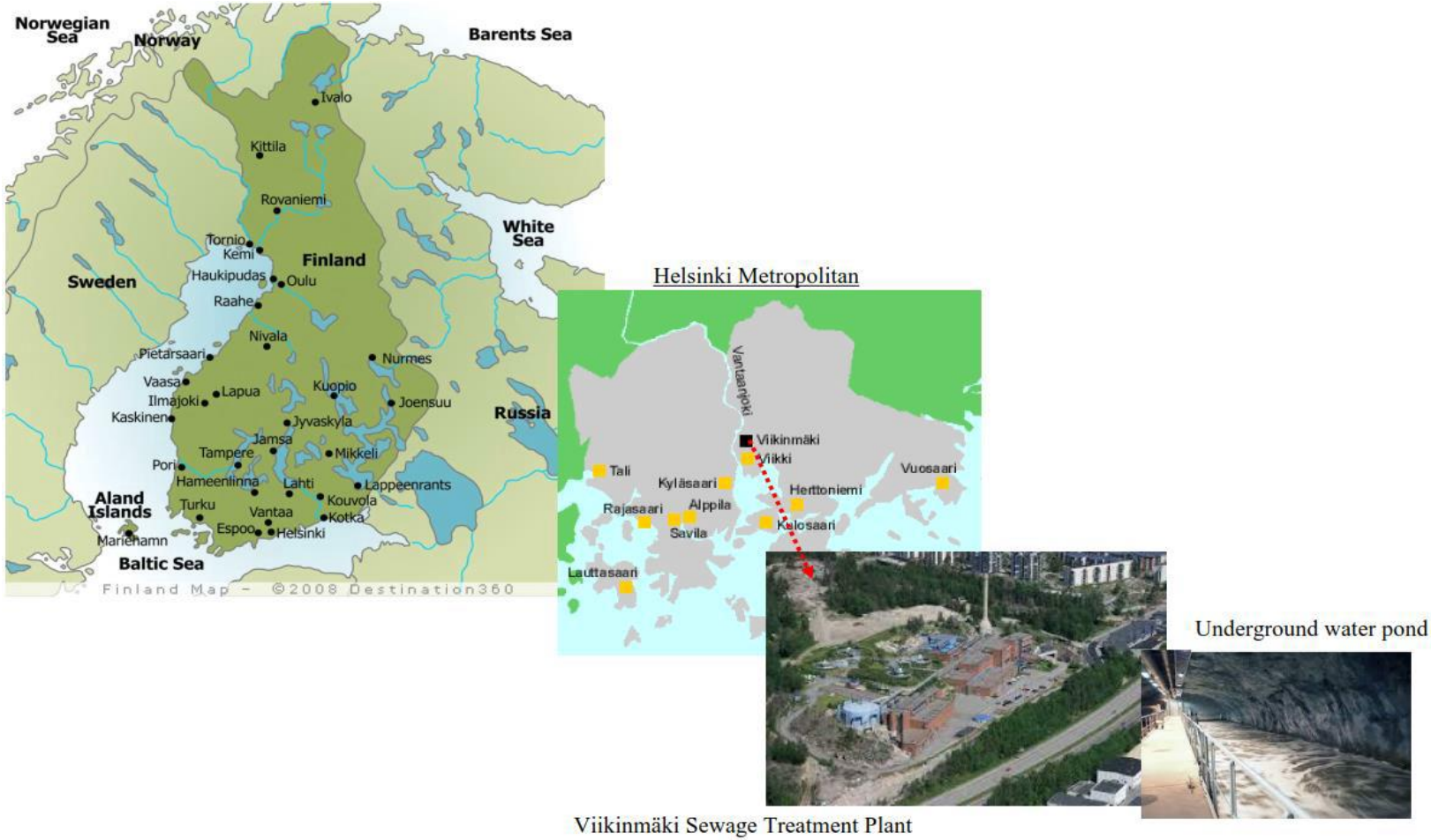
Appendix I: Study sites.
Sampling sites in Nairobi, Kenya.



Sampling site in Shkodra, Albania.



Sampling site at Helsinki metropolitan, Finland.



Viikinmäki Sewage Treatment Plant

Appendix II: Bacterial strains.

A table of bacterial strains used in the study and their origin. These bacteria are from either humans or livestock (pigs).

No.	Strains	ID	Origin
1	*Staphylococcus aureus* (MSSA)	5511	Human (blood)
2	*S. aureus* (MRSA)	5515	Human*
3	*S. aureus* (MSSA)	5523	Human (blood)
4	*S. aureus* (MSSA)	5526	Human (blood)
5	*S. aureus* (MSSA)	5527	Human (blood)
6	*S. aureus* (MSSA)	5528	Human (blood)
7	*S. aureus* (MSSA)	5530	Human (blood)
8	*S. aureus* (MSSA)	5531	Human (blood)
9	*S. aureus* (MSSA)	5535	Human (blood)
10	*S. aureus* (MSSA)	5676	Human*
11	*S. aureus* (MSSA)	5677	Human (abscesses)
12	*S. aureus* (MSSA)	5678	Human (skin wound)
13	*S. aureus* (MSSA)	5679	Human (skin wound)
14	*S. aureus* (MSSA)	5680	Human (sputum)
15	*S. aureus* (MSSA)	5681	Human *
16	*S. aureus* (MSSA)	5682	Human (skin wound)
17	*S. aureus* (MSSA)	5683	Human (abscesses)
18	*S. aureus* (MSSA)	5684	Human (skin wound)
19	*S. aureus* (MSSA)	5685	Human (genital skin)
20	*S. aureus* (MSSA)	5686	Human (finger scar)
21	*S. aureus* (MSSA)	5689	Human (skin tissue)
22	*S. aureus* (MSSA)	5690	Human (skin wound)
23	*S. aureus* (MSSA)	5691	Human *
24	*S. aureus* (MSSA)	5692	Human (skin scar)
25	*S. aureus* (MSSA)	5693	Human (skin wound)
26	*S. aureus* (MRSA)	5694	Human (decubitus)
27	*S. aureus* (MSSA)	5695	Human (skin wound)
28	*S. aureus* (MRSA)	5696	Human (skin scar)
29	*S. aureus* (MRSA)	5697	Human *
30	*S. aureus* (MRSA)	5698	Human (nose)
31	*S. aureus* (MRSA)	5699	Human (throat & nose)
32	*S. aureus* (MRSA)	5700	Human (throat & nose)
33	*S. aureus* (MRSA)	5701	Human (blood)
34	*S. aureus* (MRSA)	5702	Human (throat)

35	*S. aureus* (MRSA)	5703	Human (throat & nose)
36	*S. aureus* (MRSA)	5704	Human (throat)
37	*S. aureus* (MRSA)	5705	Human (abscess)
38	*S. aureus* (MRSA)	5849	Human (throat)
39	*S. aureus* (MRSA)	5851	Human (skin scar)
40	*S. aureus* (MRSA)	5852	Human (conjunctiva)
41	*S. aureus* (MSSA)	5853	Human (skin scar)
42	*S. aureus* (MSSA)	5854	Human (skin wound)
43	*S. aureus* (MSSA)	5855	Human (skin wound)
44	*S. aureus* (MSSA)	5856	Human (skin tissue)
45	*S. aureus* (MSSA)	5857	Human (skin scar)
46	*S. aureus* (MSSA)	5858	Human sputum
47	*S. aureus* (MSSA)	5859	Human (skin scar)
48	*S. aureus* (MSSA)	5860	Human (abscess)
49	*S. aureus* (MSSA)	5861	Human (joint fluid)
50	*S. intermedius*	6209	Human (skin scar)
51	*S. intermedius*	6210	Human (conjunctiva)
52	*S. intermedius*	6211	Human (wound)
53	*S. intermedius*	6212	Human (skin scar)
54	*S. intermedius*	6213	Human (skin scar)
55	*S. epidermidis*	6219	Human (blood)
56	*S. epidermidis*	6220	Human (blood)
57	*S. epidermidis*	6221	Human (blood)
58	*S. epidermidis*	6222	Human (blood)
59	*S. epidermidis*	6223	Human (blood)
60	*S. haemolyticus*	6224	Human (blood)
61	*S. haemolyticus*	6225	Human (blood)
62	*S. haemolyticus*	6226	Human (blood)
63	*S. haemolyticus*	6227	Human (blood)
64	*S. haemolyticus*	6228	Human (blood)
65	*S. saprophyticus*	6229	Human (urine)
66	*S. saprophyticus*	6230	Human (urine)
67	*S. saprophyticus*	6231	Human (urine)
68	*S. saprophyticus*	6232	Human (urine)
69	*S. saprophyticus*	6233	Human (urine)
70	*S. aureus* (MRSA)	6248	Pig
71	*S. aureus* (MSSA)	6249	Pig
72	*S. aureus* (MRSA)	6250	Pig
73	*S. aureus* (MRSA)	6251	Pig
74	*S. aureus* (MSSA)	6252	Pig

75	*S. aureus* (MRSA)	6253	Pig
76	*S. aureus* (MRSA)	6254	Pig
77	*S. aureus* (MRSA)	6258	Pig
79	*S. aureus* (MRSA)	6259	Pig
80	*S. aureus* (MRSA)	6260	Pig
81	*S. aureus* (MRSA)	6261	Pig
82	*S. aureus* (MRSA)	6262	Pig
83	*S. aureus* (MRSA)	6263	Pig
84	*S. aureus* (MRSA)	6264	Pig
85	*S. aureus* (MRSA)	6265	Pig
86	*S. aureus* (MRSA)	6266	Pig
87	*S. aureus* (MSSA)	6273	Pig
88	*S. aureus* (MRSA)	6274	Pig
89	*S. aureus* (MSSA)	6278	Pig
90	*S. aureus* (MRSA)	6280	Pig
91	*S. aureus* (MRSA)	6281	Pig
92	*S. aureus* (MRSA)	6283	Pig
93	*S. aureus* (MRSA)	6284	Pig
94	*S. aureus* (MRSA)	6286	Pig
95	*S. aureus* (MRSA)	6287	Pig
96	*S. aureus* (MRSA)	6288	Pig
97	*S. aureus* (MRSA)	6295	Pig
98	*S. aureus* (MRSA)	6296	Pig
99	*S. aureus* (MRSA)	6297	Pig
100	*S. aureus* (MRSA)	6298	Pig
101	**Control** (*S. xylosus*)	**DD-34**	**Food item (sausage)**

*****The exact clinical source of these isolates could not be traced though they are from patients.

Appendix III: Stab phages annotation results.
Annotation of Stab phage gene products. The LC-MS/MS identified gene products in **grey shade**.

Table 1: Putative gene products of Stab20 phage NCBI/ENA accession number (**acc. No.**): **LR215718**, and its homology to Kayvirus phages at protein level.

Stab20							
Gp	**Genomic location**	**Predicted function**	**AA**	**MW**	**Best hit (acc. no)**	**e-value (query coverage %)**	**Phage with similar gene**
Gp001	484..654	putative membrane protein	56	6298	AUV57100.1	1e-31 (100 %)	Staphylococcus phage vB_SauM_LM12
Gp002	670..963	hypothetical protein	97	11245	YP_009006863.1	6e-61 (100%)	Staphylococcus phage phiSA12
Gp003	960..1145	hypothetical protein	61	6540	YP_009099453.1	3e-33 (100%)	Staphylococcus phage P108
Gp004	1175..1399	hypothetical protein	74	8889	.....	.....	.....
Gp005	1415..1705	TreC	96	11361	AUV57038.1	9e-62 (100%)	Staphylococcus phage vB_SauM_LM12
Gp006	1705..1992	hypothetical protein	95	10858	YP_008853954.1	3e-63 (100%)	Staphylococcus phage S25-4
Gp007	1992..2285	terminal repeat-encoded protein	97	11538	YP_009099457.1	9e-62 (100%)	Staphylococcus phage P108
Gp008	2289..2537	hypothetical protein	82	9964	YP_009006868.1	2e-50 (100%)	Staphylococcus phage phiSA12
Gp009	2614..2862	hypothetical protein	82	9382	ARM69064.1	3e-24 (95%)	Staphylococcus phage vB_Sau_CG
Gp010	2859..3185	hypothetical protein"	108	13000	YP_009006870.1	2e-62 (100%)	Staphylococcus phage phiSA12
Gp011c	(3404..3742)	hypothetical protein	112	13528	YP_007002124.1	1e-71 (100%)	Staphylococcus phage GH15
Gp012	4054..4362	TreJ	102	11808	YP_007112862.1	2e-70 (100%)	Staphylococcus phage JD007
Gp013	4568..4852	hypothetical protein	94	10985	YP_009196040.1	1e-64 (100%)	Staphylococcus phage phiIPLA-RODI
Gp014	4927..5118	terminal repeat-encoded	63	7674	YP_007002127.1	1e-39 (100%)	Staphylococcus phage GH15

		protein					
Gp015	5654..5812	hypothetical protein	52	6070	YP_008853962.1	2e-28 (100%)	Staphylococcus phage S25-4
Gp016	5978..6301	hypothetical protein	107	12453	YP_241037.1	1e-73 (100%)	Staphylococcus virus G1
Gp017	6395..6907	hypothetical protein	170	20668	VEV88440.1	6e-107 (100%)	Staphylococcus phage Stab21
Gp018	6971..7357	hypothetical protein	128	14985	VEV88378.1	1e-43 (99%)	Staphylococcus phage Stab23
Gp019	7800..8021	hypothetical protein	73	8464	YP_008853966.1	1e-47 (100%)	Staphylococcus phage S25-4
Gp020	8102..8245	hypothetical protein	47	5668	YP_008853967.1	1e-25 (93%)	Staphylococcus phage S25-4
Gp021	8302..8475	hypothetical protein	57	6783	VEV88448.1	1e-31 (100%)	Staphylococcus phage Stab21
Gp022	8555..8791	hypothetical protein	78	9008	YP_009196049.1	8e-48 (100%)	Staphylococcus phage phiIPLA-RODI
Gp023	8882..9226	hypothetical protein	114	13653	ARM69507.1	2e-75 (100%)	Staphylococcus phage vB_Sau_S24
Gp024	9293..9565	hypothetical protein	90	10843	BBC69463.1	2e-52 (96%)	Staphylococcus phage phiSA039
Gp025	9569..9733	hypothetical protein	54	6237	YP_009097941.1	6e-30 (100%)	Staphylococcus phage MCE-2014
Gp026	9737..9934	hypothetical protein	65	7628	VEV88452.1	7e-35 (100%)	Staphylococcus phage Stab21
Gp027	9939..10130	hypothetical protein	63	7314	VEV88454.1	7e-33 (95%)	Staphylococcus phage Stab21
Gp028	10111..10389	TreT	92	10605	YP_009195839.1	1e-54 (96%)	Staphylococcus phage phiIPLA-RODI
Gp029	10466..10696	TreU	76	9320	VEV89584.1	1e-46 (98%)	Staphylococcus phage Stab23
Gp030c	(10977..11267)	hypothetical protein	96	11641	YP_009097946.1	2e-63 (100%)	Staphylococcus phage MCE-2014
Gp031c	(11358..11606)	BofL	82	9992	ARM69513.1	2e-50 (100%)	Staphylococcus phage vB_Sau_S24
Gp032c	(11622..11867)	hypothetical protein	81	9612	YP 008853977.1	8e-53 (100%)	Staphylococcus phage S25-4
Gp033c	(11867..12112)	hypothetical protein	81	9979	YP_009097950.1	2e-50 (100%)	Staphylococcus phage MCE-2014
Gp034c	(12112..12303)	putative membrane protein	63	7912	YP_008853979.1	8e-38 (100%)	Staphylococcus phage S25-4
Gp035c	(12300..12785)	putative membrane protein	161	18130	YP_009097951.1	6e-110 (100%)	Staphylococcus phage MCE-2014

Gp036c	(12778..13218)	hypothetical protein	146	17215	YP_009097952.1	5e-102 (100%)	Staphylococcus phage MCE-2014
Gp037c	(13232..13774)	hypothetical protein	180	21527	YP_007002151.1	2e-127 (100%)	Staphylococcus phage GH15
Gp038c	(13786..14274)	hypothetical protein	162	19492	YP_009097954.1	1e-118 (100%)	Staphylococcus phage MCE-2014
Gp039c	(14289..14741)	hypothetical protein	150	17747	YP_009097955.1	1e-106 (100%)	Staphylococcus phage MCE-2014
Gp040c	(14758..15162)	hypothetical protein	134	16464	YP_009097956.1	2e-92 (100%)	Staphylococcus phage MCE-2014
Gp041c	(15165..15866)	Serine/threonine protein phosphatase	233	27262	YP_009097957.1	2e-172 (100%)	Staphylococcus phage MCE-2014
Gp042c	(16664..17212)	hypothetical protein	182	21975	ASZ78174.1	2e-111(100%)	Staphylococcus phage SA3
Gp043c	(17216..17434)	hypothetical protein	72	8368	YP_008853988.1	8e-46 (100%)	Staphylococcus phage S25-4
Gp044c	(17435..17629)	hypothetical protein	64	7641	YP_241059.1	4e-40 (100%)	Staphylococcus virus G1
Gp045c	(17619..18356)	hypothetical protein	245	28664	YP_008853990.1	6e-174 (100%)	Staphylococcus phage S25-4
Gp046c	(18532..18771)	hypothetical protein	79	9377	YP_007002161.1	4e-48 (100%)	Staphylococcus phage GH15
Gp047c	(18773..19162)	hypothetical protein	129	14773	YP_007002162.1	4e-85 (100%)	Staphylococcus phage GH15
Gp048c	(19256..19429)	hypothetical protein	57	6819	YP_007002163.1	2e-34 (100%)	Staphylococcus phage GH15
Gp049c	(19470..19952)	hypothetical protein	160	19000	YP_008853994.1	4e-111 (100%)	Staphylococcus phage S25-4
Gp050c	(20002..20544)	hypothetical protein	180	20462	YP_009195864.1	1e-124 (100%)	Staphylococcus phage phiIPLA-RODI
Gp051c	(20544..21074)	hypothetical protein	176	20542	YP_009195865.1	2e-125 (100%)	Staphylococcus phage phiIPLA-RODI
Gp052c	(21077..21241)	putative membrane protein	54	6153	YP_009195866.1	3e-31 (100%)	Staphylococcus phage phiIPLA-RODI
Gp053c	(21244..21531)	putative membrane protein	95	11294	YP_009195867.1	1e-59 (100%)	Staphylococcus phage phiIPLA-RODI
Gp054c	(21531..22376)	hypothetical protein	281	31774	YP_009195868.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp055c	(22390..23508)	AAA family ATPase	372	42215	YP_009099502.1	0.0 (100%)	Staphylococcus phage P108
Gp056c	(23660..24001)	hypothetical protein	108	13348	ARQ96019.1	5e-71 (100%)	Staphylococcus phage qdsa002
Gp057c	(23979..24395)	hypothetical protein	138	15993	YP_007002172.1	1e-97 (100%)	Staphylococcus phage GH15

Gp058c	(24528..24830)	NTP pyrophosphohydrolase	100	11304	YP_241074.1	1e-66 (100%)	Staphylococcus phage GH15
Gp059c	(24830..25018)	hypothetical protein	62	7321	YP_007002174.1	7e-37 (100%)	Staphylococcus phage GH15
Gp060c	(25062..25223)	hypothetical protein	53	6402	YP_007002175.1	3e-31 (100%)	Staphylococcus phage GH15
Gp061c	(25224..27275)	hypothetical protein	683	79750	YP_009195875.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp062c	(27354..27617)	hypothetical protein	87	10140	YP_008854007.1	2e-56 (100%)	Staphylococcus phage S25-4
Gp063c	(27634..27807)	hypothetical protein	57	6630	YP_009099510.1	7e-34 (100%)	Staphylococcus phage P108
Gp064c	(27814..28392)	putative membrane protein	192	21480	ASZ77976.1	1e-132 (100%)	Staphylococcus phage SA3
Gp065c	(28385..29011)	nucleoside 2-deoxyribosyltransferase	208	23648	YP_009006709.1	1e-140 (100%)	Staphylococcus phage phiSA12
Gp066c	(29001..29897)	RNA ligase	298	34738	YP_009195880.1	0.0 (99%)	Staphylococcus phage phiIPLA-RODI
Gp067c	(30193..30933)	PhoH-related protein	246	28533	YP_009195882.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp068c	(30985..31599)	hypothetical protein	204	23020	YP_008854014.1	1e-147 (100%)	Staphylococcus phage S25-4
Gp069c	(31615..32040)	ribonuclease H	141	15795	YP_007002186.1	3e-97 (100%)	Staphylococcus phage GH15
Gp070c	(32030..32221)	hypothetical protein	63	7472	YP_241086.1	1e-39 (100%)	Staphylococcus virus G1
Gp071c	(32244..32885)	hypothetical protein	213	24587	YP_009099518.1	3e-146 (100%)	Staphylococcus phage P108
Gp072c	(32875..33105)	transcriptional regulator	76	8832	YP_241088.1	3e-47 (100%)	Staphylococcus virus G1
Gp073c	(33108..33335)	hypothetical protein	75	9261	YP_009195888.1	4e-47 (100%)	Staphylococcus phage phiIPLA-RODI
Gp074c	(33444..34136)	putative transglycosylase	230	24934	YP_007002191.1	3e-169 (100%)	Staphylococcus phage GH15
Gp075c	(34334..35128)	putative membrane protein	264	29296	YP_007002192.1	0.0 (100%)	Staphylococcus phage GH15
Gp076c	(35128..35436)	putative membrane protein	102	12173	YP_007002193.1	1e-67 (100%)	Staphylococcus phage GH15
Gp077c	(35550..37037)	endolysin	495	54734	YP_009195893.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp078c	(37037..37540)	holin	167	18110	YP_009195894.1	7e-119 (100%)	Staphylococcus phage phiIPLA-RODI
Gp079c	(37625..37810)	hypothetical protein	61	7066	YP_241098.1	5e-36 (100%)	Staphylococcus virus G1

Gp080c	(39353..39571)	hypothetical protein	72	8679	YP_241099.1	1e-47 (100%)	Staphylococcus virus G1
Gp081c	(40058..40267)	hypothetical protein	69	7761	YP_007002198.1	4e-43 (100%)	Staphylococcus phage GH15
Gp082c	(40280..40612)	putative membrane protein	110	12505	YP_007002199.1	1e-69 (100%)	Staphylococcus phage GH15
Gp083c	(40625..40951)	hypothetical protein	108	13056	YP_007002200.1	6e-73 (100%)	Staphylococcus phage GH15
Gp084	41511..41777	hypothetical protein	88	10364	YP_009195900.1	2e-56 (100%)	Staphylococcus phage phiIPLA-RODI
Gp085	41755..42033	hypothetical protein	92	10579	YP_241104.1	4e-63 (100%)	Staphylococcus virus G1
Gp086	42030..42440	hypothetical protein	136	15626	YP_241105.1	9e-94 (100%)	Staphylococcus virus G1
Gp087	42455..44272	terminase large subunit	605	70243	YP_007112786.1	0.0 (100%)	Staphylococcus phage JD007
Gp088	44286..45104	hypothetical protein	272	30484	YP_009195906.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp089	45091..45264	hypothetical protein	57	6687	YP_009099539.1	9e-31 (100%)	Staphylococcus phage P108
Gp090	45261..45740	hypothetical protein	159	18540	YP_007002208.1	6e-112 (100%)	Staphylococcus phage GH15
Gp091	45782..47005	hypothetical protein	407	44868	YP_009195908.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp092	47090..47431	hypothetical protein	113	12826	YP_007002210.1	2e-74 (100%)	Staphylococcus phage GH15
Gp093	47450..47821	hypothetical protein	123	14479	YP_009195910.1	2e-85 (100%)	Staphylococcus phage phiIPLA-RODI
Gp094	47825..49516	portal protein	563	64075	YP_007002212.1	0.0 (100%)	Staphylococcus phage GH15
Gp095	49710..50483	prohead protease	257	28624	YP_007002213.1	0.0 (100%)	Staphylococcus phage GH15
Gp096	50502..51461	hypothetical protein	319	36116	YP_009098016.1	0.0 (100%)	Staphylococcus phage MCE-2014
Gp097	51577..52968	major capsid protein	463	51239	YP_009098017.1	0.0 (100%)	Staphylococcus phage MCE-2014
Gp098	53060..53356	hypothetical protein	98	11215	YP_009098018.1	9e-63 (100%)	Staphylococcus phage MCE-2014
Gp099	53369..54277	hypothetical protein	302	34161	YP_240905.1	0.0 (100%)	Staphylococcus virus G1
Gp100	54291..55169	hypothetical protein	292	33716	YP_009099551.1	0.0 (100%)	Staphylococcus phage P108
Gp101	55169..55789	hypothetical protein	206	23773	YP_009098021.1	2e-151 (100%)	Staphylococcus phage MCE-2014

Gp102	55808..56644	hypothetical protein	278	31768	YP_240908.1	0.0 (100%)	Staphylococcus virus G1
Gp103	56646..56861	hypothetical protein	71	8280	YP_240909.1	7e-48 (100%)	Staphylococcus virus G1
Gp104	56888..58651	major tail sheath protein	587	64418	YP_009195921.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp105	58724..59152	tail tube protein	142	15925	YP_240911.1	2e-102 (100%)	Staphylococcus virus G1
Gp106	59237..59401	hypothetical protein	54	6739	YP_007002224.1	5e-31 (100%)	Staphylococcus phage GH15
Gp107	59391..59531	hypothetical protein	46	5420	AFN38132.1	4e-20 (100%)	Staphylococcus phage A3R
Gp108	59573..60031	hypothetical protein	152	18111	YP_240913.1	4e-107 (100%)	Staphylococcus virus G1
Gp109	60044..60238	putative membrane protein	64	7125	BBC69542.1	2e-33 (100%)	Staphylococcus phage phiSA039
Gp110	60254..60406	hypothetical protein	50	5818	YP_009098028.1	3e-30 (100%)	Staphylococcus phage MCE-2014
Gp111	60474..60785	hypothetical protein	103	12238	YP_007002227.1	1e-68 (100%)	Staphylococcus phage GH15
Gp112	60917..61375	hypothetical protein	152	18108	YP_007002228.1	5e-108 (100%)	Staphylococcus phage GH15
Gp113	61419..61955	tail morphogenetic protein	178	20963	YP_009098031.1	1e-128 (100%)	Staphylococcus phage MCE-2014
Gp114	62008..66066	tail tape measure protein	1352	143788	AUV56888.1	0.0 (100%)	Staphylococcus phage vB_SauM_LM12
Gp115	66145..68571	N-acetylmuramoyl-L-alanine amidase	808	91281	ASZ78029.1	0.0 (100%)	Staphylococcus phage SA3
Gp116	68585..69472	protease	295	34503	YP_009098034.1	0.0 (100%)	Staphylococcus phage MCE-2014
Gp117	69472..72018	Glycerophosphoryl diester phosphodiesterase	848	95994	YP_009098035.1	0.0 (100%)	Staphylococcus phage MCE-2014
Gp118	72125..72916	hypothetical protein	263	29329	YP_009099345.1	0.0 (100%)	Staphylococcus phage P108
Gp119	72916..73440	hypothetical protein	174	19953	YP_240925.1	4e-124 (100%)	Staphylococcus virus G1
Gp120	73440..74144	baseplate wedge subunit protein	234	26584	YP_240926.1	5e-174 (100%)	Staphylococcus virus G1
Gp121	74159..75205	putative tail protein	348	39179	YP_007002237.1	0.0 (100%)	Staphylococcus phage GH15
Gp122	75226..78291	hypothetical protein	1021	116293	YP_009098040.1	0.0 (100%)	Staphylococcus phage MCE-2014

Gp123	78402..78923	hypothetical protein	173	19239	YP_240929.1	5e-125 (100%)	Staphylococcus virus G1
Gp124	78944..82402	adsorption-associated tail protein	1152	129264	YP_009195940.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp125	82451..82609	hypothetical protein	52	6305	YP_009099353.1	1e-27 (100%)	Staphylococcus phage P108
Gp126	82610..84532	carbohydrate binding domain-containing protein	640	72571	YP_009099354.1	0.0 (100%)	Staphylococcus phage P108
Gp127	84546..84920	hypothetical protein	124	14650	YP_009098045.1	1e-87 (100%)	Staphylococcus phage MCE-2014
Gp128	84927..86303	putative capsid and scaffold protein	458	50436	YP_009098046.1	0.0 (100%)	Staphylococcus phage MCE-2014
Gp129	86394..88142	DNA helicase A	582	67202	YP_009098047.1	0.0 (100%)	Staphylococcus phage MCE-2014
Gp130	88154..89767	putative Rep protein	537	63147	YP_009098048.1	0.0 (100%)	Staphylococcus phage MCE-2014
Gp131	89760..91202	DNA helicase B	480	54613	YP_009195947.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp132	91281..91700	hypothetical protein	139	16206	YP_009195948.1	4e-98 (100%)	Staphylococcus phage phiIPLA-RODI
Gp133	91700..92725	exonuclease	341	39342	YP_009195949.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp134	92725..93102	hypothetical protein	125	15027	YP_009195950.1	2e-84 (100%)	Staphylococcus phage phiIPLA-RODI
Gp135	93102..95021	putative recombination exonuclease B	639	73264	YP_009098052.1	0.0 (100%)	Staphylococcus phage MCE-2014
Gp136	95021..95602	HNH homing endonuclease	193	22894	ANH50485.1	7e-132 (100%)	Staphylococcus phage pSco-10
Gp137	95602..96198	hypothetical protein	198	23207	YP_009098053.1	7e-145 (100%)	Staphylococcus phage MCE-2014
Gp138	96213..97280	DNA primase	355	41040	YP_009195953.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp139	97346..97684	hypothetical protein	112	12964	YP_240943.1	3e-74 (100%)	Staphylococcus virus G1
Gp140	97684..98136	hypothetical protein	150	17044	AUV56979.1	2e-103 (100%)	Staphylococcus phage vB_SauM_LM12
Gp141	98123..98731	resolvase	202	23640	YP_009195956.1	2e-150 (100%)	Staphylococcus phage phiIPLA-RODI
Gp142	98748..99140	ribonucleotide reduction protein NrdI	130	14737	YP_009098058.1	1e-90 (100%)	Staphylococcus phage MCE-2014

Gp143	99155..101269	ribonucleotide reductase large subunit	704	80259	YP_009195958.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp144	101283..102332	ribonucleotide reductase small subunit	349	40444	YP_009195959.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp145	102350..102679	hypothetical protein	109	12458	AUV57013.1	1e-74 (100%)	Staphylococcus phage vB_SauM_LM12
Gp146	102663..102983	thioredoxin	106	12045	YP_009195961.1	2e-71 (100%)	Staphylococcus phage phiIPLA-RODI
Gp147	103190..103786	hypothetical protein	198	23600	YP_007002262.1	2e-143 (100%)	Staphylococcus phage GH15
Gp148	103796..104101	integration host factor	101	11928	YP_240952.1	9e-69 (100%)	Staphylococcus virus G1
Gp149	104177..107395	DNA polymerase A	1072	124594	YP_009195964.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp150	107423..107707	hypothetical protein	94	10843	YP_008854095.1	2e-51 (100%)	Staphylococcus phage S25-4
Gp151	107724..108206	hypothetical protein	160	18919	YP_007002266.1	3e-117 (100%)	Staphylococcus phage GH15
Gp152	108293..109600	hypothetical protein	435	48352	YP_009195967.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp153	109660..110916	DNA repair protein	418	46764	YP_009195968.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp154	110920..111273	hypothetical protein	117	13379	YP_240963.1	1e-81 (100%)	Staphylococcus virus G1
Gp155	111260..111922	RNA polymerase sigma factor	220	26610	YP_008873651.1	1e-158 (100%)	Staphylococcus phage Sb1
Gp156	112049..112681	hypothetical protein	210	23169	ASZ78069.1	3e-152 (100%)	Staphylococcus phage SA3
Gp157	112696..113217	tail protein	173	18161	AEA36766.1	2e-116 (100%)	Staphylococcus phage GH15
Gp158	113232..113459	Ig-like protein	75	7829	YP_007002273.1	6e-47 (100%)	Staphylococcus phage GH15
Gp159	113554..113814	hypothetical protein	86	10273	YP_007002274.1	2e-57 (100%)	Staphylococcus phage GH15
Gp160	113818..114573	hypothetical protein	251	29179	YP_007002275.1	4e-180 (100%)	Staphylococcus phage GH15
Gp161	114566..115816	metallophosphoesterase	416	47606	YP_007002276.1	0.0 (100%)	Staphylococcus phage GH15
Gp162	115830..116198	membrane protein	122	14010	YP_007002277.1	7e-83 (100%)	Staphylococcus phage GH15
Gp163	116185..116496	hypothetical protein	103	12038	YP_009099393.1	3e-70 (100%)	Staphylococcus phage P108

Gp164	116560..117096	hypothetical protein	178	20762	YP_009098082.1	1e-130 (100%)	Staphylococcus phage MCE-2014
Gp165	117089..117856	hypothetical protein	255	30073	YP_009098083.1	0.0 (100%)	Staphylococcus phage MCE-2014
Gp166	117834..118280	hypothetical protein	148	17367	YP_009099396.1	8e-106 (100%)	Staphylococcus phage P108
Gp167	118280..119143	hypothetical protein	287	32316	YP_007002282.1	0.0 (100%)	Staphylococcus phage GH15
Gp168	119515..120246	hypothetical protein	243	28351	ARQ96133.1	1e-175 (100%)	Staphylococcus phage qdsa002
Gp169	120264..120722	hypothetical protein	152	17850	YP_007002284.1	3e-108 (100%)	Staphylococcus phage GH15
Gp170	120787..121230	hypothetical protein	147	17537	YP_007002285.1	3e-101 (100%)	Staphylococcus phage GH15
Gp171	121247..121951	hypothetical protein	234	27445	YP_009098089.1	5e-170 (100%)	Staphylococccus phage MCE-2014
Gp172	122014..122412	putative membrane protein	132	15428	YP_009006819.1	9e-93 (100%)	Staphylococcus phage phiSA12
Gp173	122559..122801	hypothetical protein	80	9423	YP_009098091.1	3e-50 (100%)	Staphylococccus phage MCE-2014
Gp174	122806..123363	putative membrane protein	185	21691	YP_009099404.1	2e-133 (100%)	Staphylococcus phage P108
Gp175	123399..123575	hypothetical protein	58	6988	YP_009041391.1	7e-35 (100%)	Staphylococcus virus K
Gp176	123565..123816	putative membrane protein	83	9246	VEV88755.1	1e-50 (100%)	Staphylococcus phage Stab21
Gp177	123809..124042	hypothetical protein	77	8960	YP_009099407.1	2e-48 (100%)	Staphylococcus phage P108
Gp178	124124..124768	putative membrane protein	214	25205	YP_007002293.1	5e-151 (100%)	Staphylococcus phage GH15
Gp179	125044..125220	hypothetical protein	58	7005	YP_009006827.1	5e-33 (100%)	Staphylococcus phage phiSA12
Gp180	125213..125509	hypothetical protein	98	11451	YP_009195996.1	2e-64 (100%)	Staphylococcus phage phiIPLA-RODI
Gp181	125548..125739	putative membrane protein	63	7497	ACB89144.1	1e-34 (100%)	Staphylococcus phage A5W
Gp182	125752..126120	hypothetical protein	122	14160	YP_009098101.1	4e-83 (100%)	Staphylococcus phage MCE-2014
Gp183	126133..126480	hypothetical protein	115	12973	YP_009098102.1	2e-77 (100%)	Staphylococcus phage MCE-2014
Gp184	126486..126758	membrane protein	90	9942	YP_009196000.1	2e-53 (100%)	Staphylococcus phage phiIPLA-RODI]
Gp185	126819..127133	hypothetical protein	104	12493	VEV88776.1	1e-66 (100%)	Staphylococcus phage Stab21

Gp186	127148..127498	hypothetical protein	116	13682	YP_009099417.1	6e-77 (100%)	Staphylococcus phage P108
Gp187	127498..128100	hypothetical protein	200	23383	YP_007002302.1	2e-145 (100%)	Staphylococcus phage GH15
Gp188	128114..128293	hypothetical protein	59	7277	YP_009196003.1	8e-35 (100%)	Staphylococcus phage phiIPLA-RODI
Gp189	128296..128862	HNH endonuclease	188	21514	YP_009007668.1	1e-34 (96%)	Staphylococcus phage vB_SepS_SEP9
Gp190	129030..129440	membrane protein	136	15408	YP_009196004.1	9e-90 (100%)	Staphylococcus phage phiIPLA-RODI
Gp191	129442..129735	hypothetical protein	97	11644	AUV57037.1	1e-62 (100%)	Staphylococcus phage vB_SauM_LM12
Gp192	129752..130039	putative membrane protein	95	10554	YP_007112901.1	2e-59 (100%)	Staphylococcus phage JD007
Gp193	130050..130163	hypothetical protein	37	4422	YP_007002307.1	2e-13 (100%)	Staphylococcus phage GH15
Gp194	130156..130428	hypothetical protein	90	10423	AUV57045.1	1e-52 (100%)	Staphylococcus phage vB_SauM_LM12
Gp195	130443..131108	hypothetical protein	221	24930	AUV56940.1	1e-155 (100%)	Staphylococcus phage vB_SauM_LM12
Gp196	131185..131490	hypothetical protein	101	11684	AUV57027.1	3e-64 (100%)	Staphylococcus phage vB_SauM_LM12
Gp197	131490..131894	putative membrane protein	134	15205	AUV56992.1	6e-86 (100%)	Staphylococcus phage vB_SauM_LM12
Gp198	131899..132135	hypothetical protein	78	9159	AUV57061.1	2e-49 (100%)	Staphylococcus phage vB_SauM_LM12
Gp199	132132..132659	putative metallophosphatase	175	20607	AUV56964.1	7e-124 (100%)	Staphylococcus phage vB_SauM_LM12
Gp200	132640..132951	hypothetical protein	103	12523	AUV57022.1	1e-67 (100%)	Staphylococcus phage vB_SauM_LM12
Gp201	132997..133176	putative membrane protein	59	6342	YP_007002315.1	9e-28 (100%)	Staphylococcus phage GH15
Gp202	133191..133454	hypothetical protein	87	10223	AUV57050.1	1e-51 (100%)	Staphylococcus phage vB_SauM_LM12
Gp203	133457..133762	hypothetical protein	101	11520	YP_007002317.1	9e-41 (100%)	Staphylococcus phage GH15
Gp204	133840..133998	putative membrane protein	52	5706	YP_009098109.1	3e-23 (100%)	Staphylococcus phage MCE-2014
Gp205	134014..134238	hypothetical protein	74	8515	YP_009098110.1	9e-46 (100%)	Staphylococcus phage MCE-2014
Gp206	134251..134451	hypothetical protein	66	7600	YP_008873669.1	1e-41 (100%)	Staphylococcus phage Sb1
Gp207	134452..134742	putative membrane protein	96	11134	YP_007002323.1	1e-59 (100%)	Staphylococcus phage GH15

Gp208	134835..135128	hypothetical protein	97	11414	YP_009099439.1	2e-64 (100%)	Staphylococcus phage GH15
Gp209	135125..136033	Ribose-phosphate pyrophosphokinase	302	34960	YP_009196009.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp210	136051..138441	Nicotinamide phosphoribosyltransferase	796	92251	ARM69254.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp211	138520..138765	hypothetical protein	81	9863	YP_009098116.1	1e-49 (100%)	Staphylococcus phage MCE-2014]
Gp212	138785..139189	hypothetical protein	134	16100	YP_009196017.1	5e-84 (100%)	Staphylococcus phage phiIPLA-RODI]
Gp213	139194..139448	hypothetical protein	84	9899	YP_009099444.1	1e-47 (100%)	Staphylococcus phage P108
Gp214	139469..139666	Hypothetical protein	65	7833	YP_009098118.1	1e-38 (100%)	Staphylococcus phage MCE-2014
Gp215	139732..140043	hypothetical protein	103	11624	YP_009098119.1	1e-66 (100%)	Staphylococcus phage MCE-2014
Gp216	140046..140555	hypothetical protein	169	20300	YP_009098120.1	4e-119 (100%)	Staphylococcus phage MCE-2014
Gp217	140557..140892	hypothetical protein	111	12900	YP_009098121.1	3e-70 (100%)	Staphylococcus phage MCE-2014
Gp218	140892..141110	hypothetical protein	72	8650	YP_007002335.1	7e-42 (100%)	Staphylococcus phage GH15
Gp219	141212..141436	hypothetical protein	74	8869	AUV57111.1	6e-15 (50%)	Staphylococcus phage vB_SauM_LM12
Gp220	141436..141630	hypothetical protein	64	7840	YP_008873679.1	7e-25 (100%)	Staphylococcus phage Sb1
Gp221	141654..141968	hypothetical protein	104	12080	AEJ79800.1	4e-61 (100%)	Staphylococcus phage Sb1
Gp222	141984..142274	hypothetical protein	96	11446	….	…	….
Gp223	142290..142460	hypothetical protein	56	6800	….	….	…
Gp001	143008..143178	Putative membrane protein	56	6298	AUV57100.1	1e-29 (100 %)	Staphylococcus phage vB_SauM_LM12
Gp002	143194..143487	Terminal repeat-encoded protein	97	11245	YP_009006863.1	5e-59 (100%)	Staphylococcus phage phiSA12
Gp003	143484..143669	hypothetical protein	61	6540	YP_009099453.1	2e-31 (100%)	Staphylococcus phage P108
Gp004	143699..143923	putative membrane protein	74	8889	TAG94958.1	0.70 (64%)	…..
Gp005	143939..144229	TreC	96	11361	AUV57038.1	7e-60 (100%)	Staphylococcus phage vB_SauM_LM12

Gp006	144229..144516	Terminal repeat-encoded protein	95	10858	YP_008853954.1	2e-61 (100%)	Staphylococcus phage S25-4
Gp007	144516..144809	Terminal repeat-encoded protein	97	11538	YP_009099457.1	7e-60 (100%)	Staphylococcus phage P108
Gp008	144813..145061	Terminal repeat-encoded protein	82	9964	YP_009006868.1	1e-48 (100%)	Staphylococcus phage phiSA12
Gp009	145138..145386	hypothetical protein	82	9382	YP_009099460.1	4e-38 (91%)	Staphylococcus phage vB_Sau_CG
Gp010	145383..145709	terminal repeat-encoded protein	108	13000	AUG85650.1	9e-63 (99%)	Staphylococcus phage phiSA12
Gp011c	(145928..146266)	hypothetical protein	112	13528	YP_007002124.1	1e-69 (100%)	Staphylococcus phage GH15
Gp012	146578..146886	TreJ	102	11808	YP_007112862.1	1e-68 (100%)	Staphylococcus phage JD007
Gp013	147092..147376	TreK	94	10985	YP_009196040.1	1e-62 (100%)	Staphylococcus phage phiIPLA-RODI
Gp014	147451..147642	Terminal repeat-encoded protein	63	7674	YP_007002127.1	9e-38 (100%)	Staphylococcus phage GH15
Gp015	148178..148336	hypothetical protein	52	6070	YP_008853962.1	1e-26 (100%)	Staphylococcus phage S25-4
Gp016	148502..148825	TreP	107	12453	YP_241037.1	9e-72 (100%)	Staphylococcus virus G1
Gp017	148919..149431	hypothetical protein	170	20668	AXU40178.1	2e-108 (98%)	Staphylococcus phage Stab21
Gp018	149495..149881	hypothetical protein	128	14985	YP_008853965.1	8e-85 (98%)	Staphylococcus phage Stab23
Gp019	150324..150545	hypothetical protein	73	8464	YP_008853966.1	1e-45 (100%)	Staphylococcus phage S25-4
Gp020	150626..150769	hypothetical protein	47	5668	YP_008853967.1	7e-24 (93%)	Staphylococcus phage S25-4
Gp021	150826..150999	hypothetical protein	57	6783	VEV88448.1	1e-31 (100%)	Staphylococcus phage Stab21
Gp022	151037..151315	hypothetical protein	78	9008	YP_009196049.1	6e-46 (100%)	Staphylococcus phage phiIPLA-RODI
Gp023	151406..151750	hypothetical protein	114	13653	ARM69507.1	2e-73 (100%)	Staphylococcus phage vB_Sau_S24
Gp024	151817..152089	hypothetical protein	90	10843	BBC69463.1	1e-50 (96%)	Staphylococcus phage phiSA039
Gp025	152093..152257	hypothetical protein	54	6237	YP_009097941.1	4e-28 (100%)	Staphylococcus phage MCE-2014

Gp026	152270..152458	hypothetical protein	65	7628	VEV88452.1	5e-33 (100%)	Staphylococcus phage Stab21
Gp027	152472..152654	terminal repeat-encoded protein	63	7314	YP_009097942.1	6e-31 (90%)	Staphylococcus phage Stab21
Gp028	152638..152913	TreT	92	10605	YP_009195839.1	1e-52 (96%)	Staphylococcus phage phiIPLA-RODI
Gp029	152990..153220	TreU	76	9320	VEV89584.1	1e-44 (98%)	Staphylococcus phage Stab23

Table 2: Putative gene products of Stab21 phage NCBI/ENA accession number (**acc.No.**): **LR215719**, and its homology to Kayvirus phages at protein level.

Stab21							
Gp	**Genomic location**	**Predicted function**	**AA**	**MW**	**Best hit (acc no)**	**e-value (query coverage %)**	**phage with similar gene**
Gp001	485..646	hypothetical protein	53	6072	YP_007112871.1	9e-31 (100%)	Staphylococcus phage JD007
Gp002	741..1049	hypothetical protein	102	11792	YP_007112870.1	6e-67 (100%)	Staphylococcus phage JD007
Gp003	1061..1360	hypothetical protein	99	11562	YP_007112869.1	1e-63 (100%)	Staphylococcus phage JD007
Gp004	1376..1561	TreB	61	6855	YP_007112868.1	3e-31 (100%)	Staphylococcus phage JD007
Gp005	1669..1989	hypothetical protein	106	12354	AVX47357.1	4e-46 (100%)	Staphylococcus phage vB_SauM_0414_108
Gp006	2004..2297	hypothetical protein	97	11604	BBC69665.1	7e-64 (100%)	Staphylococcus phage phiSA039
Gp007	2301..2558	TreF	85	10310	YP_009196035.1	1e-53 (100%)	Staphylococcus phage phiIPLA-RODI
Gp008	2672..2911	hypothetical protein	79	8973	YP_009196036.1	1e-41 (100%)	Staphylococcus phage phiIPLA-RODI
Gp009	2922..3269	hypothetical protein	115	13689	YP_009098142.1	4e-77 (100%)	Staphylococcus phage Team1
Gp010c	c(3476..3814)	hypothetical protein	112	13472	YP_009196038.1	1e-71 (100%)	Staphylococcus phage phiIPLA-RODI
Gp011	4125..4433	TreJ	102	11768	AFN37829.1	5e-69 (100%)	Staphylococcus phage Staph1N
Gp012	4640..4927	hypothetical protein	95	11102	YP_007112861.1	1e-63 (100%)	Staphylococcus phage JD007
Gp013	4977..5249	hypothetical protein	90	10539	ARM69069.1	1e-57 (100%)	Staphylococcus phage vB_Sau_CG
Gp014	5773..5931	hypothetical protein	52	6070	YP_007112859.1	9e-27 (100%)	Staphylococcus phage JD007
Gp015	6098..6421	TreP	107	12352	YP_007112857.1	9e-72 (100%)	Staphylococcus phage JD007
Gp016	6515..7027	hypothetical protein	170	20780	AXU40178.1	1e-108 (98%)	Staphylococcus phage VB_SavM_JYL01
Gp017	7091..7447	hypothetical protein	118	13865	VEV88121.1	7e-71 (98%)	Staphylococcus phage Stab20

Gp018	7977..8198	hypothetical protein	73	8469	YP_007112855.1	2e-45 (100%)	Staphylococcus phage JD007
Gp019	8279..8416	hypothetical protein	45	5459	YP_007112854.1	4e-24 (100%)	Staphylococcus phage JD007
Gp020	8478..8651	hypothetical protein	57	6765	VEV88124.1	1e-31 (100%)	Staphylococcus phage Stab20
Gp021	8904..9374	hypothetical protein	156	17885	AVX47376.1	5e-103 (100%)	Staphylococcus phage vB_SauM_0414_108
Gp022	9434..9631	hypothetical protein	65	7481	VEV88129.1	5e-33 (100%)	Staphylococcus phage Stab20
Gp023	9637..9819	hypothetical protein	60	6962	VEV88130.1	5e-31 (100%)	Staphylococcus phage Stab20
Gp024	9819..10475	hypothetical protein	218	25574	AUV56944.1	6e-72 (97%)	Staphylococcus phage vB_SauM_LM12
Gp025	10483..10758	TreT	91	10518	YP_009195839.1	4e-23 (100%)	Staphylococcus phage phiIPLA-RODI
Gp026	10834..11022	hypothetical protein	62	7512	YP_007002139.1	1e-35 (100%)	Staphylococcus phage GH15
Gp027c	c(11360..11596)	hypothetical protein	78	9557	AVX47381.1	1e-49 (100%)	Staphylococcus phage vB_SauM_0414_108
Gp028c	c(11598..12083)	hypothetical protein	161	19058	YP_241045.1	4e-111 (100%)	Staphylococcus virus G1
Gp029c	c(12096..12503)	hypothetical protein	135	16464	YP_008873525.1	4e-94 (100%)	Staphylococcus phage Sb1
Gp030c	c(12503..12934)	hypothetical protein	143	17323	AFN38052.1	2e-94 (100%)	Staphylococcus phage A3R
Gp031c	c(13125..13373)	hypothetical protein	82	9987	QAU05802.1	3e-49 (100%)	Staphylococcus virus Sa87
Gp032c	c(13373..13855)	membrane protein	160	18385	YP_007112840.1	7e-106 (100%)	Staphylococcus phage JD007
Gp033c	c(13848..14279)	hypothetical protein	143	16785	YP_008854159.1	4e-96 (100%)	Staphylococcus phage S25-3
Gp034c	c(14293..14835)	hypothetical protein	180	21586	YP_007112838.1	3e-125 (100%)	Staphylococcus phage JD007
Gp035c	c(14847..15335)	hypothetical protein	162	19434	QAU05805.1	4e-118 (100%)	Staphylococcus virus Sa87
Gp036c	c(15348..15746)	hypothetical protein	132	16141	YP_007112836.1	3e-89 (100%)	Staphylococcus phage JD007
Gp037c	c(15743..16450)	Serine/threonine protein phosphatase	235	27697	YP_007112835.1	7e-172 (100%)	Staphylococcus phage JD007
Gp038c	c(16541..18391)	lipase acylhydrolase domain protein	616	68944	YP_007112834.1	0.0 (100%)	Staphylococcus phage JD007

Gp039c	c(19301..19849)	hypothetical protein	182	21954	YP_007112833.1	1e-124 (100%)	Staphylococcus phage JD007
Gp040c	c(19853..20071)	hypothetical protein	72	8425	YP_007002157.1	8e-44 (100%)	Staphylococcus phage GH15
Gp041c	c(20072..20266)	hypothetical protein	64	7641	YP_241059.1	3e-38 (100%)	Staphylococcus virus G1
Gp042c	c(20256..20993)	hypothetical protein	245	28633	YP_007112830.1	1e-174 (100%)	Staphylococcus phage JD007
Gp043c	c(21172..21411)	hypothetical protein	79	9369	AFN38067.1	2e-51 (100%)	Staphylococcus phage A3R
Gp044c	c(21413..21802)	hypothetical protein	129	15153	YP_008854169.1	2e-89 (100%)	Staphylococcus phage S25-3
Gp045c	C (21901..22074)	hypothetical protein	57	6819	YP_007002163.1	2e-34 (100%)	Staphylococcus phage GH15
Gp046c	C (22115..22597)	hypothetical protein	160	18855	YP_008873543.1	4e-110 (100%)	Staphylococcus phage Sb1
Gp047c	c(22647..23189)	hypothetical protein	180	20415	ARM69103.1	1e-123 (100%)	Staphylococcus phage vB_Sau_CG
Gp048c	c(23189..23722)	hypothetical protein	177	20707	YP_241067.1	2e-124 (100%)	Staphylococcus virus G1
Gp049c	c(23725..23889)	hypothetical protein	54	6196	YP_008854174.1	5e-30 (100%)	Staphylococcus phage S25-3
Gp050c	c(23892..24170)	putative membrane protein	92	10955	AFN37865.1	4e-54 (100%)	Staphylococcus phage Staph1N
Gp051c	c(24170..25015)	hypothetical protein	281	31748	YP_241070.1	0.0 (100%)	Staphylococcus virus G1
Gp052c	c(25027..26154)	AAA family ATPase	375	42599	YP_007112820.1	0.0 (99%)	Staphylococcus phage JD007
Gp053c	c(26298..26624)	hypothetical protein	108	12980	YP_241072.1	4e-73 (100%)	Staphylococcus virus G1
Gp054c	c(26617..27033)	hypothetical protein	138	15979	YP_241073.1	8e-96 (100%)	Staphylococcus virus G1
Gp055c	c(27166..27468)	nucleoside triphosphate pyrophosphohydrolase	100	11304	ARM69324.1	1e-64 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp056c	c(27468..27656)	hypothetical protein	62	7309	YP_007112816.1	3e-35 (100%)	Staphylococcus phage JD007
Gp057c	c(27700..27861)	hypothetical protein	53	6370	YP_241076.1	1e-29 (100%)	Staphylococcus virus G1
Gp058c	c(27861..29909)	hypothetical protein	682	79800	AZB49981.1	0.0 (100%)	Staphylococcus phage 812h1
Gp059c	c(29987..30250)	hypothetical protein	87	10147	YP_007112813.1	6e-54 (100%)	Staphylococcus phage JD007
Gp060c	c(30267..30440)	hypothetical protein	57	6670	YP_008854008.1	1e-31 (100%)	Staphylococcus phage S25-4

Gp061c	c(30447..31025)	hypothetical protein	192	21478	YP_007112811.1	4e-131 (100%)	Staphylococcus phage JD007
Gp062c	c(31018..31641)	hypothetical protein	207	23650	VEV89232.1	4e-129 (100%)	Staphylococcus phage Stab22
Gp063c	c(31641..32201)	HNH homing endonuclease	186	21870	AXY83933.1	1e-100 (100%)	Staphylococcus phage Terranova
Gp064c	c(32241..33137)	RNA ligase	298	35071	YP_008854188.1	0.0 (100%)	Staphylococcus phage S25-3
Gp065c	c(33137..33361)	hypothetical protein	74	8165	YP_008873561.1	2e-40 (100%)	Staphylococcus phage Sb1
Gp066c	c(33430..34170)	Phosphate starvation-inducible protein PhoH, predicted ATPase	246	28575	YP_009097984.1	0.0 (100%)	Staphylococcus phage MCE-2014
Gp067c	c(34222..34836)	hypothetical protein	204	23008	YP_008854191.1	1e-145 (100%)	Staphylococcus phage S25-3
Gp068c	c(34852..35277)	ribonuclease H	141	15795	YP_007112805.1	2e-95 (100%)	Staphylococcus phage JD007
Gp069c	c(35267..35458)	hypothetical protein	63	7444	YP_008854193.1	2e-37 (100%)	Staphylococcus phage S25-3
Gp070c	c(35481..36122)	hypothetical protein	213	24573	YP_007112803.1	2e-144 (100%)	Staphylococcus phage JD007
Gp071c	c(36112..36342)	transcriptional regulator protein	76	8831	YP_007112802.1	3e-47 (100%)	Staphylococcus phage JD007
Gp072c	c(36345..36572)	hypothetical protein	75	9225	YP_008854019.1	2e-45 (100%)	Staphylococcus phage S25-4
Gp073c	c(36681..37373)	transglycosylase	230	24826	YP_009098202.1	2e-167 (100%)	Staphylococcus phage Team1
Gp074c	c(37571..38365)	membrane protein	264	29309	YP_007112798.1	0.0 (100%)	Staphylococcus phage JD007
Gp075c	c(38365..38673)	hypothetical protein	102	12135	YP_007112797.1	4e-65 (100%)	Staphylococcus phage JD007
Gp076c	c(38786..40273)	N-acetylmuramoyl-L-alanine amidase	495	54754	YP_007112796.1	0.0 (100%)	Staphylococcus phage JD007
Gp077c	c(40273..40776)	holin	167	18096	AUV56969.1	5e-117 (100%)	Staphylococcus phage vB_SauM_LM12
Gp078c	c(40861..41046)	hypothetical protein	61	7066	YP_007002196.1	3e-34 (100%)	Staphylococcus phage GH15
Gp079c	c(42594..42812)	hypothetical protein	72	8691	YP_009097998.1	2e-45 (100%)	Staphylococcus phage MCE-2014
Gp080c	c(43292..43501)	hypothetical protein	69	8019	YP_008873578.1	5e-43 (100%)	Staphylococcus phage Sb1
Gp081c	c(43514..43846)	hypothetical protein	110	12477	YP_008854029.1	3e-69 (100%)	Staphylococcus phage S25-4

Gp082c	c(43859..44185)	hypothetical protein	108	13056	YP_007002200.1	5e-71 (100%)	Staphylococcus phage GH15
Gp083c	c(44218..44484)	hypothetical protein	88	10121	YP_241102.1	1e-50 (100%)	Staphylococcus virus G1
Gp084	44625..45011	membrane protein	128	14819	YP_008854032.1	3e-82 (100%)	Staphylococcus phage S25-4
Gp085	44989..45267	hypothetical protein	92	10579	YP_008873583.1	3e-61 (100%)	Staphylococcus phage Sb1
Gp086	45264..45674	hypothetical protein	136	15626	YP_007112787.1	7e-92 (100%)	Staphylococcus phage JD007
Gp087	45689..47506	Terminase, large subunit	605	70243	YP_009041305.1	0.0 (100%)	Staphylococcus virus K
Gp088	47499..48320	hypothetical protein	273	30649	YP_009098222.1	0.0 (100%)	Staphylococcus phage Team1
Gp089	48307..48480	hypothetical protein	57	6674	YP_240894.1	1e-29 (100%)	Staphylococcus virus G1
Gp090	48477..48956	hypothetical protein	159	18524	YP_007112783.1	2e-110 (100%)	Staphylococcus phage JD007
Gp091	48999..50189	hypothetical protein	396	43647	YP_008854038.1	0.0 (100%)	Staphylococcus phage S25-4
Gp092	50275..50616	hypothetical protein	113	12852	YP_240898.1	4e-72 (100%)	Staphylococcus G1
Gp093	50634..51005	hypothetical protein	123	14479	YP_009195910.1	2e-83 (100%)	Staphylococcus phage philPLA-RODI
Gp094	51009..52700	Portal protein	563	64051	YP_240900.1	0.0 (100%)	Staphylococcus virus G1
Gp095	52894..53667	Prohead protease	257	28624	YP_007002213.1	0.0 (100%)	Staphylococcus phage GH15
Gp096	53686..54642	hypothetical protein	318	36016	YP_007112777.1	0.0 (100%)	Staphylococcus phage JD007
Gp097	54758..56149	Major capsid protein	463	51211	YP_007112776.1	0.0 (100%)	Staphylococcus phage JD007
Gp098	56241..56537	hypothetical protein	98	11257	YP_007112775.1	5e-60 (100%)	Staphylococcus phage JD007
Gp099	56550..57458	hypothetical protein	302	34161	YP_240905.1	0.0 (100%)	Staphylococcus virus G1
Gp100	57472..58350	hypothetical protein	292	33758	YP_007112773.1	0.0 (100%)	Staphylococcus phage JD007
Gp101	58350..58970	hypothetical protein	206	23746	YP_240907.1	5e-149 (100%)	Staphylococcus virus G1
Gp102	58989..59825	hypothetical protein	278	31782	YP_240908.1	0.0 (100%)	Staphylococcus virus G1
Gp103	59827..60042	hypothetical protein	71	8280	YP_240909.1	6e-46 (100%)	Staphylococcus virus G1

Gp104	60069..61832	major tail sheath protein	587	64458	YP_007112769.1	0.0 (100%)	Staphylococcus phage JD007
Gp105	61905..62333	tail tube protein	142	15925	YP_009041323.1	1e-100 (100%)	Staphylococcus virus K
Gp106	62430..62570	hypothetical protein	46	5408	YP_008873604.1	2e-23 (100%)	Staphylococcus phage Sb1
Gp107	62613..63071	hypothetical protein	152	18131	EF136582.1	3e-97 (100%)	Staphylococcus phage 812 strain phi812
Gp108	63084..63278	hypothetical protein	64	7157	YP_240914.1	7e-35 (100%)	Staphylococcus virus G1
Gp109	63360..63671	hypothetical protein	103	12252	YP_008854056.1	1e-6 (100%)	Staphylococcus phage S25-4
Gp110	63803..64261	hypothetical protein"	152	18122	YP_008854057.1	5e-106 (100%)	Staphylococcus phage S25-4
Gp111	64305..64841	tail morphogenetic protein	178	20915	YP_009195929.1	2e-125 (100%)	Staphylococcus phage philPLA-RODI
Gp112	64894..68952	putative tail lysin	1352	143895	YP_007002230.1	0.0 (100%)	Staphylococcus phage GH15
Gp113	69031..71457	tail lysin	808	91180	YP_007112760.1	0.0 (100%)	Staphylococcus phage JD007
Gp114	71471..72358	protease	295	34593	YP_009041332.1	0.0 (100%)	Staphylococcus virus K
Gp115	72358..74904	Glycerophosphoryl diester phosphodiesterase	848	96085	YP_007112758.1	0.0 (100%)	Staphylococcus phage JD007
Gp116	75011..75802	hypothetical protein	263	29343	YP_240924.1	0.0 (100%)	Staphylococcus virus G1
Gp117	75802..76326	hypothetical protein	174	19953	YP_007002235.1	3e-122 (100%)	Staphylococcus phage GH15
Gp118	76326..77030	putative baseplate protein	234	26584	YP_008873616.1	4e-172 (100%)	Staphylococcus phage Sb1
Gp119	77045..78091	baseplate	348	39179	ARM68952.1	0.0 (100%)	Staphylococcus phage vB_Sau_CG
Gp120	78112..81171	hypothetical protein	1019	116346	YP_009041338.1	0.0 (100%)	Staphylococcus virus K
Gp121	81282..81803	hypothetical protein	173	19239	YP_007002239.1	4e-123 (100%)	Staphylococcus phage GH15
Gp122	81824..85282	adsorption-associated tail protein	1152	129184	ARM68955.1	0.0 (100%)	Staphylococcus phage vB_Sau_CG
Gp123	85331..85489	hypothetical protein	52	6208	YP_240931.1	3e-27 (100%)	Staphylococcus virus G1
Gp124	85490..87412	hypothetical protein	640	72602	YP_007112749.1	0.0 (100%)	Staphylococcus phage JD007
Gp125	87435..87806	hypothetical protein	123	14496	YP_007112748.1	9e-84 (100%)	Staphylococcus phage JD007

Gp126	87813..89189	hypothetical protein	458	50465	YP_008854075.1	0.0 (100%)	Staphylococcus phage S25-4
Gp127	89280..91028	DNA helicase A	582	67219	AKC02275.1	0.0 (100%)	Staphylococcus phage IME-SA1
Gp128	91040..92653	putative Rep protein	537	63159	YP_007002246.1	0.0 (100%)	Staphylococcus phage GH15
Gp129	92646..94088	DNA helicase B	480	54558	AVX47482.1	0.0 (100%)	Staphylococcus phage vB_SauM_0414_108
Gp130	94167..95192	putative exonuclease	341	39267	YP_007002248.1	0.0 (100%)	Staphylococcus phage GH15
Gp131	95192..95569	hypothetical protein	125	14912	YP_007112960.1	2e-86 (100%)	Staphylococcus phage JD007
Gp132	95569..97488	exonuclease	639	73446	YP_007112959.1	0.0 (100%)	Staphylococcus phage JD007
Gp133	97488..98084	hypothetical protein	198	23180	YP_007112958.1	3e-143 (100%)	Staphylococcus phage JD007
Gp134	98099..99166	DNA primase	355	40927	YP_007112957.1	0.0 (100%)	Staphylococcus phage JD007
Gp135	99232..99570	hypothetical protein	112	12964	YP_240943.1	2e-72 (100%)	Staphylococcus virus G1
Gp136	99570..100022	hypothetical protein	150	17012	YP_008854085.1	2e-101 (100%)	Staphylococcus phage S25-4
Gp137	100009..100617	resolvase	202	23613	YP_008873634.1	1e-148 (100%)	Staphylococcus phage Sb1
Gp138	100634..101026	Ribonucleotide reduction protein NrdI	130	14734	ABL87151.1	2e-88 (100%)	Staphylococcus phage 812
Gp139	101041..103155	Ribonucleotide reductase of class Ib (aerobic), alpha subunit	704	80291	YP_009006789.1	0.0 (100%)	Staphylococcus phage phiSA12
Gp140	103169..104218	Ribonucleotide reductase of class Ib (aerobic), beta subunit	349	40446	YP_007002259.1	0.0 (100%)	Staphylococcus phage GH15
Gp141	104236..104565	hypothetical protein	109	12384	YP_007112950.1	5e-73 (100%)	Staphylococcus phage JD007
Gp142	104549..104869	thioredoxin-like protein	106	12059	YP_008873639.1	1e-69 (100%)	Staphylococcus phage Sb1
Gp143	105112..105672	hypothetical protein	186	22107	YP_007002262.1	1e-132 (100%)	Staphylococcus phage GH15
Gp144	105682..105987	integration host factor	101	11928	YP_009098279.1	7e-67 (100%)	Staphylococcus phage Team1
Gp145	106063..109281	DNA polymerase I	1072	124537	YP_009006795.1	0.0 (100%)	Staphylococcus phage phiSA12
Gp146	109351..109593	hypothetical protein	80	9026	VEV88253.1	4e-50 (100%)	Staphylococcus phage Stab20

Gp147	109610..110092	hypothetical protein	160	18947	YP_009098069.1	1e-115 (100%)	Staphylococcus phage MCE-2014
Gp148	110179..111450	hypothetical protein	423	46908	YP_008854097.1	0.0 (100%)	Staphylococcus phage S25-4
Gp149	111510..112766	recombinase protein	418	46793	AZB49858.1	0.0 (100%)	Staphylococcus phage 812
Gp150	112770..113123	hypothetical protein	117	13379	YP_009041370.1	9e-80 (100%)	Staphylococcus virus K
Gp151	113110..113772	RNA polymerase sigma factor	220	26610	AQT25578.1	8e-157 (100%)	Staphylococcus phage pSa-3
Gp152	113899..114531	hypothetical protein	210	23172	YP_008854277.1	2e-150 (100%)	Staphylococcus phage S25-3
Gp153	114545..115066	Ig-like protein	173	18147	AEA36766.1	4e-116 (100%)	Staphylococcus phage GH15
Gp154	115081..115308	major tail protein	75	7787	YP_007112935.1	5e-45 (100%)	Staphylococcus phage JD007
Gp155	115403..115663	hypothetical protein	86	10273	YP_007002274.1	2e-55 (100%)	Staphylococcus phage GH15
Gp156	115667..116422	hypothetical protein	251	29112	YP_007112933.1	2e-180 (100%)	Staphylococcus phage JD007
Gp157	116415..117665	DNA polymerase	416	47534	YP_007112932.1	0.0 (100%)	Staphylococcus phage JD007
Gp158	117679..118047	membrane protein	122	14008	YP_007112931.1	2e-81 (100%)	Staphylococcus phage JD007
Gp159	118034..118345	hypothetical protein	103	12010	YP_008873658.1	1e-68 (100%)	Staphylococcus phage Sb1
Gp160	118409..118945	hypothetical protein	178	20824	YP_240973.1	6e-128 (100%)	Staphylococcus virus G1
Gp161	118938..119705	hypothetical protein	255	30046	YP_007112928.1	0.0 (100%)	Staphylococcus phage JD007
Gp162	119683..120129	hypothetical protein	148	17337	YP_008854111.1	4e-104 (100%)	Staphylococcus phage S25-4
Gp163	120129..120992	hypothetical protein	287	32356	YP_008854112.1	0.0 (100%)	Staphylococcus phage S25-4
Gp164	121364..122095	hypothetical protein	243	28351	ARQ96133.1	8e-174 (100%)	Staphylococcus phage qdsa002
Gp165	122113..122571	hypothetical protein	152	17850	YP_007002284.1	3e-106 (100%)	Staphylococcus phage GH15
Gp166	122636..123079	hypothetical protein	147	17497	YP_007112923.1	3e-99 (100%)	Staphylococcus phage JD007
Gp167	123096..123800	hypothetical protein	234	27400	YP_007112922.1	9e-169 (100%)	Staphylococcus phage JD007
Gp168	123862..124260	putative membrane protein	132	15429	YP_009098309.1	7e-91 (100%)	Staphylococcus phage Team1

Gp169	124407..124649	hypothetical protein	80	9421	YP_007112920.1	7e-49 (100%)	Staphylococcus phage JD007
Gp170	124654..125211	hypothetical protein	185	21702	ARM69003.1	9e-132 (100%)	Staphylococcus phage vB_Sau_CG
Gp171	125247..125423	hypothetical protein	58	6988	YP_240984.1	5e-33 (100%)	Staphylococcus virus G1
Gp172	125413..125664	hypothetical protein	83	9190	ARQ96141.1	5e-50 (100%)	Staphylococcus phage qdsa002
Gp173	125657..125890	hypothetical protein	77	8840	YP_008854298.1	8e-48 (100%)	Staphylococcus phage S25-3
Gp174	125971..126615	hypothetical protein	214	25138	YP_008854123.1	3e-148 (100%)	Staphylococcus phage S25-4
Gp175	126630..126878	hypothetical protein	82	9067	AVX47531.1	2e-45 (100%)	Staphylococcus phage vB_SauM_0414_108
Gp176	126890..127066	hypothetical protein	58	6991	YP_008854125.1	1e-31 (100%)	Staphylococcus phage S25-4
Gp177	127059..127355	hypothetical protein	98	11342	YP_240987.1	3e-63 (100%)	Staphylococcus virus G1
Gp178	127394..127585	Putative membrane protein	63	7511	ACB89144.1	4e-35 (100%)	Staphylococcus phage A5W
Gp179	127598..127966	hypothetical protein	122	14272	YP_007112910.1	1e-82 (100%)	Staphylococcus phage JD007
Gp180	127979..128326	hypothetical protein	115	13029	AFN38826.1	5e-77 (100%)	Staphylococcus phage MSA6
Gp181	128326..128604	hypothetical protein	92	10184	YP_009098321.1	1e-56 (100%)	Staphylococcus phage Team1
Gp182	128665..128979	hypothetical protein	104	12520	YP_008854307.1	1e-67 (97%)	Staphylococcus phage S25-3
Gp183	128994..129344	hypothetical protein	116	13711	YP_009041401.1	7e-77 (100%)	Staphylococcus virus K
Gp184	129344..129946	hypothetical protein	200	23356	YP_240994.1	2e-145 (100%)	Staphylococcus virus G1
Gp185	129960..130139	hypothetical protein	59	7275	YP_007112904.1	8e-35 (100%)	Staphylococcus phage JD007
Gp186	130366..130776	hypothetical protein	136	15400	YP_007112903.1	4e-91 (100%)	Staphylococcus phage JD007
Gp187	130778..131071	hypothetical protein	97	11672	YP_007112902.1	7e-63 (100%)	Staphylococcus phage JD007
Gp188	131088..131375	hypothetical protein	95	10540	YP_240999.1	1e-59 (100%)	Staphylococcus virus G1
Gp189	131492..131755	hypothetical protein	87	9918	YP_007112899.1	1e-53 (100%)	Staphylococcus phage JD007
Gp190	131833..132138	hypothetical protein	101	11777	YP_008854311.1	2e-65 (100%)	Staphylococcus phage S25-3

Gp191	132138..132542	hypothetical protein	134	15169	YP_007112897.1	3e-88 (100%)	Staphylococcus phage JD007
Gp192	132547..132783	hypothetical protein	78	9192	YP_007112896.1	2e-48 (100%)	Staphylococcus phage JD007
Gp193	132780..133307	Phosphoesterase	175	20597	YP_009006842.1	7e-126 (100%)	Staphylococcus phage phiSA12
Gp194	133288..133608	hypothetical protein	106	12898	YP_008854315.1	5e-68 (100%)	Staphylococcus phage S25-3
Gp195	133608..133838	hypothetical protein	76	8860	YP_008854316.1	3e-44 (100%)	Staphylococcus phage S25-3
Gp196	133891..134070	hypothetical protein	59	6422	AFN38011.1	1e-29 (100%)	Staphylococcus phage Staph1N
Gp197	134085..134348	hypothetical protein	87	10251	YP_007112891.1	7e-56% (100%)	Staphylococcus phage JD007
Gp198	134351..134668	hypothetical protein	105	11992	YP_007112890.1	1e-68 (100%)	Staphylococcus phage JD007
Gp199	134669..135349	hypothetical protein	226	25749	YP_007112889.1	8e-160 (100%)	Staphylococcus phage JD007
Gp200	135427..135585	membrane protein	52	5686	YP_007112888.1	2e-23 (100%)	Staphylococcus phage JD007
Gp201	135601..135825	hypothetical protein	74	8573	YP_007112887.1	2e-46 (100%)	Staphylococcus phage JD007
Gp202	135838..136038	hypothetical protein	66	7699	YP_008854323.1	9e-42 (100%)	Staphylococcus phage S25-3
Gp203	136039..136329	Putative membrane protein	96	11082	YP_009006850.1	2e-58 (100%)	Staphylococcus phage phiSA12
Gp204	136423..136731	hypothetical protein	102	12049	YP_007112884.1	5e-63 (100%)	Staphylococcus phage JD007
Gp205	136728..137636	Ribose-phosphate pyrophosphokinase	302	35262	YP_008854326.1	0.0 (100%)	Staphylococcus phage S25-3
Gp206	137651..138046	hypothetical protein	131	15360	BBC69643.1	2e-87 (100%)	Staphylococcus phage phiSA039
Gp207	138050..139519	Nicotinamide phosphoribosyltransferase	489	56176	YP_009006853.1	0.0 (100%)	Staphylococcus phage phiSA12
Gp208	139598..139843	hypothetical protein	81	9830	YP_008854328.1	2e-50 (100%)	Staphylococcus phage S25-3
Gp209	139860..140252	hypothetical protein	130	15352	YP_007112880.1	1e-86 (100%)	Staphylococcus phage JD007
Gp210	140254..140451	hypothetical protein	65	7838	YP_007112879.1	2e-39 (100%)	Staphylococcus phage JD007
Gp211	140516..140812	hypothetical protein	98	11330	YP_007112878.1	9e-64 (100%)	Staphylococcus phage JD007
Gp212	140816..141127	hypothetical protein	103	11715	YP_009006858.1	1e-66 (100%)	Staphylococcus phage phiSA12

Gp213	141130..141369	hypothetical protein	79	9779	BBC69651.1	2e-49 (100%)	Staphylococcus phage phiSA039
Gp214	141359..141514	hypothetical protein	51	6141	YP_007112875.1	2e-29 (100%)	Staphylococcus phage JD007
Gp215	141518..141712	hypothetical protein	64	7717	YP_009041432.1	3e-37 (100%)	Staphylococcus virus K
Gp216	141729..142082	hypothetical protein	117	13906	YP_007112873.1	4e-77 (100%)	Staphylococcus phage JD007
Gp217	142101..142487	hypothetical protein	128	15650	YP_007112872.1	5e-85 (100%)	Staphylococcus phage JD007
Gp001	143133..143294	hypothetical protein	53	6072	YP_007112871.1	9e-31 (100%)	Staphylococcus phage JD007
Gp002	143389..143697	hypothetical protein	102	11792	YP_007112870.1	6e-67 (100%)	Staphylococcus phage JD007
Gp003	143709..144008	hypothetical protein	99	11562	YP_007112869.1	1e-63 (100%)	Staphylococcus phage JD007
Gp004	144024..144209	TreB	61	6855	YP_007112868.1	3e-31 (100%)	Staphylococcus phage JD007
Gp005	144317..144637	hypothetical protein	106	12354	AVX47357.1	4e-46 (100%)	Staphylococcus phage vB_SauM_0414_108
Gp006	144652..144945	hypothetical protein	97	11604	BBC69665.1	7e-64 (100%)	Staphylococcus phage phiSA039
Gp007	144949..145206	hypothetical protein	85	10310	YP_009196035.1	1e-53 (100%)	Staphylococcus phage phiIPLA-RODI
Gp008	145320..145559	hypothetical protein	79	8973	YP_009196036.1	1e-41 (100%)	Staphylococcus phage phiIPLA-RODI
Gp009	145570..145917	hypothetical protein	115	13689	YP_009098142.1	4e-77 (100%)	Staphylococcus phage Team1
Gp010c	c(146124..146462)	hypothetical protein	112	13472	YP_009196038.1	1e-71 (100%)	Staphylococcus phage phiIPLA-RODI
Gp011	146773..147081	TreJ	102	11768	AFN37829.1	5e-69 (100%)	Staphylococcus phage Staph1N
Gp012	147288..147575	hypothetical protein	95	11102	YP_007112861.1	1e-63 (100%)	Staphylococcus phage JD007
Gp013	147625..147897	hypothetical protein	90	10539	ARM69069.1	1e-57 (100%)	Staphylococcus phage vB_Sau_CG
Gp014	148421..148579	hypothetical protein	52	6070	YP_007112859.1	9e-27 (100%)	Staphylococcus phage JD007
Gp015	148746..149069	TreP	107	12352	YP_007112857.1	9e-72 (100%)	Staphylococcus phage JD007
Gp016	149163..149675	hypothetical protein	170	20780	AXU40178.1	1e-108 (98%)	Staphylococcus phage VB_SavM_JYL01
Gp017	149739..150095	hypothetical protein	118	13865	VEV88121.1	7e-71 (98%)	Staphylococcus phage Stab20

Gp018	150625..150846	hypothetical protein	73	8469	YP_007112855.1	2e-45 (100%)	Staphylococcus phage JD007
Gp019	150927..151064	hypothetical protein	45	5459	YP_007112854.1	4e-24 (100%)	Staphylococcus phage JD007
Gp020	151126..151299	hypothetical protein	57	6765	VEV88124.1	1e-31 (100%)	Staphylococcus phage Stab20
Gp021	151552..152022	hypothetical protein	156	17885	AVX47376.1	5e-103 (100%)	Staphylococcus phage vB_SauM_0414_108
Gp022	152082..152279	hypothetical protein	65	7481	VEV88129.1	5e-33 (100%)	Staphylococcus phage Stab20
Gp023	152285..152467	terminal repeat-encoded protein	60	6962	VEV88130.1	5e-31 (100%)	Staphylococcus phage Stab20
Gp024	152467..153123	hypothetical protein	218	25574	AUV56944.1	6e-72 (97%)	Staphylococcus phage vB_SauM_LM12
Gp025	153131..153406	Terminal repeat-encoded protein	91	10518	YP_009195839.1	4e-23 (100%)	Staphylococcus phage phiIPLA-RODI
Gp026	153482..153670	hypothetical protein	62	7512	YP_007002139.1	1e-35 (100%)	Staphylococcus phage GH15

Table 3: Putative gene products of Stab22 phage NCBI/ENA accession number (**acc. No.**): **LR215720**, and its homology to Kayvirus phages at protein level.

Stab22							
Gp	**Genomic location**	**Predicted function**	**AA**	**MW**	**Best hit acc. No.**	**e-value (query coverage %)**	**phage with similar gene**
Gp001	312..491	hypothetical protein	59	7075	YP_008854130.1	3e-15 (96%)	Staphylococcus phage S25-4
Gp002	715..1239	hypothetical protein	174	19562	AVP40463.1	4e-69 (98%)	Staphylococcus phage phiSA_BS1
Gp003	1307..1519	hypothetical protein	70	7958	….	….	…..
Gp004	1869..2162	TreA	97	11137	ARM69483.1	2e-56 (100%)	Staphylococcus phage vB_Sau_S24
Gp005	2159..2344	membrane protein	61	7046	YP_008853952.1	8e-17 (98%)	Staphylococcus phage S25-4
Gp006	2347..2520	membrane protein	57	6631	ARM69272.1	5e-17 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp007	2532..2828	hypothetical protein	98	11140	AXU40163.1	1e-50 (100%)	Staphylococcus phage VB_SavM_JYL01
Gp008	3043..3246	putative membrane protein	67	7957	…..	…..	……
Gp009	3259..3573	hypothetical protein	104	11887	ARM69275.1	2e-72 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp010	3587..3886	hypothetical protein	99	11591	ARM69488.1	8e-63 (100%)	Staphylococcus phage vB_Sau_S24
Gp011	3903..4190	TreC	95	10992	ARM69276.1	4e-51 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp012	4190..4486	TreE	98	11398	ARM69278.1	3e-34 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp013	4511..4738	TreF	75	8713	ARM69279.1	1e-44 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp014	4775..4985	terminal repeat-encoded protein	76	9014	YP_009099458.1	3e-21 (100%)	Staphylococcus phage P108
Gp015	5069..5332	hypothetical protein	87	10195	YP_008853956.1	1e-37 (91%)	Staphylococcus phage S25-4
Gp016	5332..5583	terminal repeat-encoded protein	83	9443	BBC69667.1	2e-18 (100%)	Staphylococcus phage phiSA039
Gp017c	(5834..6118)c	hypothetical protein	94	11231	ASZ78147.1	1e-54 (100%)	Staphylococcus phage SA3

Gp018	6518..6832	TreJ	104	12109	ARM69283.1	4e-60 (98%)	Staphylococcus phage vB_Sau_Clo6
Gp019	6939..7409	hypothetical protein	156	18824	AVP40358.1	4e-92 (100%)	Staphylococcus phage phiSA_BS1
Gp020	7483..7737	hypothetical protein	84	9973	AVR55650.1	5e-40 (100%)	Staphylococcus phage phiSA_BS2
Gp021	8265..8555	hypothetical protein	96	11308	…	….	….
Gp022	8892..9143	hypothetical protein	83	9758	AVP40364.1	2e-37 (100%)	Staphylococcus phage phiSA_BS1
Gp023	9214..9621	hypothetical protein	135	15710	YP_009097937.1	1e-83 (98%)	Staphylococcus phage MCE-2014
Gp024	10117..10434	hypothetical protein	105	11917	BBC69674.1	1e-49 (98%)	Staphylococcus phage phiSA039
Gp025	10512..10748	hypothetical protein	78	9068	ARM69294.1	6e-37 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp026	10836..11318	terminal repeat-encoded protein	160	18554	YP_009195837.1	4e-76 (99%)	Staphylococcus phage phiIPLA-RODI
Gp027	11399..11587	hypothetical protein	62	7258	VEV88129.1	2e-33 (100%)	Staphylococcus phage Stab20
Gp028	11600..11869	TreT protein	89	10190	VEV88131.1	1e-48 (100%)	Staphylococcus phage Stab20
Gp029	11956..12186)	TreU protein	76	9177	VEV88132.1	2e-36 (100%)	Staphylococcus phage Stab20
Gp030c	c(12448..12711)	BofL	87	10640	YP_007002141.1	1e-24 (100%)	Staphylococcus phage GH15
Gp031c	c(12727..12972)	hypothetical protein	81	9652	AXU40190.1	4e-52 (100%)	Staphylococcus phage VB_SavM_JYL01
Gp032c	c(12972..13403)	hypothetical protein	143	17363	YP_009195845.1	2e-86 (100%)	Staphylococcus phage phiIPLA-RODI
Gp033c	c(13400..13837)	hypothetical protein	145	16823	AVR55457.1	2e-92 (97%)	Staphylococcus phage phiSA_BS2
Gp034c	c(13851..14393)	hypothetical protein	180	21507	YP_007002151.1	3e-124 (100%)	Staphylococcus phage GH15
Gp035c	c(14405..14893)	GTP cyclohydrolase II	162	19487	ARM69517.1	6e-116 (100%)	Staphylococcus phage vB_Sau_S24
Gp036c	c(15045..15755)	Serine/threonine phosphatase protein	236	27927	YP_009006684.1	8e-158 (100%)	Staphylococcus phage phiSA12
Gp037c	c(16912..17460)	hypothetical protein	182	21968	YP_008853987.1	1e-108 (100%)	Staphylococcus phage S25-4
Gp038c	c(17591..17830)	hypothetical protein	79	9442	YP_007002161.1	7e-48 (100%)	Staphylococcus phage GH15
Gp039c	c(17832..18218)	hypothetical protein	128	14789	ASZ78180.1	8e-78 (100%)	Staphylococcus phage SA3
Gp040c	c(18318..18491)	hypothetical protein	57	6819	YP_007002163.1	3e-36 (100%)	Staphylococcus phage GH15

Gp041c	c(18532..19014)	hypothetical protein	160	19046	VEV88152.1	2e-107 (100%)	Staphylococcus phage Stab20
Gp042c	c(19064..19600)	hypothetical protein	178	20718	ARM69316.1	6e-79 (99%)	Staphylococcus phage vB_Sau_Clo6
Gp043c	c(19600..20133)	hypothetical protein	177	20637	ARM69317.1	8e-117 (98%)	Staphylococcus phage vB_Sau_Clo6
Gp044c	c(20136..20300)	putative membrane protein	54	6286	YP_007002167.1	3e-26 (100%)	Staphylococcus phage GH15
Gp045c	c(20300..20599)	putative membrane protein	98	11605	ARM69530.1	2e-35 (100%)	Staphylococcus phage vB_Sau_S24
Gp046c	c(20599..21444)	hypothetical protein	281	31668	ARM69531.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp047c	c(21457..22575)	AAA family ATPase	372	42054	ARM69321.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp048c	c(22729..23070)	hypothetical protein	113	13285	VEV88159.1	6e-72 (100%)	Staphylococcus phage Stab20
Gp049c	c(23048..23464)	hypothetical protein	138	15941	YP_007002172.1	3e-95 (100%)	Staphylococcus phage GH15
Gp050c	c(23598..23900)	NTP pyrophosphohydrolase	100	11304	YP_007002173.1	4e-66 (99%)	Staphylococcus phage GH15
Gp051c	c(23900..24088)	hypothetical protein	62	7292	ARM69325.1	1e-35 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp052c	c(24132..24293)	hypothetical protein	53	6447	ARM69537.1	9e-31 (100%)	Staphylococcus phage vB_Sau_S24
Gp053c	c(24294..26345)	hypothetical protein	683	79762	YP_008854006.1	0.0 (100%)	Staphylococcus phage S25-4
Gp054c	c(26422..26685)	hypothetical protein	87	10190	ARM69328.1	1e-55 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp055c	c(26702..26875)	LysM domain-containing protein	57	6656	YP_007002178.1	5e-33 (100%)	Staphylococcus phage GH15
Gp056c	c(26882..27460)	membrane protein	192	21498	ARM69541.1	3e-130 (100%)	Staphylococcus phage vB_Sau_S24
Gp057c	c(27453..28055)	nucleoside 2-deoxyribosyltransferase protein	200	22430	VEV88168.1	4e-116 (100%)	Staphylococcus phage Stab20
Gp058c	c(28055..28192)	hypothetical protein	45	4936	VEV88417.1	6e-23 (100%)	Staphylococcus phage Stab23
Gp059c	c(28194..28604)	hypothetical protein	136	15636	AVP40314.1	3e-90 (100%)	Staphylococcus phage phiSA_BS1
Gp060c	c(28604..28828)	putative membrane protein	74	8129	ARM69543.1	1e-36 (100%)	Staphylococcus phage vB_Sau_S24
Gp061c	c(28896..29636)	PhoH-related protein	246	28646	ARM69333.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp062c	c(29690..30265)	hypothetical protein	191	21477	ARM69334.1	6e-129 (100%)	Staphylococcus phage vB_Sau_Clo6

Gp063c	c(30283..30708)	ribonuclease H	141	15844	ARM69546.1	5e-96 (100%)	Staphylococcus phage vB_Sau_S24
Gp064c	c(30701..30889)	hypothetical protein	62	7442	ARM69336.1	2e-36 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp065c	c(30912..31553)	hypothetical protein	213	24475	ARM69337.1	7e-144 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp066c	c(31543..31773)	transcriptional regulator	76	8831	YP_007112802.1	4e-49 (100%)	Staphylococcus phage JD007
Gp067c	c(31776..32003)	hypothetical protein	75	9231	YP_007002190.1	4e-44 (100%)	Staphylococcus phage GH15
Gp068c	c(32113..32811)	transglycosylase	232	25289	ARM69551.1	3e-168 (100%)	Staphylococcus phage vB_Sau_S24
Gp069c	c(33001..33795)	putative membrane protein	264	29353	VEV89254.1	0.0 (100%)	Staphylococcus phage Stab23
Gp070c	c(33796..34104)	putative membrane protein	102	12254	AUV57026.1	2e-63 (100%)	Staphylococcus phage vB_SauM_LM12
Gp071c	c(34219..34514)	hypothetical protein	98	11766	VEV89258.1	8e-65 (100%)	Staphylococcus phage Stab23
Gp072c	c(34616..36106)	N-acetylmuramoyl-L-alanine amidase	496	54904	YP_009097995.1	0.0 (100%)	Staphylococcus phage MCE-2014
Gp073c	c(36106..36609)	holin	167	18068	YP_009195894.1	2e-116 (100%)	Staphylococcus phage phiIPLA-RODI
Gp074c	c(36695..36880)	hypothetical protein	61	7052	YP_007002196.1	7e-36 (100%)	Staphylococcus phage GH15
Gp075c	c(38219..38437)	hypothetical protein	72	8709	YP_007002197.1	5e-47 (100%)	Staphylococcus phage GH15
Gp076c	c(38906..39115)	hypothetical protein	69	7871	ANH50542.1	1e-41 (100%)	Staphylococcus phage pSco-10
Gp077c	c(39128..39460)	hypothetical protein	110	12563	YP_007002199.1	1e-69 (100%)	Staphylococcus phage GH15
Gp078c	c(39473..39799)	putative membrane protein	108	13157	ARM69350.1	1e-71 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp079	40239..40625	membrane protein	128	14809	YP_009195900.1	2e-57 (100%)	Staphylococcus phage phiIPLA-RODI
Gp080	40603..40881	hypothetical protein	92	10610	ANH50538.1	4e-61 (100%)	Staphylococcus phage pSco-10
Gp081	40878..41288	hypothetical protein	136	15698	ANH50537.1	8e-93 (100%)	Staphylococcus phage pSco-10
Gp082	41303..43120	terminase, large subunit	605	70430	ARM69355.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp083	43134..43934	hypothetical protein	266	29765	ARM69356.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp084	43921..44094	hypothetical protein	57	6755	ARM69144.1	1e-28 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp085	44091..44570	hypothetical protein	159	18521	YP_007002208.1	1e-109 (100%)	Staphylococcus phage GH15

Gp086	44663..45823	hypothetical protein	386	42608	AUV56911.1	4e-146 (100%)	Staphylococcus phage vB_SauM_LM12
Gp087	45962..46252	membrane protein	96	11038	ARM69147.1	3e-59 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp088	46258..46629	hypothetical protein	123	14479	YP_009195910.1	2e-85 (100%)	Staphylococcus phage phiIPLA-RODI
Gp089	46633..48324	portal protein	563	63969	ARM69149.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp090	48518..49282	prohead protease	254	28019	ARM69150.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp091	49301..50260	hypothetical protein	319	36065	ARM69364.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp092	50376..51767	major capsid protein	463	51298	ANH50522.1	0.0 (100%)	Staphylococcus phage pSco-10
Gp093	51859..52131	hypothetical protein	90	10280	ANH50521.1	1e-42 (100%)	Staphylococcus phage pSco-10
Gp094	52144..53052	hypothetical protein	302	34108	ARM69154.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp095	53066..53944	capsid protein	292	33715	ARM69368.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp096	53944..54564	hypothetical protein	206	23735	ANH50518.1	2e-147 (100%)	Staphylococcus phage pSco-10
Gp097	54583..55419	hypothetical protein	278	31816	YP_007112771.1	0.0 (100%)	Staphylococcus phage JD007
Gp098	55421..55636	hypothetical protein	71	8252	YP_007002221.1	3e-47 (100%)	Staphylococcus phage GH15
Gp099	55663..57426	major tail sheath	587	64225	ASZ78017.1	0.0 (100%)	Staphylococcus phage SA3
Gp100	57499..57909	tail tube protein	136	15202	AFN38130.1	3e-90 (100%)	Staphylococcus phage A3R
Gp101	58431..59729	hypothetical protein	432	50246	YP_238556.1	5e-156 (99%)	Staphylococcus virus Twort
Gp102	59784..59942	hypothetical protein	52	6502	YP_009006753.1	1e-29 (100%)	Staphylococcus phage phiSA12
Gp103	59932..60069	hypothetical protein	45	5334	AFN38132.1	8e-19 (100%)	Staphylococcus phage A3R
Gp104	60103..60564	hypothetical protein	153	18043	ARM68939.1	4e-104 (98%)	Staphylococcus phage vB_Sau_CG
Gp105	60577..60771	membrane protein	64	6992	ANH50510.1	3e-34 (100%)	Staphylococcus phage pSco-10
Gp106	60842..61153	hypothetical protein	106	12160	YP_007002227.1	2e-62 (100%)	Staphylococcus phage GH15
Gp107	61285..61740	hypothetical protein	151	17978	ARM69381.1	2e-104 (100%)	Staphylococcus phage vB_Sau_S24
Gp108	61775..62320	tail morphogenetic protein	181	21234	AXU40054.1	3e-129 (100%)	Staphylococcus phage VB_SavM_JYL01

Gp109	62373..66422	tail length tape-measure protein	1349	143659	ARM69383.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp110	66502..68925	tail lysin	807	91433	ASZ78029.1	0.0 (100%)	Staphylococcus phage SA3
Gp111	68939..69826	protease	295	34633	YP_009195932.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp112	69826..72372	Glycerophosphoryl diester phosphodiesterase	848	96077	ARM69386.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp113	72479..73270	hypothetical protein	263	29292	ARM69174.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp114	73270..73794	hypothetical protein	174	19948	ARM69175.1	2e-123 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp115	73794..74498	baseplate wedge subunit protein	234	26539	ARM69389.1	5e-172 (100%)	Staphylococcus phage vB_Sau_S24
Gp116	74513..75559	baseplate morphogenetic protein	348	39121	ARM69177.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp117	75580..77820	hypothetical protein	746	85246	YP_008854069.1	0.0 (100%)	Staphylococcus phage S25-4
Gp118	77928..78449	structural protein	173	19298	BBC69556.1	2e-124 (100%)	Staphylococcus phage phiSA039
Gp119	78470..81946	adsorption-associated tail protein	1158	129844	ARM69393.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp120	81995..82153	hypothetical protein	52	6277	YP_008854072.1	3e-29 (100%)	Staphylococcus phage S25-4
Gp121	82154..84073	hypothetical protein	639	73210	ARM69182.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp122	84087..84452	hypothetical protein	121	14388	ARM69183.1	5e-74 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp123	84459..85832	tail fiber protein	457	50960	ARM69184.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp124	85921..87669	DNA helicase A	582	67211	ARM69399.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp125	87681..89294	Rep protein	537	63201	ARM69186.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp126	89287..90729	DNA helicase B	480	54531	ARM69187.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp127	90808..91089	hypothetical protein	93	10916	AVP40297.1	4e-54 (100%)	Staphylococcus phage phiSA_BS1
Gp128	91089..92114	recombination exonuclease A	341	39505	YP_008854079.1	0.0 (100%)	Staphylococcus phage S25-4
Gp129	92114..94033	recombination exonuclease B	639	73035	ARM69190.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp130	94033..94629	anti-sigma factor	198	23365	ARM69192.1	2e-133 (100%)	Staphylococcus phage vB_Sau_Clo6

Gp131	94644..95711	DNA primase	355	40979	ANH50483.1	0.0 (100%)	Staphylococcus phage pSco-10
Gp132	95777..96115	hypothetical protein	112	12964	YP_240943.1	2e-72 (100%)	Staphylococcus virus G1
Gp133	96115..96567	hypothetical protein	150	17155	ANH50481.1	5e-99 (100%)	Staphylococcus phage pSco-10
Gp134	96554..97162	resolvase	202	23617	ANH50480.1	1e-147 (100%)	Staphylococcus phage pSco-10
Gp135	97179..97571	Ribonucleotide reduction protein Class Ib, NrdI	130	14764	ARM69197.1	1e-90 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp136	97586..99700	Ribonucleotide reductase, large subunit	704	80063	ARM69198.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp137	99714..100763	Ribonucleotide reductase, small subunit	349	40410	YP_009099373.1	0.0 (100%)	Staphylococcus phage P108
Gp138	100781..101110	hypothetical protein	109	12401	ARM69413.1	5e-75 (100%)	Staphylococcus phage vB_Sau_S24
Gp139	101094..101414	thioredoxin	106	12048	ARM69201.1	3e-70 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp140	101622..102218	hypothetical protein	198	23602	YP_007002262.1	2e-141 (100%)	Staphylococcus phage GH15
Gp141	102228..102533	integration host factor	101	11839	ANH50469.1	2e-67 (100%)	Staphylococcus phage pSco-10
Gp142	102609..105827	DNA polymerase A	1072	124521	ARM69417.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp143	105896..106138	hypothetical protein	80	9144	ARM69418.1	1e-49 (100%)	Staphylococcus phage vB_Sau_S24
Gp144	106155..106637	hypothetical protein	160	18974	ANH50464.1	3e-117 (100%)	Staphylococcus phage pSco-10
Gp145	106724..107902	hypothetical protein	392	43625	ARM69420.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp146	107962..109209	DNA repair recombinase protein	415	46753	YP_007002268.1	0.0 (100%)	Staphylococcus phage GH15
Gp147	109213..109566	hypothetical protein	117	13421	YP_007002269.1	2e-80 (99%)	Staphylococcus phage GH15
Gp148	109553..110215	RNA polymerase sigma factor	220	26600	ARM69210.1	2e-157 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp149	110342..110974	hypothetical protein	210	23241	YP_008873652.1	2e-149 (100%)	Staphylococcus phage Sb1
Gp150	110987..111508	tail morphogenetic protein	173	18261	AEA36766.1	5e-113 (99%)	Staphylococcus phage GH15
Gp151	111523..111759	Ig-like domain	78	8101	AVX47510.1	1e-40 (92%)	Staphylococcus phage vB_SauM_0414_108

Gp152	111856..112116	hypothetical protein	86	10205	ARM69214.1	7e-57 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp153	112120..112875	hypothetical protein	251	29132	ARM69215.1	1e-180 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp154	112868..114118	metallophosphoesterase	416	47610	ARM69429.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp155	114132..114500	membrane protein	122	14023	YP_009006809.1	4e-82 (100%)	Staphylococcus phage phiSA12
Gp156	114487..114798	hypothetical protein	103	11967	ARM69431.1	2e-70 (100%)	Staphylococcus phage vB_Sau_S24
Gp157	114864..115400	hypothetical protein	178	20826	ARM69219.1	5e-129 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp158	115393..116160	hypothetical protein	255	30047	ARM69220.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp159	116138..116584	hypothetical protein	148	17405	ANH50449.1	2e-130 (100%)	Staphylococcus phage pSco-10
Gp160	116584..117447	hypothetical protein	287	32298	VEV88618.1	0.0 (100%)	Staphylococcus phage Stab23
Gp161	117806..118537	hypothetical protein	243	28342	YP_007002283.1	9e-175 (100%)	Staphylococcus phage GH15
Gp162	118555..119013	hypothetical protein	152	17823	YP_007002284.1	2e-106 (100%)	Staphylococcus phage GH15
Gp163	119078..119521	hypothetical protein	147	17443	ARM69438.1	2e-99 (100%)	Staphylococcus phage vB_Sau_S24
Gp164	19538..120242	hypothetical protein	234	27570	ARM69439.1	6e-161 (100%)	Staphylococcus phage vB_Sau_S24
Gp165	120305..120703	hypothetical protein	132	15381	ARM69227.1	5e-79 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp166	120851..121096	hypothetical protein	81	9504	ARM69228.1	2e-48 (97%)	Staphylococcus phage vB_Sau_Clo6
Gp167	121166..121342	hypothetical protein	58	7052	YP_007002290.1	1e-31 (100%)	Staphylococcus phage GH15
Gp168	121335..121583	putative membrane protein	82	9125	VEV88279.1	3e-48 (100%)	Staphylococcus phage Stab20
Gp169	121576..121809	hypothetical protein	77	8869	ARM69232.1	4e-47 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp170	121889..122533	Ribulose 1,5-biphosphate carboxylase/oxygenase small subunit	214	25071	ARM69446.1	1e-144 (100%)	Staphylococcus phage vB_Sau_S24
Gp171	122808..122984	hypothetical protein	58	6924	ARM69448.1	1e-32 (100%)	Staphylococcus phage vB_Sau_S24
Gp172	122977..123273	hypothetical protein	98	11449	YP_009098099.1	1e-61 (100%)	Staphylococcus phage MCE-2014
Gp173	123312..123503	membrane protein	63	7398	ACB89144.1	6e-32 (100%)	Staphylococcus phage A5W

Gp174	123515..123901	hypothetical protein	128	15095	YP_009195998.1	3e-64 (100%)	Staphylococcus phage phiIPLA-RODI
Gp175	123914..124261	hypothetical protein	115	13047	ANH50431.1	5e-74 (100%)	Staphylococcus phage pSco-10
Gp176	1246267..124539	membrane protein	90	9959	ARM69240.1	5e-53 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp177	124599..124919	hypothetical protein	106	12690	VEV88288.1	1e-65 (97%)	Staphylococcus phage Stab20
Gp178	124942..126000	hypothetical protein	352	41190	YP_009098107.1	5e-70 (99%)	Staphylococcus phage MCE-2014
Gp179	125979..126356	hypothetical protein	125	14672	ARM69242.1	6e-71 (92%)	Staphylococcus phage vB_Sau_Clo6
Gp180	126356..126958	hypothetical protein	200	23377	YP_007002302.1	5e-145 (100%)	Staphylococcus phage GH15
Gp181	126978..127370	hypothetical protein	130	15125	AZB66577.1	4e-15 (100%)	Staphylococcus phage phiSP38-1
Gp182	127371..127559	hypothetical protein	62	7570	ANH50426.1	3e-30 (95%)	Staphylococcus phage pSco-10
Gp183	127885..128334	putative membrane protein	149	16749	ARM69244.1	5e-80 (91%)	Staphylococcus phage vB_Sau_Clo6
Gp184	128336..128626	hypothetical protein	97	11624	ARM69458.1	2e-60 (100%)	Staphylococcus phage vB_Sau_S24
Gp185	128646..128873	putative membrane protein	75	8216	YP_238658.1	8.6	Staphylococcus virus Twort
Gp186	128889..129176	hypothetical protein	95	10902	ARM69460.1	3e-45 (100%)	Staphylococcus phage vB_Sau_S24
Gp187	129178..129843	hypothetical protein	221	25004	ARM69461.1	1e-150 (100%)	Staphylococcus phage vB_Sau_S24
Gp188	129920..130225	hypothetical protein	101	11643	ARM69248.1	9e-63 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp189	130225..130638	hypothetical protein	137	15305	ARM69249.1	1e-50 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp190	130641..131165	metallophosphoesterase	174	20477	YP_007002312.1	3e-122 (100%)	Staphylococcus phage GH15
Gp191	131258..131437	putative membrane protein	59	6360	AUV57092.1	2e-30 (100%)	Staphylococcus phage vB_SauM_LM12
Gp192	131452..131715	hypothetical protein	87	10295	YP_009041416.1	8e-50 (98%)	Staphylococcus virus K
Gp193	131718..132035	hypothetical protein	105	12066	ARM69464.1	9e-67 (100%)	Staphylococcus phage vB_Sau_S24
Gp194	132036..132716	hypothetical protein	226	25789	ARM69036.1	2e-130 (100%)	Staphylococcus phage vB_Sau_CG
Gp195	132794..133018	hypothetical protein	74	8477	ARM69252.1	9e-48 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp196	133034..133294	membrane protein	86	9708	BBC69640.1	1e-27 (97%)	Staphylococcus phage phiSA039

Gp197	13310..134218	ribose-phosphate pyrophosphokinase	302	34752	ARM69253.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp198	134236..136674	nicotinamide phosphoribosyl transferase	812	93458	ARM69254.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp199	136754..137008	hypothetical protein	84	9939	ARM69469.1	7e-47 (100%)	Staphylococcus phage vB_Sau_S24
Gp200	137031..137342	hypothetical protein	103	11707	ARM69256.1	1e-66 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp201	137367..137801	hypothetical protein	144	16853	AVR55483.1	1e-77 (97%)	Staphylococcus phage phiSA_BS2
Gp202	137794..137938	hypothetical protein	48	5772	.....	.....	....
Gp203	138187..138336	hypothetical protein	49	6062	AUV57107.1	1e-11 (100%)	Staphylococcus phage vB_SauM_LM12
Gp204	138365..138628	hypothetical protein	88	10778	ATP66760.1	.....	.....
Gp205	138660..138971	hypothetical protein	103	12128	ARM69266.1	2e-52 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp206	138986..139333	hypothetical protein	115	13463	AVP40379.1	6e-59 (98%)	Staphylococcus phage phiSA_BS1
Gp207	139348..139827	hypothetical protein	159	18930	ANH50414.1	6e-22 (39%)	Staphylococcus phage pSco-10
Gp208	139901..140023	hypothetical protein	40	4852	ARM69258.1	3e-17 (92%)	Staphylococcus phage vB_Sau_Clo6
Gp209	140055..140228	hypothetical protein	57	7022	ANT44694.1	4e-24 (100%)	Staphylococcus phage vB_SscM-1
Gp210	140297..140494	hypothetical protein	66	7636	AXF38435.1	4e-15 (92%)	Staphylococcus phage Quidividi
Gp211	140773..140997	hypothetical protein	74	8632	YP_006561216.1	3e-32 (100%)	Staphylococcus virus IPLA7
Gp212	141043..141444	hypothetical protein	133	16128	AVP40385.1	3-87 (100%)	Staphylococcus phage phiSA_BS1
Gp213	141479..141814	hypothetical protein	111	12917	VEV88320.1	9e-69 (100%)	Staphylcoccus phage Stab20
Gp214	141814..142221	hypothetical protein	135	15478	AVR55468.1	1e-71 (100%)	Staphylococcus phage phiSA_BS2
Gp215	142306..142581	hypothetical protein	91	10492	YP_009196021.1	2e-42 (100%)	Staphylococcus phage phiIPLA-RODI
Gp216	142600..142830	hypothetical protein	76	9027	....	....	....
Gp217	142971..143378	hypothetical protein	136	16229	YP_009196025.1	1e-79 (97%)	Staphylococcus phage phiIPLA-RODI
Gp218	143412..143549	hypothetical protein	46	5679	....	.....	.....

Gp001	143970..144149	hypothetical protein	59	7075	YP_008854130.1	3e-15 (96%)	Staphylococcus phage S25-4
Gp002	149269..149427	hypothetical protein	174	19562	AVP40463.1	4e-69 (98%)	Staphylococcus phage phiSA_BS1
Gp003	149501..149809	hypothetical protein	70	7958	….	….	…..
Gp004	149972..150298	TreA	97	11137	ARM69483.1	2e-56 (100%)	Staphylococcus phage vB_Sau_S24
Gp005	150397..150714	membrane protein	61	7046	YP_008853952.1	8e-17 (98%)	Staphylococcus phage S25-4
Gp006	150794..151195	membrane protein	57	6631	ARM69272.1	5e-17 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp007	151771..152025	hypothetical protein	98	11140	AXU40163.1	1e-50 (100%)	Staphylococcus phage VB_SavM_JYL01
Gp008	152239..152516	putative membrane protein	67	7957	…..	…..	…….
Gp009	152609..153082	hypothetical protein	104	11887	ARM69275.1	2e-72 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp010	153161..153325	hypothetical protein	99	11591	ARM69488.1	8e-63 (100%)	Staphylococcus phage vB_Sau_S24
Gp011	15338..1535601	TreC	95	10992	ARM69276.1	4e-51 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp012	153605..153793	TreE	98	11398	ARM69278.1	3e-34 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp013	153830..154075	TreF	75	8713	ARM69279.1	1e-44 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp014	148413..148643	terminal repeat-encoded protein	76	9014	YP_009099458.1	3e-21 (100%)	Staphylococcus phage P108
Gp015	148727..148987	hypothetical protein	87	10195	YP_008853956.1	1e-37 (91%)	Staphylococcus phage S25-4
Gp016	148990..149238	terminal repeat-encoded protein	83	9443	BBC69667.1	2e-18 (100%)	Staphylococcus phage phiSA039
Gp017	149495..149776 c	hypothetical protein	94	11231	ASZ78147.1	1e-54 (100%)	Staphylococcus phage SA3
Gp018	150176..150490	TreJ	104	12109	ARM69283.1	4e-60 (98%)	Staphylococcus phage vB_Sau_Clo6
Gp019	150597..151064	hypothetical protein	156	18824	AVP40358.1	4e-92 (100%)	Staphylococcus phage phiSA_BS1
Gp020	151141..151392	hypothetical protein	84	9973	AVR55650.1	5e-40 (100%)	Staphylococcus phage phiSA_BS2
Gp021	151923..152210	hypothetical protein	96	11308	…	….	….
Gp022	152550..152798	hypothetical protein	83	9758	AVP40364.1	2e-37 (100%)	Staphylococcus phage phiSA_BS1
Gp023	152872..153276	hypothetical protein	135	15710	YP_009097937.1	1e-83 (98%)	Staphylococcus phage MCE-2014

Gp024	153766..154092	hypothetical protein	105	11917	BBC69674.1	1e-49 (98%)	Staphylococcus phage phiSA039
Gp025	154170..154406	hypothetical protein	78	9068	ARM69294.1	6e-37 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp026	154494..154976	terminal repeat-encoded protein	160	18554	YP_009195837.1	4e-76 (99%)	Staphylococcus phage phiIPLA-RODI
Gp027	155057..155245	hypothetical protein	62	7258	VEV88129.1	2e-33 (100%)	Staphylococcus phage Stab20
Gp028	155258..155527	TreT protein	89	10190	VEV88131.1	1e-48 (100%)	Staphylococcus phage Stab20
Gp029	155614..155844	TreU protein	76	9177	VEV88132.1	2e-36 (100%)	Staphylococcus phage Stab20

Table 4: Putative gene products of Stab23 phage NCBI/ENA accession number (**acc. No.**): **LR215721**, and its homology to Kayvirus phages at protein level.

Stab23 putative gene products (Gp)							
Gp	**Genomic location**	**Predicted function**	**AA**	**MW**	**Best hit acc. No.**	**e-value (query coverage %)**	**phage with similar gene**
Gp001	553..1536	hypothetical protein	327	36796	ARM69482.1	1e-119 (79%)	Staphylococcus phage vB_Sau_S24
Gp002	1766..2059	hypothetical protein	97	11159	ARM69271.1	6e-58 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp003	2056..2217	putative membrane protein	53	5951	ARM69484.1	2e-26 (98%)	Staphylococcus phage vB_Sau_S24
Gp004	2314..2499	putative membrane protein	61	7238	…	….	….
Gp005	2515..2829	hypothetical protein	104	11954	ARM69275.1	1e-70 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp006	2843..3136	hypothetical protein	97	11232	ARM69277.1	5e-55 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp007	3140..3391	hypothetical protein	83	9746	ARM69279.1	2e-52 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp008	3479..3727	hypothetical protein	82	9860	ARM69280.1	4e-37 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp009	3740..3976	hypothetical protein	78	8862	ARM69493.1	2e-50 (100%)	Staphylococcus phage vB_Sau_S24
Gp010c	(4219..4551)c	hypothetical protein	110	13307	ARM69282.1	8e-61 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp011	4862..5170	TreJ	102	11938	ARM69283.1	8e-64 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp012	5362..5835	hypothetical protein	157	18855	ARM69285.1	3e-101 (98%)	Staphylococcus phage vB_Sau_Clo6
Gp013	5892..6137	hypothetical protein	81	9901	AVP40359.1	3e-36 (96%)	Staphylococcus phage phiSA_BS1
Gp014	6995..7153	TreN	52	5985	ARM69287.1	2e-27 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp015	7227..7535	hypothetical protein	102	11944	ARM69500.1	2e-68 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp016	7698..8024	TreP	108	12401	ARM69501.1	1e-71 (100%)	Staphylococcus phage vB_Sau_S24
Gp017	8123..8440	hypothetical protein	105	12181	BBC69674.1	8e-33 (100%)	Staphylococcus phage phiSA039
Gp018	8514..8921	hypothetical protein	135	15740	ARM69291.1	1e-84 (98%)	Staphylococcus phage vB_Sau_Clo6

Gp019	9497..9751	hypothetical protein	84	9730	ARM69292.1	3e-56 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp020	9965..10243	hypothetical protein	92	10789	VEV88348.1	2e-51 (100%)	Staphylococcus phage Stab20
Gp021	10335..10808	hypothetical protein	157	18016	ARM69078.1	8e-87 (100%)	Staphylococcus phage vB_Sau_CG
Gp022	10887..11051	hypothetical protein	54	6181	ARM69297.1	1e-29 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp023	11064..11327	hypothetical protein	87	10250	ARM69079.1	6e-55 (100%)	Staphylococcus phage vB_Sau_CG
Gp024	11331..11519	hypothetical protein	62	7173	VEV88129.1	2e-33 (100%)	Staphylococcus phage Stab20
Gp025	11532..11801	TreT	89	10112	ARM69081.1	6e-55 (100%)	Staphylococcus phage vB_Sau_CG
Gp026	11886..12107	TreU	73	8944	VEV88132.1	6e-45 (100%)	Staphylococcus phage Stab20
Gp027c	(12388..12678)c	hypothetical protein	96	11594	VEV88133.1	5e-62 (100%)	Staphylococcus phage Stab20
Gp028c	(12775..14487)c	putative tail fiber protein	570	64852	YP_009097947.1	2e-61 (24%)	Staphylococcus phage MCE-2014
Gp029c	(14555..14806)c	BofL	83	10015	ARM69301.1	6e-51 (97%)	Staphylococcus phage vB_Sau_Clo6
Gp030c	(14822..15067)c	hypothetical protein	81	9681	YP_008853977.1	7e-52 (100%)	Staphylococcus phage S25-4
Gp031c	(15067..15588)c	hypothetical protein	173	20327	YP_009099479.1	2e-80 (99%)	Staphylococcus phage P108
Gp032c	(15594..16073)c	putative membrane protein	159	17910	ARM69303.1	5e-105 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp033c	(16066..16320)c	hypothetical protein	84	9675	ASZ78168.1	8e-49 (100%)	Staphylococcus phage SA3]
Gp034c	(16320..16754)c	hypothetical protein	144	16762	YP_007002150.1	1e-87 (100%)	Staphylococcus phage GH15
Gp035c	(16769..17263)c	GTP cyclohydrolase II	164	19751	ARM69306.1	3e-118 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp036c	(17278..17730)c	hypothetical protein	150	17704	YP_009097955.1	2e-92 (100%)	Staphylococcus phage MCE-2014
Gp037c	(18836..19381)c	hypothetical protein	181	21873	YP_008853987.1	6e-100 (92%)	Staphylococcus phage S25-4
Gp038c	(19385..19600)c	hypothetical protein	71	8347	ARM69310.1	4e-41 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp039c	(19597..20346)c	hypothetical protein	249	29109	YP_009195858.1	8e-151 (98%)	Staphylococcus phage phiIPLA-RODI
Gp040c	(20483..20722)c	hypothetical protein	79	9398	YP_007002161.1	8e-47 (100%)	Staphylococcus phage GH15

Gp041c	(20724..21110)c	hypothetical protein	128	14679	ARM69313.1	7e-84 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp042c	(21208..21381)c	hypothetical protein	57	6861	ARM69314.1	3e-34 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp043c	(21422..21904)c	hypothetical protein	160	19093	ARM69525.1	1e-110 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp044c	(21954..22490)c	hypothetical protein	178	20200	YP_008853995.1	5e-108 (100%)	Staphylococcus phage S25-4
Gp045c	(22490..23023)c	hypothetical protein	177	20640	ARM69317.1	3e-118 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp046c	(23193..23474)c	putative membrane protein	93	11124	ARM69530.1	7e-58 (100%)	Staphylococcus phage vB_Sau_S24
Gp047c	(23474..24319)c	hypothetical protein	281	31672	ARM69320.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp048c	(24331..25461)c	AAA family ATPase	376	42536	ARM69321.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp049c	(25612..25938)c	hypothetical protein	108	12813	ARM69322.1	3e-71 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp050c	(25931..26347)c	hypothetical protein	138	16029	ARM69323.1	3e-97 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp051c	(26482..26784)c	nucleoside triphosphate pyrophosphohydrolase	100	11290	YP_007002173.1	7e-66 (100%)	Staphylococcus phage GH15
Gp052c	(26784..26972)c	hypothetical protein	62	7293	YP_007112816.1	3e-35 (100%)	Staphylococcus phage JD007
Gp053c	(27016..27177)c	hypothetical protein	53	6336	ARM69326.1	8e-25 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp054c	(27178..29229)c	hypothetical protein	683	80127	ARM69327.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp055c	(29307..29570)c	hypothetical protein	87	10232	ARM69539.1	2e-56 (100%)	Staphylococcus phage vB_Sau_S24
Gp056c	(29587..29760)c	Lysin	57	6628	YP_007002178.1	1e-33 (100%)	Staphylococcus phage GH15
Gp057c	(29767..30345)c	putative membrane protein	192	21438	ARM69541.1	8e-133 (100%)	Staphylococcus phage vB_Sau_S24
Gp058c	(30338..30961)c	nucleoside 2-deoxyribosyltransferase	207	23457	VEV88533.1	5e-131 (100%)	Staphylococcus phage Stab21
Gp059c	(30961..31098)c	hypothetical protein	45	5041	AVP40312.1	4e-17 (97%)	Staphylococcus phage phiSA_BS1
Gp060c	(31100..31324)c	putative membrane protein	74	8080	ARM69119.1	5e-37 (100%)	Staphylococcus phage vB_Sau_CG
Gp061c	(31392..32132)c	PhoH-related protein	246	28760	ARM69333.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp062c	(32184..32894)c	hypothetical protein	236	27107	ARM69545.1	2e-167 (100%)	Staphylococcus phage vB_Sau_S24

Gp063c	(32912..33337)c	ribonuclease H	141	15774	ARM69546.1	2e-98 (100%)	Staphylococcus phage vB_Sau_S24
Gp064c	(33330..33518)c	hypothetical protein	62	7505	ARM69336.1	6e-39 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp065c	(33541-34182)c	hypothetical protein	213	24475	ARM69548.1	4e-146 (100%)	Staphylococcus phage vB_Sau_S24
Gp066c	(34172..34402)c	transcriptional regulator	76	8833	YP_007002189.1	2e-48 (100%)	Staphylococcus phage GH15
Gp067c	(34405..34632)c	hypothetical protein	75	9235	BBC69504.1	2e-47 (100%)	Staphylococcus phage phiSA039
Gp068c	(34741..35430)c	transglycosylase	229	25065	BBC69505.1	3e-149 (100%)	Staphylococcus phage phiSA039
Gp069c	(35625..36419)c	putative membrane protein	264	29297	ARM69552.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp070c	(36420..36728)c	hypothetical protein	102	12173	YP_009099524.1	1e-67 (100%)	Staphylococcus phage P108
Gp071c	(36842..37462)c	hypothetical protein	206	24604	YP_009097994.1	2e-144 (100%)	Staphylococcus phage MCE-2014
Gp072c	(37525..39015)c	N-acetylmuramoyl-L-alanine amidase	496	54981	ARM69554.1	0.0 (99%)	Staphylococcus phage vB_Sau_S24
Gp073c	(39015..39518)c	holin	167	18111	ARM69345.1	1e-118 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp074c	(39603..39788)c	hypothetical protein	61	7052	ARM69556.1	4e-36 (100%)	Staphylococcus phage vB_Sau_S24
Gp075c	(41015..41233)c	hypothetical protein	72	8665	ARM69347.1	4e-47 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp076c	(41697..41906)c	hypothetical protein	69	7804	ARM69348.1	2e-42 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp077c	(41919..42251)c	hypothetical protein	110	12491	ANH50541.1	1e-71 (100%)	Staphylococcus phage pSco-10
Gp078c	(42264..42590)c	hypothetical protein	108	13100	ARM69350.1	2e-73 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp079	43149..43424	hypothetical protein	91	10695	ARM69352.1	6e-55 (94%)	Staphylococcus phage vB_Sau_Clo6
Gp080	43393..43680	hypothetical protein	95	10934	ANH50538.1	1e-62 (95%)	Staphylococcus phage pSco-10
Gp081	43677..44087	hypothetical protein	136	15726	ANH50537.1	2e-93 (100%)	Staphylococcus phage pSco-10
Gp082	44102..45919	terminase, large subunit	605	70485	ARM69142.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp083	45912..46733	hypothetical protein	273	30512	YP_007002206.1	0.0 (100%)	Staphylococcus phage GH15
Gp084	46890..47369	hypothetical protein	159	18524	ARM69145.1	1e-110 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp085	47412..48707	hypothetical protein	431	47006	ARM69359.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24

Gp086	48789..49136	hypothetical protein	115	13154	ARM69147.1	1e-75 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp087	49142..49513	hypothetical protein	123	14479	YP_009195910.1	2e-85 (100%)	Staphylococcus phage phiIPLA-RODI
Gp088	49517..51208	portal protein	563	64038	ARM69149.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp089	51402..52166	prohead protease	254	28062	ARM69150.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp090	52185..53135	hypothetical protein	316	35836	ARM69364.1	3e-159 (100%)	Staphylococcus phage vB_Sau_S24
Gp091	53251..54642	major capsid porotein	463	51260	ARM69152.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp092	54734..55006	hypothetical protein	90	10382	ARM69153.1	8e-56 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp093	55020..55928	hypothetical protein	302	34082	ARM69154.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp094	55942..56820	capsid protein	292	33741	ARM69155.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp095	56820..57440	hypothetical protein	206	23748	ARM69156.1	4e-151 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp096	57459..58295	hypothetical protein	278	31794	ARM69157.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp097	58297..58512	hypothetical protein	71	8280	YP_007002221.1	7e-48 (100%)	Staphylococcus phage GH15
Gp098	58539..60302	major tail sheath	587	64491	ARM69159.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp099	60375..60803	tail tube protein	142	15871	ARM69160.1	5e-102 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp100	60902..61039	hypothetical protein	45	5307	ANH50512.1	3e-24 (100%)	Staphylococcus phage pSco-10
Gp101	61073..61534	hypothetical protein	153	17945	ARM69163.1	3e-97 (98%)	Staphylococcus phage vB_Sau_Clo6
Gp102	61547..61789	hypothetical protein	85	8865	ARM68940.1	2e-50 (94%)	Staphylococcus phage vB_Sau_CG
Gp103	61789..61983	putative membrane protein	64	6950	ANH50510.1	6e-36 (100%)	Staphylococcus phage pSco-10
Gp104	61999..62151	hypothetical protein	50	5859	ARM69379.1	6e-30 (100%)	Staphylococcus phage vB_Sau_S24
Gp105	62219..62530	hypothetical protein	103	12190	ARM69380.1	3e-68 (100%)	Staphylococcus phage vB_Sau_S24
Gp106	62219..62530	hypothetical protein	151	18036	ARM69381.1	1e-106 (100%)	Staphylococcus phage vB_Sau_S24
Gp107	63161..63697	tail morphogenetic protein	178	21023	ARM69382.1	1e-128 (100%)	Staphylococcus phage vB_Sau_S24

Gp108	63751..67809	tail length tape-measure protein	1352	143711	ARM69383.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp109	67889..70312	tail lysin	807	91344	ARM69171.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp110	70326..71213	peptidoglycan hydrolase	295	34650	ARM69172.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp111	71213..73759	Glycerophosphoryl diester phosphosdiesterase	848	95925	ARM69173.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp112	73866..74657	hypothetical protein	263	29277	ARM69174.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp113	74657..75181	hypothetical protein	174	19939	ARM69388.1	4e-123 (100%)	Staphylococcus phage vB_Sau_S24
Gp114	75181..75885	baseplate wedge subunit	234	26526	ARM69176.1	1e-172 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp115	75900..76946	baseplate morphogenetic protein	348	39110	ARM69177.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp116	76967..80512	tail morphogenetic protein	1181	134933	ARM69391.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp117	80623..81144	baseplate morphogenetic protein	173	19121	ARM68954.1	3e-124 (100%)	Staphylococcus phage vB_Sau_CG
Gp118	81165..84641	adsorption-associated tail protein	1158	129758	ARM69393.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp119	84690..84848	hypothetical protein	52	6305	YP_009099353.1	2e-29 (100%)	Staphylococcus phage P108
Gp120	84849..86762	hypothetical protein	637	72509	ASK86679.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp121	86776..87147	hypothetical protein	123	14553	ARM69397.1	2e-87 (100%)	Staphylococcus phage vB_Sau_S24
Gp122	87154..88527	tail fiber protein	457	50699	ARM69398.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp123	88616..90364	DNA helicase A	582	67212	ARM69399.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp124	90376..91989	replication protein	537	63285	ANH50490.1	0.0 (100%)	Staphylococcus phage pSco-10
Gp125	91982..93424	DNA helicase B	480	54588	ANH50489.1	0.0 (100%)	Staphylococcus phage pSco-10
Gp126	93504..93785	hypothetical protein	93	10859	ARM69188.1	1e-52 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp127	93785..94810	recombination exonuclease A	341	39336	YP_009195949.1	0.0 (100%)	Staphylococcus phage phiIPLA-RODI
Gp128	94810..95187	hypothetical protein	125	15133	YP_009099363.1	7e-87 (100%)	Staphylococcus phage P108
Gp129	95187..97106	recombination exonuclease B	639	73229	ARM69404.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24

Gp130	97106..97702	hypothetical protein	198	23207	YP_009098053.1	1e-143 (100%)	Staphylococcus phage MCE-2014
Gp131	97717..98784	DNA primase	355	40951	ANH50483.1	0.0 (100%)	Staphylococcus phage pSco-10
Gp132	98849..99187	hypothetical protein	112	12947	ARM69194.1	2e-73 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp133	99187..99639	hypothetical protein	150	17128	ARM69195.1	3e-102 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp134	99626..100234	resolvase	202	23692	ARM69196.1	1e-147 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp135	100251..100643	ribonucleotide reduction protein NrdI	130	14738	ARM69410.1	2e-91 (100%)	Staphylococcus phage vB_Sau_S24
Gp136	100658..102772	ribonucleotide reductase, large subunit	704	79967	ARM69411.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp137	102786..103835	ribonucleotide reductase, small subunit	349	40472	ARM69199.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp138	103853..104182	hypothetical protein	109	12387	ARM69413.1	3e-74 (100%)	Staphylococcus phage vB_Sau_S24
Gp139	104166..104486	thioredoxin	106	12018	ARM69201.1	2e-70 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp140	104694..105290	hypothetical protein	198	23582	ANH50470.1	4e-143 (100%)	Staphylococcus phage pSco-10
Gp141	105300..105605	DNA binding protein	101	11909	ARM69203.1	2e-68 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp142	105681..108863	DNA polymerase A	1060	122811	ARM69417.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp143	108934..109176	hypothetical protein	80	9198	ARM69418.1	5e-51 (100%)	Staphylococcus phage vB_Sau_S24
Gp144	109193..109675	hypothetical protein	160	18932	ARM69206.1	3e-117 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp145	109761..110933	hypothetical protein	390	43495	ARM69207.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp146	110993..112249	repair recombinase	418	46734	ARM69208.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp147	112253..112606	hypothetical protein	117	13352	ANH50461.1	1e-81 (100%)	Staphylococcus phage pSco-10
Gp148	112593..113255	RNA polymerase sigma factor	220	26594	ARM69210.1	7e-159 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp149	113382..114014	hypothetical protein	210	23198	ARM69211.1	2e-151 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp150	114036..114548	tail morphogenetic protein	170	17429	ARM69425.1	7e-114 (100%)	Staphylococcus phage vB_Sau_S24
Gp151	114563..114781	Ig-like domain	72	7399	YP_007002273.1	1e-37 (95%)	Staphylococcus phage GH15
Gp152	114877..115137	hypothetical protein	86	10232	ARM69214.1	2e-57 (100%)	Staphylococcus phage vB_Sau_Clo6

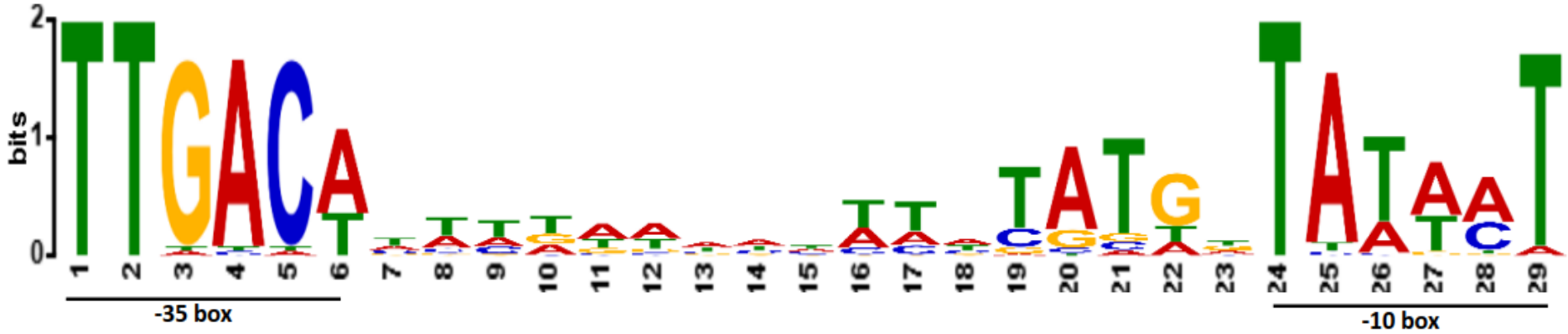
Gp153	115141..115896	hypothetical protein	251	29423	ARM69428.1	1e-178 (100%)	Staphylococcus phage vB_Sau_S24
Gp154	115889..117139	metallophosphoesterase	416	47594	ARM69429.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp155	117153..117521	membrane protein	122	14068	ANH50453.1	2e-83 (100%)	Staphylococcus phage pSco-10
Gp156	117508..117819	hypothetical protein	103	11981	ARM69431.1	3e-70 (100%)	Staphylococcus phage vB_Sau_S24
Gp157	117883..118419	hypothetical protein	178	20830	ARM69219.1	2e-130 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp158	118412..119179	hypothetical protein	255	30033	ANH50450.1	0.0 (100%)	Staphylococcus phage pSco-10
Gp159	119157..119603	hypothetical protein	148	17427	ARM69434.1	3e-105 (100%)	Staphylococcus phage vB_Sau_S24
Gp160	119603..120466	hypothetical protein	287	32318	ARM69222.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp161	120825..121556	hypothetical protein	243	28351	ARQ96133.1	1e-175 (100%)	Staphylococcus phage qdsa002
Gp162	121574..122032	hypothetical protein	152	17907	ARM69437.1	2e-108 (100%)	Staphylococcus phage vB_Sau_S24
Gp163	122097..122540	hypothetical protein	147	17398	ARM69438.1	1e-100 (100%)	Staphylococcus phage vB_Sau_S24
Gp164	122557..123279	hypothetical protein	240	28131	ARM69439.1	2e-152 (100%)	Staphylococcus phage vB_Sau_S24
Gp165	123340..123738	putative membrane protein	132	15345	ARM69227.1	8e-81 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp166	123886..124128	hypothetical protein	80	9393	ARM69441.1	4e-51 (100%)	Staphylococcus phage vB_Sau_S24
Gp167	124133..124690	putative membrane protein	185	21584	ARM69229.1	6e-129 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp168	124726..124902	hypothetical protein	58	6935	ARM69443.1	6e-34 (100%)	Staphylococcus phage vB_Sau_S24
Gp169	124895..125143	putative membrane protein	82	9103	ARM69231.1	9e-51 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp170	125136..125369	hypothetical protein	77	8916	ARM69445.1	2e-48 (100%)	Staphylococcus phage vB_Sau_S24
Gp171	125450..126094	ribulose 1, 5-biphosphate carboxylase/oxygenase small subunit	214	25158	ARM69446.1	6e-141 (100%)	Staphylococcus phage vB_Sau_S24
Gp172	126109..126357	hypothetical protein	82	8829	ARM69447.1	2e-45 (100%)	Staphylococcus phage vB_Sau_S24
Gp173	126369..126545	hypothetical protein	58	7009	YP_009098098.1	2e-33 (100%)	Staphylococcus phage MCE-2014
Gp174	126538..126834	hypothetical protein	98	11243	ARM69236.1	2e-60 (100%)	Staphylococcus phage vB_Sau_Clo6

Gp175	126882..127064	putative membrane protein	60	7068	ARM69237.1	3e-34 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp176	127077..127445	hypothetical protein	122	14063	ARM69451.1	2e-84 (100%)	Staphylococcus phage vB_Sau_S24
Gp177	127458..127805	hypothetical protein	115	13026	ARM69239.1	1e-76 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp178	127805..128083	putative membrane protein	92	10180	ARM69240.1	1e-56 (68%)	Staphylococcus phage vB_Sau_Clo6
Gp179	128153..128458	hypothetical protein	101	12163	YP_007002300.1	3e-70 (100%)	Staphylococcus phage GH15
Gp180	128473..128823	hypothetical protein	116	13666	YP_007112906.1	3e-77 (100%)	Staphylococcus phage JD007
Gp181	128823..129206	hypothetical protein	127	15075	ANH50427.1	2e-19 (100%)	Staphylococcus phage pSco-10
Gp182	129207..129386	hypothetical protein	59	7220	ANH50426.1	2e-31 (100%)	Staphylococcus phage pSco-10
Gp183	129612..130022	putative membrane protein	136	15168	ARM69244.1	2e-90 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp184	130024..130317	hypothetical protein	97	11641	ARM69458.1	5e-63 (100%)	Staphylococcus phage vB_Sau_S24
Gp185	130334..130621	putative membrane protein	95	10554	ARM69459.1	2e-61 (100%)	Staphylococcus phage vB_Sau_S24
Gp186	130669..131334	hypothetical protein	221	25046	ARM69247.1	1e-153 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp187	131411..131716	hypothetical protein	101	11646	ARM69248.1	1e-66 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp188	131716..132123	hypothetical protein	135	15378	ARM69249.1	6e-85 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp189	132126..132443	hypothetical protein	105	12152	ARM69464.1	3e-70 (100%)	Staphylococcus phage vB_Sau_S24
Gp190	132521..132724	hypothetical protein	60	7771	ARM69251.1	4e-37 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp191	132758..132982	hypothetical protein	74	8477	ARM69252.1	1e-47 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp192	132999..133907	ribose-phosphate pyrophosphokinase	302	35002	ARM69253.1	0.0 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp193	133926..135395	Nicorinamide phosphoribosyltransferase	489	56102	ARM69468.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp194	135476..135730	hypothetical protein	84	9866	ARM69469.1	1e-47 (100%)	Staphylococcus phage vB_Sau_S24
Gp195	135754..136065	hypothetical protein	103	11704	ARM69256.1	3e-69 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp196	136144..136434	hypothetical protein	96	11568	ARM69471.1	6e-65 (100%)	Staphylococcus phage vB_Sau_S24
Gp197	136431..136544	hypothetical protein	37	4458	ARM69258.1	5e-17 (100%)	Staphylococcus phage vB_Sau_Clo6

Gp198	136575..136757	hypothetical protein	60	7226	….	….	…..
Gp199	136799..136960	hypothetical protein	53	6171	….	……	……
Gp200	137003..137284	hypothetical protein	93	10814	ARM69261.1	8e-57 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp201	137342..139615	RNA ligase	757	89137	ARM69476.1	0.0 (100%)	Staphylococcus phage vB_Sau_S24
Gp202	139715..139984	hypothetical protein	89	10321	ARM69263.1	7e-59 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp203	140013..140402	hypothetical protein	129	15203	ARM69264.1	3e-90 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp204	140429..140578	hypothetical protein	49	5846	ARM69265.1	1e-26 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp205	141030..142235	hypothetical protein	103	11972	ARM69266.1	1e-68 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp206	141030..142235	hypothetical protein	401	47057	ANT44859.1	2e-91 (87%)	Staphylococcus phage vB_SscM-1
Gp001	553..1536	hypothetical protein	327	36796	ARM69482.1	1e-119 (79%)	Staphylococcus phage vB_Sau_S24
Gp002	1766..2059	hypothetical protein	97	11159	ARM69271.1	6e-58 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp003	2056..2217	putative membrane protein	53	5951	ARM69484.1	2e-26 (98%)	Staphylococcus phage vB_Sau_S24
Gp004	2314..2499	putative membrane protein	61	7238	…	….	….
Gp005	2515..2829	hypothetical protein	104	11954	ARM69275.1	1e-70 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp006	2843..3136	hypothetical protein	97	11232	ARM69277.1	5e-55 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp007	3140..3391	hypothetical protein	83	9746	ARM69279.1	2e-52 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp008	3479..3727	hypothetical protein	82	9860	ARM69280.1	4e-37 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp009	3740..3976	hypothetical protein	78	8862	ARM69493.1	2e-50 (100%)	Staphylococcus phage vB_Sau_S24
Gp010c	(4219..4551)c	hypothetical protein	110	13307	ARM69282.1	8e-61 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp011	4862..5170	TreJ	102	11938	ARM69283.1	8e-64 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp012	5362..5835	hypothetical protein	157	18855	ARM69285.1	3e-101 (98%)	Staphylococcus phage vB_Sau_Clo6
Gp013	5892..6137	hypothetical protein	81	9901	AVP40359.1	3e-36 (96%)	Staphylococcus phage phiSA_BS1
Gp014	6995..7153	TreN	52	5985	ARM69287.1	2e-27 (100%)	Staphylococcus phage vB_Sau_Clo6

Gp015	7227..7535	hypothetical protein	102	11944	ARM69500.1	2e-68 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp016	7698..8024	TreP	108	12401	ARM69501.1	1e-71 (100%)	Staphylococcus phage vB_Sau_S24
Gp017	8123..8440	hypothetical protein	105	12181	BBC69674.1	8e-33 (100%)	Staphylococcus phage phiSA039
Gp018	8514..8921	hypothetical protein	135	15740	ARM69291.1	1e-84 (98%)	Staphylococcus phage vB_Sau_Clo6
Gp019	9497..9751	hypothetical protein	84	9730	ARM69292.1	3e-56 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp020	9965..10243	hypothetical protein	92	10789	VEV88348.1	2e-51 (100%)	Staphylococcus phage Stab20
Gp021	10335..10808	hypothetical protein	157	18016	ARM69078.1	8e-87 (100%)	Staphylococcus phage vB_Sau_CG
Gp022	10887..11051	hypothetical protein	54	6181	ARM69297.1	1e-29 (100%)	Staphylococcus phage vB_Sau_Clo6
Gp023	11064..11327	hypothetical protein	87	10250	ARM69079.1	6e-55 (100%)	Staphylococcus phage vB_Sau_CG
Gp024	11331..11519	hypothetical protein	62	7173	VEV88129.1	2e-33 (100%)	Staphylococcus phage Stab20
Gp025	11532..11801	TreT	89	10112	ARM69081.1	6e-55 (100%)	Staphylococcus phage vB_Sau_CG
Gp026	11886..12107	TreU	73	8944	VEV88132.1	6e-45 (100%)	Staphylococcus phage Stab20

Appendix IV: Putative promoters of the Stab phages.
Consensus motif of predicted Stab20 promoters



A table of Stab20 putative promoter sequences.

No.	Upstream of gene	Strand	*p*-value	Promoter sequence				
					-35 box	Spacer	-10 box	
1.	*g001*	+	9.71×10^{-5}	T	TGACAA	CTATGAAGCGGTTATGG	TATACT	
2.	*g011c*	+	1.00×10^{-6}		TTGACT	TCTGAATAACTATACTG	TAATAT	
3.	*g012*	+	1.67×10^{-6}		TTGACT	TATTAATCATATGGTAG	TAATAT	
4.	*g013*	+	1.33×10^{-8}		TTGACA	CCTTACAAGATACATGT	TATTAT	
5.	*g015*	+	4.82×10^{-7}		TTGACT	TATGTTTATTCTTATAG	TAATAT	

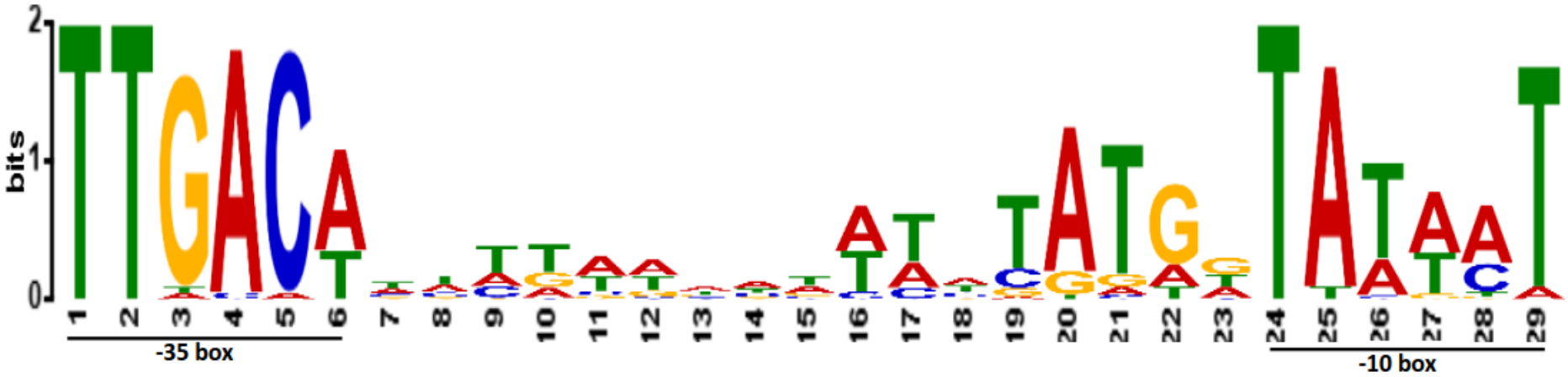
6.	*g016*	+	1.57×10^{-7}		TTGACA	GTCACTTGAAACCATGA	TATTAT	
7.	*g017*	+	8.51×10^{-9}		TTGACT	TTCAAGCCCTGCCATGT	TATTAT	
8.	*g018*	+	1.58×10^{-9}		TTGACA	CTTTCAAGCCCTCATGA	TATACT	
9.	*g019*	+	2.40×10^{-7}		TTGACA	AACTTCAAACCACATGT	TAATAT	
10.	*g020*	+	7.30×10^{-9}		TTGACA	TTCAACCCCTACCATGT	TAATAT	
11.	*g021*	+	4.40×10^{-8}		TTGACA	AACTAACCGCTTCATGA	TAATAT	
12.	*g022*	+	3.82×10^{-9}		TTGACA	CTTAGCCCCTTAGATGT	TATTAT	
13.	*g023*	+	2.40×10^{-7}		TTGACT	TCCAAGCCCTACAATGG	TAATAT	
14.	*g029*	+	6.08×10^{-5}	T	TTACAA	CTATTTAATTTGTATGC	TATAAT	
15.	*g030c*	-	7.01×10^{-7}		TTGACA	TTCTAATTATTATCCTT	TATACT	
16.	*g031c*	-	7.01×10^{-7}		TTGACA	TTCTAATTATTATCCTT	TATACT	
17.	*g041c*	-	4.82×10^{-7}		TTGACT	TTTATAAATAAGTATGG	TAAGAT	
18.	*g045c*	-	8.01×10^{-8}		TTGACA	TTAATAAACATATGTGT	TAATAT	
19.	*g051c*	-	2.64×10^{-8}		TTGACT	TTTTCACTAACTTGTGT	TATACT	
20.	*g055c*	-	1.94×10^{-7}		TTGACA	AATGAAAATACTTGTAT	TATAAT	
21.	*g061c*	-	1.75×10^{-7}		TTGACA	AATATTACTTACTATGA	TATGAT	
22.	*g073c*	-	4.40×10^{-8}		TTGACT	TCATAAGTTAACTATGC	TATAAT	

23.	*g080c*	-	5.68×10^{-6}		TTGACT	TATTTATCAATATAGTA	TATAGT	
24.	*g106*	+	8.51×10^{-9}		TTGACA	TTATAAAATTTATATGC	TATTAT	
26.	*g111*	+	2.13×10^{-6}		TTGACA	AATTAAAACTAATAAAC	TATAAT	
27.	*g115*	+	2.31×10^{-8}		TTGACA	CAAGAGTAGTATCATAG	TATACT	
28.	*g123*	+	7.30×10^{-9}		TTGACA	GAAAGTTAATAATATGG	TATACT	
29.	*g129*	+	2.31×10^{-8}		TTGACT	TGGAGAGTATTATGTGG	TATACT	
30.	*g131*	+	2.37×10^{-4}		TTGACA	AAAGAGGGTATGTTGGA	TTATAA	T
31.	*g132*	+	1.15×10^{-8}		TTGACA	TTTTATATGTTAGGTGG	TATAAT	
32.	*g150*	+	1.33×10^{-8}		TTGACA	ATATGTTTAACTTATGT	TATACT	
33.	*g152*	+	4.40×10^{-8}		TTGACA	AATATAAAAAACTATGT	TATAAT	
34.	*g159*	+	6.33×10^{-8}		TTGACA	ATTTATAATATCTATGA	TACACT	
35.	*g164*	+	3.89×10^{-8}		TTGACT	CTTTTTACTATATATGG	TATATT	
36.	*g166*	+	1.24×10^{-4}		TTTACA	AGAGGTGTTATTTATGG	TTATAA	T
38.	*g173*	+	6.33×10^{-8}		TTGACT	CTCTTTTTGTTTTATGG	TATATT	
39.	*g181*	+	4.37×10^{-7}		TTGACA	GATGAAGCATTTTAATA	TATACT	
40.	*g185*	+	1.26×10^{-7}		TTGACA	CTTCTAAACTTTTGTAT	TATACT	
41.	*g190*	+	8.99×10^{-8}		TTGACA	AATGAGTGTGCATAGGT	TATACT	

42.	*g196*	+	2.31×10^{-8}		TTGACA	TTAGGTTTCTTTTATTA	TATACT	
43.	*g204*	+	2.02×10^{-8}		TTGACA	GCAGGTATTTTTTATAG	TATACT	
44.	*g215*	+	6.39×10^{-7}		TTGACT	TGGGTAGATATCTATTA	TATAAT	

***** Spacer region has 17 nucleotides.

Consensus motif of predicted Stab21 promoters



A table of Stab21 putative promoter sequences.

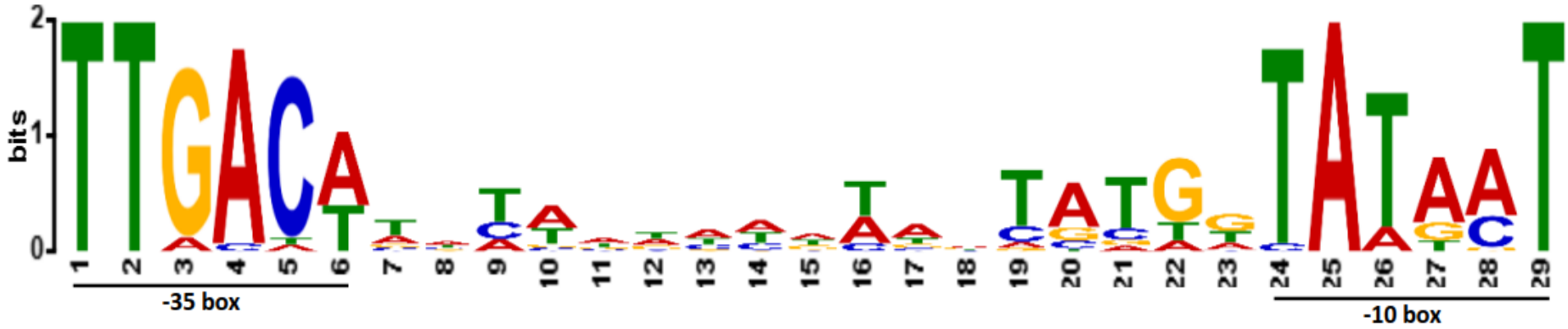
No.	Upstream of gene	Strand	*p*-value	Putative promoter sequence				
					-35 box	Spacer*	-10 box	
1.	*g010c*	+	1.30×10^{-6}		TTGACT	TCTGAATAACTATACTG	TAATAT	
2.	*g011*	+	1.75×10^{-6}		TTGACT	TTTGTATTATATGGTAG	TAATAT	
3.	*g012*	+	1.00×10^{-9}		TTGACA	CCTTATAAGATACATGT	TATTAT	
4.	*g014*	+	4.20×10^{-8}		TTGACT	TGTGTTTCTTTCTATAG	TAATAT	
5.	*g015*	+	4.70×10^{-8}		TTGACA	GTCACTTGAAACCATGA	TATTAT	

6.	*g016*	+	3.34×10^{-9}		TTGACT	TCCAAGCCCTACCATGT	TATTAT	
7.	*g017*	+	3.34×10^{-8}		TTGACA	CTCTCAAGCCTTAATGG	TATACT	
8.	*g018*	+	8.07×10^{-8}		TTGACA	AACTTCCAAATACATGA	TAATAT	
9.	*g019*	+	3.34×10^{-8}		TTGACA	TTCAACCCCTACCATGT	TAATAT	
10.	*g020*	+	1.61×10^{-8}		TTGACA	AACTAACCGCTTCATGA	TAATAT	
11.	*g021*	+	3.76×10^{-10}		TTGACA	CCCTAGCATATAGATGG	TAATAT	
12.	*g026*	+	5.00×10^{-5}	T	TTACAA	TCTTTTAATTTGTATGA	TATAAT	
13.	*g037c*	-	4.20×10^{-8}		TTGACT	TTTTTTACTAAGTATGG	TAAGAT	
14.	*g042c*	-	1.08×10^{-8}		TTGACA	TTATTATCAATATATGT	TATTAT	
15.	*g048c*	-	1.82×10^{-8}		TTGACT	TTTTCACTAACTTATGT	TATACT	
16.	*g052c*	-	3.19×10^{-7}		TTGACA	AATTCAAATACTTGTAA	TATAAT	
17.	*g058c*	-	3.34×10^{-8}		TTGACA	AATATTATTTACTATGG	TATGAT	
18.	*g072c*	-	3.74×10^{-8}		TTGACT	TCATAAGTTAACTATGC	TATAAT	
19.	*g079c*	-	3.31×10^{-6}		TTGACT	TATTTATCAATATAGTA	TATAGT	
20.	*g106*	+	1.72×10^{-9}		TTGACA	CTTTAAAATTTATATGT	TATTAT	
21.	*g109*	+	1.51×10^{-6}		TTGACA	AATTAAAACTAATAAAT	TATAAT	
22.	*g113*	+	1.82×10^{-8}		TTGACA	CAAGAGTAGTATCATAG	TATACT	

23.	*g121*	+	3.74×10^{-8}		TTGACA	GAAAGTTAATAATATGG	TATACT	
24.	*g127*	+	3.74×10^{-8}		TTGACT	TGAAAAGGATTATGTGG	TATACT	
25.	*g129*	+	5.25×10^{-5}		TTGACA	AAAGAGGGTATGTTGGA	TTATAA	T
26.	*g130*	+	9.44×10^{-9}		TTGACA	TTTTATATGTTAGGTGG	TATAAT	
27.	*g146*	+	2.00×10^{-7}		TTGACA	ATACATTTAACTTATGT	TATACT	
28.	*g148*	+	2.34×10^{-8}		TTGACA	AATATAAAAAACTATGT	TATAAT	
29.	*g155*	+	1.41×10^{-8}		TTGACA	ATTTATAATATCTATGA	TACACT	
30.	*g160*	+	1.08×10^{-8}		TTGACT	CTTTTTACTATATATGG	TATATT	
31.	*g162*	+	3.01×10^{-5}		TTTACA	AGAGGTGTTATCTATGG	TTATAA	T
32.	*g169*	+	4.70×10^{-8}		TTGACT	CTCTTTTTGTTTTATGG	TATATT	
33.	*g178*	+	4.55×10^{-7}		TTGACA	GATGAAGCATTTTAATA	TATACT	
34.	*g182*	+	3.74×10^{-8}		TTGACA	CCTTTGTACTTTTGTAT	TATACT	
35.	*g186*	+	6.16×10^{-9}		TTGACA	ATTGAGTATACATAGGT	TATACT	
36.	*g200*	+	2.97×10^{-8}		TTGACA	GCAGGTATTTTTTATAG	TATACT	
37.	*g211*	+	9.56×10^{-7}		TTGACT	TAGGTAGATACTTATTA	TATAAT	

***** Spacer region has 17 nucleotides.

Consensus motif of predicted Stab22 promoters



A table of Stab22 putative promoter sequences

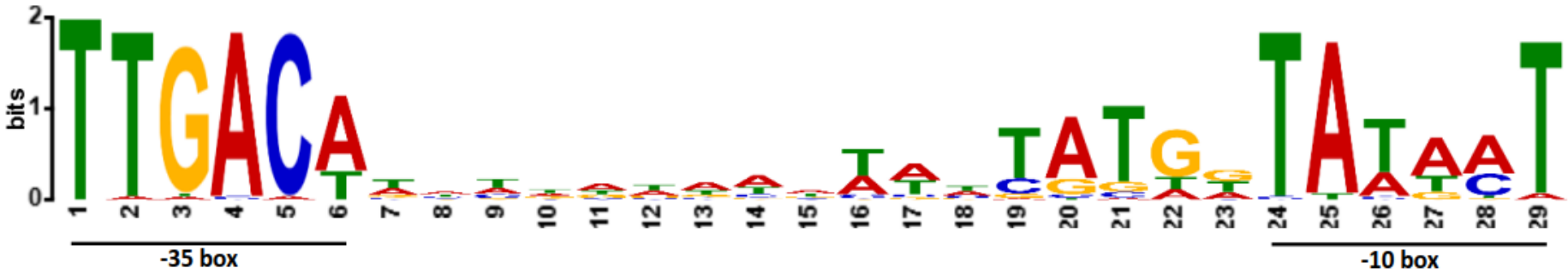
	Upstream of gene	Strand	*p*-Value	Promoter sequences			
					-35 box	Spacer*	-10 box
1.	*g001*	+	7.11×10^{-10}		TTGACA	GCTATGAAGCGGTATGG	TAAGAT
2.	*g002*	+	1.52×10^{-10}		TTGACA	TTAAGTAAGTAGTATGG	TATGAT
3.	*g003*	+	1.87×10^{-10}		TTGACA	AATAGTAAGTAGTATGT	TATACT
4.	*g004*	+	2.93×10^{-11}		TTGACA	AGTAGTAAGTAGTGTGG	TATGAT
6.	*g006*	+	4.55×10^{-5}	T	TTAATA	TTTACTTTACAGGAAGT	TATAAT
7.	*g016*	+	2.19×10^{-7}		TTGACT	TCTTATATGAGACTTGG	CATAAT

9.	*g018*	+	4.33×10^{-6}		TTGACT	TATTAGTCATTATCCTT	TAATAT
10.	*g021*	+	1.92×10^{-5}		TTGACT	TATCTCTTATTATGGTT	TAATAT
11.	*g022*	+	5.51×10^{-8}		TTGACA	GTCACTTGAAACCATGA	TATAAT
12.	*g023*	+	2.30×10^{-10}		TTGACT	TCCAAGCCCTACCATGA	TATACT
13.	*g024*	+	8.35×10^{-8}		TTGACA	CACTAACCGCTTCATGA	TATTAT
14.	*g025*	+	6.59×10^{-7}		TTGACT	TTCAAGCCCTAAACCTT	TATAAT
15.	*g026*	+	2.86×10^{-7}		TTGACT	TCCAAGCCTTAAACCTT	TATAAT
16.	*g029*	+	6.83×10^{-5}	T	TTACAA	CTATTTAATTTGTATGC	TATAAT
17.	*g036c*	-	7.16×10^{-9}		TTGACA	TTTATAAATAAGTATGG	TAAGAT
18.	*g043c*	-	2.53×10^{-8}		TTGACT	TTTTCACTAACTTATGT	TATAAT
19.	*g047c*	-	2.00×10^{-7}		TTGACA	AATGCAAATACTTGTAG	TATACT
20.	*g053c*	-	3.56×10^{-8}		TTGACA	AATATTATTACCTGTGA	TATGAT
21.	*g060c*	-	5.43×10^{-9}		TTGACA	AGCCTCCTTAGTTATGG	TATACT
22.	*g067c*	-	7.11×10^{-10}		TTGACT	TCCTAAGTTAACTATGG	TATAAT
23.	*g075c*	-	3.37×10^{-6}		TTGACT	TATTTATCAATATAGTA	TATAGT
24.	*g104*	+	1.92×10^{-9}		TTGACA	AGTATAATTAGATACGG	TATACT
25.	*g106*	+	1.13×10^{-6}		TTGACA	AATTAAAAATAATAAAT	TATAAT

26.	*g110*	+	1.13×10^{-7}		TTGACA	CAAGAGTAGTATCATAG	TATACT
27.	*g118*	+	1.40×10^{-9}		TTGACA	GGAAGTTAATAATATGG	TATACT
28.	*g124*	+	1.07×10^{-8}		TTGACT	TAATAAGTATTCTGTGG	TATACT

***** Spacer region has 17 nucleotides.

Consensus motif of predicted Stab23 promoters



A table of Stab23 putative promoter sequences.

No.	Upstream of gene	Strand	*p*-Value	Promoter sequences				
					-35 box	Spacer*	-10 box	
1.	*g001*	+	3.61×10^{-10}		TTGACA	TTTAGTAAGTAGTATGG	TAAGAT	
2.	*g002*	+	7.38×10^{-10}		TTGACA	AGTAGTAAGTAGTGTGG	TAAGAT	
3.	*g010c*	-	1.56×10^{-6}		TTGACT	TCTGAATAACTATACTG	TAATAT	
4.	*g011*	+	2.47×10^{-6}		TTGACT	TATTAATCATATGGTAG	TAATAT	
5.	*g012*	+	2.54×10^{-8}		TTGACA	CATTACAAGATACATGT	TATTAT	

6.	*g014*	+	4.29×10^{-8}		TTGACA	GTACATAAACAACATGG	TAATAT	
7.	*g015*	+	5.49×10^{-8}		TTGACA	ACTTAGAAACAACGTGT	TAATAT	
8.	*g016*	+	1.69×10^{-7}		TTGACA	GTCACTTGAAACCATGA	TATTAT	
9.	*g017*	+	8.99×10^{-9}		TTGACT	TCCAAGCCCTACCATGT	TATTAT	
10.	*g018*	+	2.14×10^{-9}		TTGACT	TCCAAGCCCTAGCATGA	TATACT	
11.	*g019*	+	3.32×10^{-8}		TTGACA	ACCTTCCAAATACATGT	TATTAT	
12.	*g020*	+	1.92×10^{-8}		TTGACA	TCCAACCCCTATCATGT	TAATAT	
13.	*g021*	+	1.44×10^{-6}		TTGACT	TCCAAGCCCTATAATGA	TAATAT	
14.	*g026*	+	3.40×10^{-4}	T	TTACAA	CTATTTAATTTGTATGT	TACAAT	
15.	*g027c*	-	5.06×10^{-6}		TTGACA	TTCTAATTACCATCCTT	TATACT	
16.	*g036c*	-	1.43×10^{-8}		TTGACA	TTTATAAATAAGTATGG	TAAGAT	
17.	*g039c*	-	5.55×10^{-7}		TAGACA	AGACGATATTGATATGG	TATAAT	
18.	*g045c*	-	4.18×10^{-7}		TTGACT	TTTCCAATAGTATGTGT	TATACT	
19.	*g048c*	-	3.80×10^{-7}		TTGACA	AATGCAAATACTTGTAT	TATAAT	
20.	*g054c*	-	1.66×10^{-8}		TTGACA	AGTATTAATTACTATGA	TATGAT	
21.	*g060c*	-	1.05×10^{-8}		TTGACA	AGCCTCCTTAGTTATGG	TATACT	
22.	*g067c*	-	6.96×10^{-8}		TTGACT	TCCTGAGTTAATTATGC	TATAAT	

23.	*g075c*	-	1.20×10^{-5}		TTGACT	TATTTATCAATATAGTA	TATAGT	
24.	*g101*	+	7.97×10^{-7}		TTGACA	AGGAATATTAAAGCTGA	TATACT	
25.	*g105*	+	2.29×10^{-6}		TTGACA	GATTAAAAATAATAAAT	TATAAT	
26.	*g109*	+	1.23×10^{-8}		TTGACA	CAAGAGTAGTATCATAG	TATACT	
27.	*g117*	+	2.16×10^{-10}		TTGACA	GAAAGTTAATAATATGG	TATACT	
28.	*g123*	+	3.16×10^{-9}		TTGACT	TAATAAGTATTCTATGG	TATACT	
29.	*g125*	+	1.62×10^{-4}		TTGACA	AAAGAGGGTATGTTGGA	TTATAA	T
30.	*g126*	+	7.63×10^{-9}		TTGACA	TTTTATATGTTAGGTGG	TATAAT	
31.	*g143*	+	2.91×10^{-8}		TTGACA	AAATGTTTAACTTATGT	TATACT	
32.	*g145*	+	1.05×10^{-8}		TTGACA	AATACAAAAAACTATGT	TATAAT	
33.	*g152*	+	2.54×10^{-8}		TTGACA	ATTTATAATAACTATGT	TACACT	
34.	*g157*	+	3.44×10^{-7}		TTGACT	CTTTTTACTATATATGG	TATATT	
35.	*g159*	+	8.21×10^{-5}		TTTACA	AGAGGTGTTATTTATGG	TTATAA	T
37.	*g166*	+	2.82×10^{-7}		TTGACT	CTCTTTTTGTTTTATGG	TATATT	
38.	*g169*	+	2.50×10^{-5}		TTGACT	ACATTCAAGAGTTAGAA	CAAAAT	
39.	*g175*	+	1.36×10^{-7}		TTGACA	GATGGAATATTTTAGTA	TATACT	
40.	*g179*	+	1.52×10^{-7}		TTGACA	TTTCTAAACTTTTGTAT	TATACT	

41.	*g183*	+	4.29×10^{-8}		TTGACA	AATGAGTGTACATAGGT	TATACT	
42.	*g187*	+	1.92×10^{-8}		TTGACA	TTAGGTTTCTTTTATTG	TATACT	
43.	*g190*	+	3.80×10^{-9}		TTGACA	GCAGGTATTTATTATAG	TATACT	
44.	*g194*	+	1.69×10^{-7}		TTGACA	AATAGGGGTTTCTATTA	TATAAT	
45.	*g196*	+	1.10×10^{-7}		TTGACT	TAGGTAGAGTTTTATTG	TATAAT	
46.	*g201*	+	5.49×10^{-8}		TTGACA	TTAAATAAATAACGTGT	TAAGAT	
47.	*g202*	+	4.29×10^{-8}		TTGACA	TTAAATAAATAATGTGT	TAAGAT	
48.	*g206*	+	5.05×10^{-7}		TTGACA	TAGGTAGAGTTTTACTA	TATACT	

***** Spacer region has 17 nucleotides.

Appendix V: Predicted rho-independent terminator sequences of the Stab phages.

Table 1: Putative Rho–independent transcription terminators of phage Stab20.

No.	Downstream of gene	Position	Strand	Regulatory element sequence **	ΔG (kcal/mol)#
1	*g011c*	3352:3400c	-	AATTATACAATACACTAGGAATAATATCCTAGTGTaTTTATTTTTGCGG	-11.60
2	*g011c*	145876:145924c	-	AATTATACAATACACTAGGAATAATATCCTAGTGTaTTTATTTTTGCGG	-11.60
3	*g014*	5128:5174	+	AATTATACGATTCCCTGGGATTAAATTCCTAGGGATTTTTATTTGTT	-13.80
4	*g014*	147652:147698	+	AATTATACGATTCCCTGGGATTAAATTCCTAGGGATTTTTATTTGTT	-13.80
5	*g018*	7361:7411	+	ATTTATATAAACCGCTTCGGATTAAATTCTTGAAGCGGTTATTTTCTTTTA -	10.90
6	*g018*	149885:149935	+	ATTTATATAAACCGCTTCGGATTAAATTCTTGAAGCGGTTATTTTCTTTTA -	10.90
7	*g021*	8476:8525	+	AAAAATTAAAATAAGGGGTTGACACTTAGCCCCTTAgaTGTTATTATTAA	-10.80
8	*g021*	151000:151049	+	AAAAATTAAAATAAGGGGTTGACACTTAGCCCCTTAgaTGTTATTATTAA	-10.80
9	*g029*	10689:10727	+	TAGATTAAGAGGAGGGCAAACGCCCTCTTTTATTTTTAT	-11.40
10	*g029*	153213:153251	+	TAGATTAAGAGGAGGGCAAACGCCCTCTTTTATTTTTAT	-11.40
11	*g030c*	10937:10982c	-	GTATAGATAAGAGAGGGGGCATATACCTCCTCTTTTTATTTTAGA	-12.70
12	*g031c*	11335:11380c	-	ATAATACCTTAGAGGAAGAATAATATTCTTTCTCTTTTTTTTATAT	-8.20
13	*g048c*	19214:19256c	-	ATTAATTCTTAGGCTACTTTAATTAGTAGCCTTTTTTTGTTGA	-10.90
14	*g050c*	19980:20024c	-	TAGGTACAGAAGCAGACTTTTAATAAGTCTGCTTTTCTCTTATAT	-11.40

15	*g062c*	27331:27376c	-	AAACTCATTTAGAAGGACTTTAAAAAAGTTCTTCTTTTTTTGTTGA	-7.70
16	*g067c*	30119:30186c	-	ATGTTGACAAACCTCTTTAGTTATGGTATACTTATCTTATAATAACTAAGGAGGaT TTTTTATGAATT	-6.80
17	*g074c*	33401:33446c	-	TAATATATTAAGACTAAGATTAATTTCTTAGTCTTTTTTGTATATT	-10.20
18	*g075c*	34295:34338c	-	AATAATAAATTAGAGAGGTTAATACCTCTCTTTTTTTGTCTTTA	-11.90
19	*g077c*	35504:35549c	-	AATAGAAATTTAGACGGATTTTAAATCCGTCTaTTTTTTTTTGCAA	-10.70
20	*g091*	46986:47026	+	ATAAAACTGAAGAGGAGTAATTACTCCTCTTTTTTGTTTGC	-10.20
21	*g094*	49512:49556	+	ATTAATTAATAAGCCTAGAATAAATCTAGGCTTTGTTTATTTTTT	-11.50
22	*g097*	52989:53035	+	ACAAGAGAATAGGGATAAACTTAGGGTTTATCCCTTTTTTATTAAAA	-8.30
23	*g105*	59149:59191	+	TTAATATACTAGACCAACTAAAAAGTTGGTCTTTTTTTTATTG	-11.10
24	*g113*	61946:61992	+	GTATATGTAAAGGGTGGTAGGTGATACTACCATCCTTATTTTTTTAA	-11.10
25	*g117*	72014:72057	+	TTTAATATTAAAGACCTATTAATTTAGGTCTTTTTTTAGTTGTA	NA
26	*g124*	82396:82438	+	TGAATAAACTAGAGGGGTTGATTGACCCCTCTTTATTTAATAA	-13.60
27	*g128*	86292:86336	+	AATATGCCATAGACTAGGAAACTTATCCTAGTCTTTTTTTTCTTG	-11.70
28	*g148*	104079:104123	+	GACTTAATGAAGAAGAGAAATAATTCTCTTCtTTTTTTATTGACA	-8.90
29	*g152*	109596:109636	+	TATAAGATATAGAGTGCCTTAGAGCACTCTTTTATTTGAGA	-8.80
30	*g158*	113456:113498	+	ATAATAATTAAGACCAACTAAAAAGTTGGTCTTTTTTTATTGA	-11.10
31	*g163*	116485:116529	+	GATTTCTTATAGAGTCAAGTCTTTACTTGACTCTTTTTACTATAT	-10.90

32	*g203*	133765:133806	+	AAATTTGTAAATACCTGTTGACAGCAGGTATTTTTTATAGTA	-8.30
33	*g210*	138443:138441	+	AAATATTTAAACTCCCTATTGACAAAGGGAGTTTTTTATTGTA	-9.30

+ Positive/forward strand

- Negative/reverse strand

******The predicted secondary structures are indicated by colours, the stems in blue and the loops in red.

[#]**ΔG:** Free energy of stem-loop region.

Table 2: Putative Rho–independent transcription terminators of phage Stab21.

No.	Downstream of gene	Position	Strand	Regulatory element sequence **	ΔG (kcal/mol)#
1	*g001*	642:684	+	AATAGGAATATGAAGCGGTTAATTCCGCTTCTCTTACTTAGAG	-11.90
2	*g001*	642:684	+	AATAGGAATATGAAGCGGTTAATTCCGCTTCTCTTACTTAGAG	-11.90
3	*g010c*	3424:3472c	-	AATTATATAATACACTGGGAATAATATCCTAGTGTaTTTATTTTTGCGG	-11.30
4	*g010c*	146072:146120c	-	AATTATATAATACACTGGGAATAATATCCTAGTGTaTTTATTTTTGCGG	-11.30
5	*g013*	5262:5308	+	AATTATACAATTCCCTAGGATTAGATTTCTAGGGATTTTTATTTATT	-11.30
6	*g013*	147910:147956	+	AATTATACAATTCCCTAGGATTAGATTTCTAGGGATTTTTATTTATT	-11.30
7	*g017*	7487:7539	+	AATTTATATAAACCGCTTCGGATTAAATTCTTGAAGCGGTTTTTTTATGTAAA	-11.60
8	*g017*	150135:150187	+	AATTTATATAAACCGCTTCGGATTAAATTCTTGAAGCGGTTTTTTTATGTAAA	-11.60
9	*g026*	11022:11062	+	AATAACAAATAGAGGGAATAAAATCCCTCTTTTATTTTTAT	-9.40
10	*g026*	153670:153710	+	AATAACAAATAGAGGGAATAAAATCCCTCTTTTATTTTTAT	-9.40
11	*g027c*	11323:11365c	-	ACCTAAGAGGAGAGGGATTTAATTTCCCCTCTTTTTTTTATTT	-7.10
12	*g038c*	16541:18391c	-	AATTTTAATTACCTACCTACTAAGGTAGGTTTTTTATTGAC	-10.30
13	*g045c*	21857:21902c	-	AATTAATATTTAGGCTACTTTAATTAGTAGCCTTTTTTTGTTGACA	-12.00
14	*g047c*	22626:22669c	-	TAGGTAAAGAAGCAGACTTTTAATAAGTCTGCTTTTCTCTTATA	-11.40
15	*g055c*	27052:27100c	-	CTTTCCTTTTTCACCTTGCTTGTAGCCAAGCAGGGTGTTTTTTTTATAT	-11.00

16	*g059c*	29965:30009c	-	AAACTCATTTAGAAGGACTTTAAAAAGTTCTTCTTTTTTTGTTGA	-8.30
17	*g073c*	36638:36683c	-	TAATATATTAAGACTAAGATTAATTTCTTAGTCTTTTTTGTATATT	-10.20
18	*g074c*	37531:37575c	-	AATAATAAATTAGAGAGGTTAATACCTCTCTtTTTTTTGTATTTA	-11.90
19	*g076c*	38740:38785c	-	AATAGAAATTTAGACGGATTTTAAATCCGTCTaTTTTTTTTGCAAA	-10.70
20	*g091*	50170:50210	+	ATAAAACTGAAGAGGAGTAATTACTCCTCTTTTTTTGTTTG	-10.20
21	*g094*	52696:52740	+	ATTAATTAATAAGCCTAGAATAAATCTAGGCTTTGTTTATTTTTT	-11.50
22	*g097*	56170:56216	+	ACAAGAGAATAGGGATAAACTTAGGGTTTATCCCTTTTTTATTAAAA	-8.30
23	*g105*	62330:62372	+	TTAATAGATTAGACCAACTAAAAAGTTGGTCTTTTTTTATTGA	-11.10
24	*g111*	64832:64878	+	GTATATGTAAAGGGTGGTAGGTGATACTACCATCCTTATTTTTTTAA	-11.10
25	*g115*	74900:74943	+	TTTAATATTAAAGACCTATTAATTTAGGTCTTTTTTTAGTTGTA	N/A
26	*g122*	85276:85318	+	TGAATAAACTAGAGGGGTTGATTGACCCCTCTTTATTTAATAA	-13.60
27	*g126*	89178:89222	+	AATATGCCATAGACTAGGAGAAATTTCCTAGTCTTTTTTTTCTTG	-11.90
28	*g144*	105965:106009	+	GGCTTAATGAAGAAGAGAAATAATTCTCTTCtTTTTTTATTGACA	-8.90
29	*g148*	111446:111486	+	TATAAGATATAGAGTGCCTTAGAGCACTCTTTTATTTGAGA	-8.80
30	*g154*	115305:115347	+	ATAGTAATTAAGACCAACTAAAAAGTTGGTCTTTTTTTATTGA	-11.10
31	*g159*	118334:118378	+	GATTTCTTATAGAGTCAAGTCTTTACTTGACTCTTTTTACTATAT	-10.90
32	*g168*	124327:124371	+	GAACAGTGATTGAGTCAAGTTAATTCTTGACTCTCTTTTTGTTTT	-11.60

33	*g199*	135352:135393	+	AAATTTATAAATGCCTGTTGACAGCAGGTATTTTTTATAGTA	-8.30
34	*g207*	139519:139567	+	ATAAATATTTAAACTCCCTATTGACAAAGGGAGTTtTTTATTATATAGT	-11.50

+ Positive/forward strand

- Negative/reverse strand

****** The predicted secondary structures are indicated by colours, the stems in blue and the loops in red.

[#]**ΔG:** Free energy of stem-loop region

Table 3: Putative Rho–independent transcription terminators of phage Stab22.

No.	Downstream gene	Position	Strand	Regulatory element sequence**	ΔG (kcal/mol)#
1	*g003*	1760:1807	+	ATTCTATACAAACCCTCTATCGGTCAATAGAGGGTTTTTTTATTTATC	-11.50
2	*g003*	145418:145465	+	ATTCTATACAAACCCTCTATCGGTCAATAGAGGGTTTTTTTATTTATC	-11.50
3	*g016*	5594:5638	+	AATAGTAAGTAGCTAGGTATTAATTTACCTAGCTTTTCTAATTTC	-12.50
4	*g016*	149252:149296	+	AATAGTAAGTAGCTAGGTATTAATTTACCTAGCTTTTCTAATTTC	-12.50
5	*g017c*	5777:5822c	-	ATTATAGAATTCACTGGGAATAATATTCCTGGTGTATTTTTTGCGG	-9.10
6	*g017c*	149435:149480c	-	ATTATAGAATTCACTGGGAATAATATTCCTGGTGTATTTTTTGCGG	-9.10
7	*g018*	6818:6864	+	GAAATTCAAGATTAGGGGTTGCAATTCCCCCCTAATCTGTTATAATA	-8.80
8	*g018*	150476:150522	+	GAAATTCAAGATTAGGGGTTGCAATTCCCCCCTAATCTGTTATAATA	-8.80
9	*g020*	7741:7787	+	AATTATACAATTCCCTAGGATTAAATTCCTAGGGATTTTTATTTGTT	-14.10
10	*g020*	151399:151445	+	AATTATACAATTCCCTAGGATTAAATTCCTAGGGATTTTTATTTGTT	-14.10
11	*g023*	9628:9681	+	AAATTATATAAACCGCTTCGGATTAAATTCTTGAAGCGGTcTTATTTTTATTTT	-11.60
12	*g023*	153286:153339	+	AAATTATATAAACCGCTTCGGATTAAATTCTTGAAGCGGTcTTATTTTTATTTT	-11.60
13	*g026*	11341:11377	+	GACACACAGAAGCGGTTTAAACCGCTTCTATATATAA	-6.90
14	*g026*	154999:155035	+	GACACACAGAAGCGGTTTAAACCGCTTCTATATATAA	-6.90
15	*g029*	12179:12217	+	TAGATTAAGAGGAGGGCAAACGCCCTCTTTTATTTTTAT	-11.40
16	*g029*	155837:155875	+	TAGATTAAGAGGAGGGCAAACGCCCTCTTTTATTTTTAT	-11.40
17	*g030c*	12425:12470c	-	ATAATACCTCAGAGGAAGAATAATATTCTTTCTCTTTTTTATTTTA	-8.20
18	*g040c*	18273:18318c	-	ATTAATTTTTAAGGCTACTTTAAATAGTAGCCTTTTTTTGTTGACA	-12.20
19	*g042c*	19041:19087c	-	TTACATGAAAAAGCAGACTCTTAATAGGTCTGCTTTTCTCTTATATT	-10.80
20	*g050c*	23484:23532c	-	CTTTCCTTTTTCACCTTGCTTGTAACCAAGCAGGGTGTTTTTTTATATA	-11.00
21	*g068c*	32069:32115c	-	TAAAATATTAAGACTAAGATTAATTTCTTAGTCtTTTTTTGTATATT	-10.20
22	*g069c*	33001:33795c	-	GTAAATAATAGAGAGAGGTTAATACCTCTCTtTTTTTTGTTTTTA	-12.10
23	*g071c*	34172:34217c	-	AGTATAAATTTAGACGGATTTAAAATCCGTCTaTTTTTTTTTGCAA	-10.70

24	*g075c*	37403:37438c	-	TAATCAGGTTCCCCGTGAGACGGGTTATGCTTGGAT	-5.40
25	*g086*	45804:45844	+	ATACAAATGAAGAGGAGTAACTACTCCTCTTTTTTTGCTAT	-10.20
26	*g089*	48320:48364	+	ATTAATTAATAAGCCTAGAATAAATCTAGGCTTTATTTATTTTTT	-11.50
27	*g092*	51788:51834	+	ACAAGAGAATAGGGATAAACTTAGGGTTTATCCCTTTTTTATTAAAA	-8.30
28	*g100*	58328:58373	+	TATAGAATATAGACCTAACAATAAAAGTTAGGTCTTTTCTATTGAC	-10.90
29	*g108*	62311:62357	+	GTATATGTAAAGGGTGGTAGGTGATACTACCATCCTTATTTTTTTAA	-11.10
30	*g112*	72368:72411	+	TTTAATATTAAAGACCTATTAATTTAGGTCTTTTTTTAGTTGTA	NA
31	*g119*	81940:81982	+	TGAATAAACTAGAGGGGTTGATTGACCCCTCTTTATTTAATAA	-13.60
32	*g123*	85821:85864	+	AATATGCCATAGACTAGGATAAACTCCTAGTCTTTTTTTCTTGA	-11.40
33	*g141*	102511:102555	+	GACTTAACGAAGAAGAGAAATAATTCTCTTCtTTTTTTATTGACA	-8.90
34	*g151*	111757:111800	+	TAAATAATTAAGACCAACTAAAAAAGTTGGTCTTTTTTTATTGA	-11.00
35	*g169*	121794:121837	+	CAATCAATCAAGCTAACATTAATTTGTTAGCTTTTTTATTGACA	NA
36	*g198*	136677:136719	+	AATAGTTAAACTCCCTATTGACAAATAGGGGTTTCTATTATAT	-9.70
37	*g207*	139831:139877	+	AAAAGATTTAACTCTATCTATTGACATAGGTAGAGTTTTAGTGTATA	-8.80

+ Positive/forward strand

- Negative/reverse strand

****** The predicted secondary structures are indicated by colours, the stems in blue and the loops in red.

#**ΔG:** Free energy of stem-loop region

Table 4: Putative Rho–independent transcription terminators of phage Stab23.

No.	Downstream gene	Position	Strand	Regulatory element sequence**	ΔG (kcal/mol)#
1	*g001*	1658:1704	+	TTCTATACAAAACCCTCTACTGGGAATAGAGGGTTTTTTTATTTATC	-11.00
2	*g001*	143932:143978	+	TTCTATACAAAACCCTCTACTGGGAATAGAGGGTTTTTTTATTTATC	-11.00
3	*g009*	3983:4029	+	GACAAATCGTAGAGAGGGCTTAAGTAGTCCTCTCTTATTTAGGTTAG	-12.30
4	*g009*	146257:146303	+	GACAAATCGTAGAGAGGGCTTAAGTAGTCCTCTCTTATTTAGGTTAG	-12.30
5	*g010c*	4169:4214c	-	ATTATACAATACACTGGGAATAATATTCCTAGTGTATTTTTTCGGT	-10.80
6	*g010c*	146443:146488c	-	ATTATACAATACACTGGGAATAATATTCCTAGTGTATTTTTTCGGT	-10.80
7	*g013*	6152:6196	+	TTATACAATATCCCTGGGATTAAATTCCTAGGGTTTTTTATTTGT	-14.00
8	*g013*	148426:148470	+	TTATACAATATCCCTGGGATTAAATTCCTAGGGTTTTTTATTTGT	-14.00
9	*g018*	8929:8979	+	AATTATATAAACCGCTTCGGATTAAATTCTTGAAGCGGTTATTTTCTTTTA	-10.90
10	*g018*	151203:151253	+	AATTATATAAACCGCTTCGGATTAAATTCTTGAAGCGGTTATTTTCTTTTA	-10.90
11	*g026*	12098:12141	+	ATTAGATTAAGAGGAGGGCAAACGCCCTTCTTTATTTTTATTCT	-13.10
12	*g026*	154372:154415	+	ATTAGATTAAGAGGAGGGCAAACGCCCTTCTTTATTTTTATTCT	-13.10
13	*g027c*	12348:12393c	-	GTATAGATAAGAGAGGGGGCATATACCTCCTCTTTTTTATTTTAGA	-12.70
14	*g028c*	12742:12784c	-	TAAATTATAAATCACTCTTAATAGAGTGAtTTTTTTATATAAA	NA
15	*g042c*	21163:21208c	-	ATTAATTTTTAAGGCTACTTTAATTAGTAGCCTTTTTTTGTTGACA	-12.20
16	*g044c*	21932:21976c	-	TAGATACAGAAGCAGACTTTTAATAAGTCTGCTTTTCTCTTATAT	-11.40
17	*g051c*	26368:26416c	-	CTTTCCTTTTTCACCTTGCTTGTAACCAAGCAGGGTGTTTTTTTTATAT	-11.00
18	*g068c*	34698:34743c	-	TAATATATTAAGACTAAGATTAATTTCTTAGTCTTTTTTGTATATT	-10.20
19	*g069c*	35587:35630c	-	AAATAATAGAGAGAGAGGTTAATACCTCTCTTTTTTTTGTTTC	-12.10
20	*g071c*	36796:36841c	-	AATAGTAATTTAGACGGATTTTATATCCGTCTaTTTTTTTTTGCAA	-11.40
21	*g076c*	41504:41553c	-	TACGTACTTTTTCTTCTGTAAGTACTGATATAGAGGGaTTTTACTTTAGA	-5.40
22	*g085*	48688:48728	+	ATAAAATTGAAGAGGAGTAAATACTCCTCTTTTTTTGCTAT	-10.20

23	*g088*	51204:51248	+	ATTAATTAATAAGCCTAGAATAAATCTAGGCTTTATTTATTTTTT	-11.50
24	*g091*	54663:54709	+	ACAAGAGAATAGGGATAAACTTAGGGTTTATCCCTTTTTTATTAAAA	-8.30
25	*g099*	60800:60843	+	TTAATATATTAGACCAACTAAAAAAGTTGGTCTTTTTTTATTGA	-11.00
26	*g107*	63688:63734	+	GTATATGTAAAGGGTGGTAGGTGATACTACCATCCTTATTTTTTTAA	-11.10
27	*g111*	73755:73798	+	TTTAATATTAAAGACCTATTAATTTAGGTCTTTTTTTAGTTGTA	NA
28	*g118*	84635:84677	+	TGAATAAACTAGAGGGGTTGATTGACCCCTCTTTATTTAATAA	-13.60
29	*g122*	88516:88559	+	AATATGCCATAGACTAGGATAAACTCCTAGTCTTTTTTTCTTGA	-11.40
30	*g141*	105583:105627	+	GACTCAATGAAGAAGAGAAATAATTCTCTTCtTTTTTTATTGACA	-8.90
31	*g145*	110929:110969	+	TATAAGATATAGAGTGCCTTAGAGCACTCTTTTATTTAAGA	-8.80
32	*g151*	114778:114821	+	ATAATAATTAAGACCAACTAAAAAAGTTGGTCTTTTTTTATTGA	-11.00
33	*g156*	117808:11752	+	GATTTCTTATAGAGTCAAGTCTTTACTTGACTCTTTTTACTATAT	-10.90
34	*g165*	123806:123850	+	GAACAGTGATTGAGTCAAGTTAATTCTTGACTCTCTTTTTGTTTT	-11.60
35	*g170*	125354:125398	+	AAACCAATCAAGCTAACATTAATTTGTTAGCtTTTTTTATTGACA	NA

+ Positive/forward strand

- Negative/reverse strand

****** The predicted secondary structures are indicated by colours, the stems in blue and the loops in red.

[#]**ΔG:** Free energy of stem-loop region

Appendix VI: Stab phages' proteomic (LC-MS/MS) results.

Table 1: The Stab20 phage particle associated proteins identified using LC-MS/MS of tryptic peptides. The selection criteria for the proteins were a minimum of 5% sequence coverage and $\geq$ 2 unique peptides.

Gene	Predicted function of gene product	Coverage (%)	# Unique Peptides	MW [kDa]
g114	Tail tape-measure protein	46.97	47	143.7
g158	Ig-like domain containing protein	78.67	4	7.8
g124	Adsorption-associated tail protein	41.23	29	129.2
g143	Ribonucleotide reductase large subunit	49.72	28	80.2
g097	Major capsid protein	74.08	22	51.2
g149	DNA polymerase A	32.37	25	124.5
g126	Carbohydrate binding domain-containing protein	55.94	26	72.5
g153	DNA repair protein	46.65	20	46.7
g104	Major tail sheath protein	50.94	19	64.4
g157	Tail protein	33.53	8	18.2
g115	N-acetylmuramoyl-L-alanine amidase	23.51	14	91.2
g117	Glycerophosphoryl diester phosphodiesterase	35.73	14	95.9
g210	Nicotinamide phosphosribosyltransferase	23.87	13	92.2
g129	DNA helicase A	35.22	13	67.2
g094	Portal protein	41.40	13	56.8
g077c	Endolysin	34.14	11	54.7
g075c	Putative membrane protein	42.05	12	29.3
g087	Terminase large subunit	21.16	9	70.2
g123	Hypothetical protein	59.54	8	19.2
g095	Prohead protease	60.16	12	27.3
g119	Hypothetical protein	68.97	8	19.9
g118	Hypothetical protein	16.73	3	29.3
g152	Hypothetical protein	28.05	7	48.3

g195	Hypothetical protein	41.18	5	24.9
g055c	AAA family ATPase	26.13	7	42.5
g156	Hypothetical protein	62.38	9	23.2
g161	Metallophosphoesterase	21.39	7	47.6
g102	Hypothetical protein	50.72	8	31.7
g127	Hypothetical protein	50.00	6	14.6
g194	Hypothetical protein	48.89	4	10.4
g169	Hypothetical protein	26.32	3	17.8
g133	Exonuclease	23.75	6	39.3
g122	Hypothetical protein	11.87	6	112.1
g105	Tail tube protein	44.37	5	15.9
g128	Putative capsid & scaffold protein	20.96	7	50.4
g137	Hypothetical protein	45.07	6	24.9
g135	Putative recombinase exonuclease B	15.81	6	73.2
g209	Ribose-phosphate pyrophosphokinase	29.80	7	34.9
g099	Hypothetical protein	25.17	4	34.1
g168	Hypothetical protein	19.12	3	29.3
g116	Protease	10.17	3	34.5
g131	DNA helicase B	14.17	6	54.6
g167	Hypothetical protein	19.51	4	32.3
g141	Resolvase	30.20	4	23.6
g101	Hypothetical protein	15.53	4	23.8
g164	Hypothetical protein	20.22	3	20.7
g090	Hypothetical protein	17.61	3	18.5
g121	putative tail protein	15.52	4	39.2
g140	Hypothetical protein	12.00	2	17.0
g189	HNH endonuclease	18.09	4	21.5
g078c	Holin	20.36	4	18.1
g054c	Hypothetical protein	10.68	3	31.8

g112	Hypothetical protein	34.87	4	18.1
g061c	Hypothetical protein	10.83	4	79.7
g096	Hypothetical protein	12.54	3	36.1
g144	Ribonucleotide reductase small subunit	12.61	3	40.4
g111	Hypothetical protein	13.59	2	12.2
g100	Hypothetical protein	17.81	3	33.7
g067c	PhoH-related protein	15.04	3	28.5
g076c	Putative membrane protein	17.65	2	12.2
g187	Hypothetical protein	14.50	2	23.4
g038c	Hypothetical protein	25.93	3	19.5
g091	Hypothetical protein	4.10	2	43.0
g130	Putative replication protein	9.12	3	63.1
g146	Thioredoxin	15.45	2	12.5
g120	Baseplate wedge subunit	9.40	1	26.6
g098	Hypothetical protein	15.31	2	11.2
g147	Hypothetical protein	13.64	2	23.6
g041c	Serine/threonine protein phosphatase	9.87	2	27.2
g172	Putative membrane protein	18.94	2	15.4
g216	Hypothetical protein	20.12	2	20.3
g051c	Hypothetical protein	17.61	2	20.5
g069c	Ribonuclease H	13.48	2	15.8
g066c	RNA ligase	6.71	2	34.7
g062c	Hypothetical protein	28.74	2	10.1

Table 2: The Stab21 phage particle associated proteins identified using LC-MS/MS of tryptic peptides. The selection criteria for the proteins were a minimum of 5% sequence coverage and $\geq$ 2 unique peptides.

Gene	Predicted function	Coverage (%)	# Unique Peptides	MW [kDa]
g154	Major tail protein	81.58	6	7.9
g112	Putative tail lysin	47.15	52	143.9
g122	Adsorption-associated tail protein	56.25	48	129.1
g104	Major tail sheath protein	61.93	27	64.8
g139	Ribonucleotide reductase of class 1b (aerobic), alpha subunit	62.22	35	81.2
g124	Hypothetical protein	67.50	34	72.6
g094	Portal protein	55.77	23	64.0
g149	Recombinase protein	63.40	27	46.8
g153	Ig-like domain	92.57	12	18.4
g145	DNA polymerase I	34.70	30	124.5
g076c	N-acetylmuramoyl-L-alanine amidase	44.15	18	54.8
g097	Major capsid protein	61.29	18	51.5
g113	Tail lysin	31.96	18	93.0
g115	Glycerolphosphoryl diester phosphodiesterase	39.98	21	96.6
g207	Nicotinamide phosphoribosyltransferase	45.10	14	56.2
g127	DNA helicase A	37.93	18	67.9
g095	Prohead protease	61.39	16	28.8
g120	Hypothetical protein	26.27	18	116.4
g087	Terminase large subunit	36.36	13	70.2
g038c	Lipase acylhyrolase domain protein	45.08	16	70.5
g121	Hypothetical protein	60.67	9	19.8
g164	Hypothetical protein	56.57	11	29.3
g116	Hypothetical protein	27.61	4	29.9
g152	Hypothetical protein	69.91	11	23.9

g074c	Membrane protein	36.00	10	30.7
g066c	PhoH predicted ATPase	54.66	12	28.7
g052c	AAA family ATPase	31.69	10	43.8
g117	Hypothetical protein	70.86	8	20.1
g126	Tail fibre protein	33.62	10	50.4
g197	Hypothetical protein	62.07	6	10.2
g129	DNA helicase B	38.68	12	55.3
g157	DNA polymerase	28.20	8	48.2
g064c	RNA ligase	34.45	7	35.2
g048c	Hypothetical protein	39.66	5	20.9
g100	Capsid	43.00	8	33.9
g101	Hypothetical protein	28.99	8	23.8
g102	Hypothetical protein	41.73	6	31.8
g114	Protease	13.85	4	34.7
g133	Hypothetical protein	42.92	8	24.7
g137	Resolvase	52.45	8	23.9
g058c	Hypothetical protein	21.26	10	79.7
g105	Tail tube protein	43.75	5	16.2
g091	Hypothetical protein	22.98	5	43.6
g143	Hypothetical protein	28.10	5	28.8
g165	Hypothetical protein	24.39	3	19.3
g088	Hypothetical protein	32.89	8	34.2
g148	Hypothetical protein	25.12	5	47.7
g163	Hypothetical protein	25.69	4	32.4
g168	Putative membrane protein	20.13	4	18.1
g189	Hypothetical protein	20.41	2	11.2
g099	Hypothetical protein	19.21	4	34.1
g142	Thioredoxin-like protein	43.75	5	12.8
g199	Hypothetical protein	22.12	4	25.7

g205	Ribose-phosphate pyrophosphokinase	24.68	6	35.9
g077c	Holin	18.28	4	20.4
g119	Baseplate protein	15.47	4	39.3
g110	Hypothetical protein	33.33	4	19.0
g111	Tail morphogenetic protein	49.49	5	23.1
g125	Hypothetical protein	40.85	4	17.0
g130	Putative exonuclease	23.28	4	40.2
g170	Hypothetical protein	14.14	2	22.3
g174	Hypothetical protein	11.50	3	26.5
g184	Hypothetical protein	24.52	4	24.2
g147	Hypothetical protein	23.75	4	18.9
g096	Hypothetical protein	17.28	4	36.7
g128	Putative replication protein	11.71	4	63.2
g132	Exonuclease	9.53	3	73.5
g160	Hypothetical protein	21.20	3	21.5
g167	Hypothetical protein	27.31	4	27.9
g059c	Hypothetical protein	43.68	4	10.1
g047c	Hypothetical protein	23.20	3	20.5
g035c	Hypothetical protein	30.12	3	20.0
g055c	Nucleoside triphosphate pyrophosphohydrolase	20.95	2	12.0
g118	Putative baseplate protein	20.34	2	26.8
g180	Hypothetical protein	30.25	2	13.4
g070c	Hypothetical protein	11.01	3	26.3
g051c	Hypothetical protein	8.62	2	32.7
g036c	Hypothetical protein	21.05	3	16.3
g161	Hypothetical protein	13.73	2	30.0
g090	Hypothetical protein	14.47	2	18.5
g151	RNA polymerase sigma factor	15.42	2	27.3

g098	Hypothetical protein	14.56	2	11.9
g162	Hypothetical protein	25.50	2	17.4
g073c	Transglycosylase	13.22	2	26.3
g067c	Hypothetical protein	27.75	2	23.5
g054c	Hypothetical protein	16.45	2	17.6
g041c	Hypothetical protein	32.39	2	8.5

Table 3: The Stab22 phage particle associated proteins identified using LC-MS/MS of tryptic peptides. The selection criteria for the proteins were a minimum of 5% sequence coverage and $\geq$ 2 unique peptides.

Gene	Predicted functions	Coverage (%)	# Unique Peptides	MW [kDa]
g123	Tail fiber protein	70.74	25	51.0
g136	Ribonucleotide reductase, large subunit	70.22	40	80.9
g109	Tail length tape-measure protein	51.70	51	143.7
g119	Adsorption-associated tail protein	55.35	46	129.8
g142	DNA polymerase A	45.71	35	124.4
g099	Major tail sheath	52.96	24	64.6
g072c	N-acetylmuramoyl-L-alanine amidase	48.09	21	55.0
g089	Portal protein	49.02	21	63.9
g186	Hypothetical protein	33.00	2	11.5
g112	Glycerolphosphoryl diester phosphodiesterase	55.92	33	96.6
g092	Major capsid protein	61.51	19	51.5
g146	DNA repair recombinase protein	59.28	23	46.7
g082	Terminase, large subunit	43.14	18	70.4
g110	Tail lysin	33.37	17	92.5
g047c	AAA family ATPase	51.46	17	42.7
g198	Nicotinamide phosphoribosyl transferase	29.03	18	93.5
g069c	Putative membrane protein	50.76	14	29.3
g126	DNA helicase B	54.32	19	55.3
g161	Hypothetical protein	57.37	11	29.3
g124	DNA helicase A	36.05	14	67.9
g061c	PhoH-related protein	50.20	13	28.7
g149	Hypothetical protein	70.37	12	24.0
g095	Capsid protein	50.51	10	33.8
g090	Prohead protease	62.84	12	28.8
g121	Hypothetical protein	34.27	12	73.2

g150	Ig-like domain protein	44.25	6	18.4
g097	Hypothetical protein	50.72	9	31.8
g114	Hypothetical protein	65.14	7	20.0
g002	Hypothetical protein	61.24	8	20.0
g113	Hypothetical protein	14.18	3	29.8
g122	Hypothetical protein	69.42	5	14.4
g129	Recombination exonuclease B	20.74	8	73.7
g134	Resolvase	47.55	7	23.9
g145	Hypothetical protein	35.09	6	44.4
g154	Metallophosphoesterase protein	36.26	9	48.3
g118	Structural protein	52.25	8	19.9
g162	Hypothetical protein	35.98	5	19.2
g207	Hypothetical protein	58.18	8	19.5
g054	Hypothetical protein	65.52	8	10.2
g130	Anti-sigma factor	37.26	5	25.0
g117	Hypothetical protein	16.73	6	85.3
g083	Hypothetical protein	26.38	5	34.6
g100	Tail tube protein	45.65	5	15.4
g165	Hypothetical protein	33.10	7	16.9
g206	Hypothetical protein	38.79	5	13.6
g116	Baseplate morphogenetic protein	19.48	5	39.2
g197	Ribose-phosphate pyrophosphokinase	40.07	8	35.2
g053c	Hypothetical protein	16.84	6	79.7
g035c	GTP cyclohydrolase II	38.89	6	19.5
g096	Hypothetical protein	20.29	6	23.8
g133	Hypothetical protein	22.78	4	18.3
g139	Thioredoxin	58.04	5	12.8
g159	Hypothetical protein	31.54	5	17.5
g160	Hypothetical protein	24.31	5	32.4

g187	Hypothetical protein	20.52	4	25.9
g073c	Holin	17.62	4	21.1
g046c	Hypothetical protein	29.47	6	32.1
g043c	Hypothetical protein	31.84	4	20.9
g094	Hypothetical protein	25.17	4	34.1
g107	Hypothetical protein	34.81	4	18.9
g111	Protease	11.15	4	34.8
g125	Rep protein	16.36	6	63.3
g140	Hypothetical protein	28.10	5	28.8
g157	Hypothetical protein	12.50	2	21.5
g019	Hypothetical protein	30.38	5	19.0
g085	Hypothetical protein	23.27	4	18.5
g086	Hypothetical protein	12.41	3	44.4
g101	Hypothetical protein	16.48	5	50.9
g115	Baseplate wedge subunit	11.86	2	26.8
g128	Recombination exonuclease A	17.25	4	39.6
g148	RNA polymerase sigma factor	20.70	3	27.3
g180	Hypothetical protein	25.00	4	24.2
g057c	Nucleoside 2-deoxyribosyltransferase	34.83	5	22.5
g144	Hypothetical protein	13.75	2	19.0
g151	Major tail protein	86.08	3	8.3
g194	Hypothetical protein	22.57	3	25.8
g210	Hypothetical protein	25.33	2	8.6
g070c	Putative membrane protein	13.21	2	12.8
g062c	Hypothetical protein	36.41	4	23.3
g049c	Hypothetical protein	21.01	2	15.9
g079	Membrane protein	17.61	3	16.6
g093	Hypothetical protein	15.79	2	10.9
g158	Hypothetical protein	13.73	2	30.0

g163	Hypothetical protein	19.02	2	19.3
g164	Hypothetical protein	12.61	2	28.0
g189	Hypothetical protein	42.07	3	16.2
g065c	Hypothetical protein	14.54	3	26.3
g059c	Hypothetical protein	38.46	2	16.3
g032c	Hypothetical protein	13.82	2	18.5
g029	Terminal repeat encoded protein U (TreU)	26.67	2	10.9
g106	Hypothetical protein	12.96	2	12.9
g131	DNA primase	7.76	2	41.7
g147	Hypothetical protein	21.37	2	13.4
g153	Hypothetical protein	19.05	2	29.2
g182	Hypothetical protein	19.35	2	7.6
g188	Hypothetical protein	25.49	2	11.8
g190	Metallophosphatase	15.17	2	20.8
g067c	Hypothetical protein	16.00	2	9.2
g042c	Hypothetical protein	16.20	2	20.8
g036c	Serine/threonine protein phosphatase	5.08	2	27.9

Table 4: The Stab23 phage particle associated proteins identified using LC-MS/MS of tryptic peptides. The selection criteria for the proteins were a minimum of 5% sequence coverage and $\geq$ 2 unique peptides.

Gene	Predicted protein	Coverage (%)	# Unique Peptides	MW [kDa]
g136	Ribonucleoitide reductase, largel subunit	38.20	18	80.9
g072c	N-acetylmuramoyl-L-alanine amidase	40.64	15	55.0
g108	Tail length tape-measure protein	21.58	18	143.7
g109	Tail lysin	25.77	14	92.4
g088	Portal protein	22.74	9	64.0
g091	Major capsid protein	38.28	11	51.5
g111	Glycerophosphoryl diester phosphodiesterase	31.18	14	96.5
g117	Baseplate morphogenetic protein	60.67	9	19.9
g142	DNA polymerase A	20.85	15	122.7
g146	Repair recombinase	38.76	12	46.7
g096	Hypothetical protein	48.20	9	31.8
g118	Adsorption-associated tail protein	11.49	9	129.7
g098	Major tail sheath	26.90	9	64.9
g122	Tail fiber protein	29.10	11	50.7
g123	DNA helicase A	20.41	6	67.9
g054c	Hypothetical protein	10.54	7	80.1
g099	Tail tube protein	52.78	6	16.1
g193	Nicotinamide phosphoribosyltransferase	15.71	5	56.2
g028c	Putative tail fiber protein	18.60	6	64.8
g106	Hypothetical protein	27.22	3	18.9
g120	Hypothetical protein	14.13	4	72.5
g093	Hypothetical protein	18.21	3	34.1
g113	Hypothetical protein	32.57	3	20.0
g115	Baseplate morphogenetic protein	15.47	3	39.2

g149	Hypothetical protein	21.76	3	23.9
g160	Hypothetical protein	13.19	3	32.4
g048c	AAA family ATPase	13.39	5	43.2
g082	Terminase, large subunit	6.78	3	70.4
g084	Hypothetical protein	17.61	2	18.5
g154	Metallophosphoesterase	9.95	3	48.3
g162	Hypothetical protein	24.39	3	19.3
g165	Putative membrane protein	27.81	4	17.5
g069c	Putative membrane protein	9.85	3	29.3
g050c	Hypothetical protein	18.12	2	16.0
g089	Prohead protease	21.46	3	28.9
g116	Tail morphogenetic protein	3.30	3	134.9
g129	Recombinase exonuclease B	4.38	2	73.3
g130	Hypothetical protein	26.42	3	24.8
g158	Hypothetical protein	16.86	3	30.0
g061c	PhoH-related protein	19.43	3	28.9
g055c	Hypothetical protein	13.83	2	10.9
g110	Peptidoglycan hydrolase	10.47	2	34.7
g125	DNA helicase B	6.38	2	55.3
g134	Resolvase	13.73	2	24.0
g144	Hypothetical protein	16.25	2	18.9
g035c	GTP cyclohydrolase II	13.94	2	19.9

Appendix VII: Publications.

Research articles published:

- ✓ **Oduor J.M.O,** Kadija E, Mureithi.W.M, Nyachieo. A, Skurnik M (2020). **Bioprospecting Staphylococcus phages with therapeutic and bio-control potential.** *MDPI Journal of Viruses.*

Abstract

Emergence of antibiotic-resistant bacteria is a serious threat to the public health. This is also true for Staphylococcus aureus and other staphylococci. Staphylococcus phages Stab20, Stab21, Stab22, and Stab23, were isolated in Albania. Based on genomic and phylogenetic analysis, they were classified to genus Kayvirus of the subfamily Twortvirinae. In this work, we describe the in-depth characterization of the phages that electron microscopy confirmed to be myoviruses. These phages showed tolerance to pH range of 5.4 to 9.4, to maximum UV radiation energy of 25 µJ/cm^2, to temperatures up to 45 °C, and to ethanol concentrations up to 25%, and complete resistance to chloroform. The adsorption rate constants of the phages ranged between 1.0 × 10^{-9} mL/min and 4.7 × 10^{-9} mL/min, and the burst size was from 42 to 130 plaque-forming units. The phages Stab20, 21, 22, and 23, originally isolated using Staphylococcus xylosus as a host, demonstrated varied host ranges among different Staphylococcus strains suggesting that they could be included in cocktail formulations for therapeutic or bio-control purpose. Phage particle proteomes, consisting on average of ca 60–70 gene products, revealed, in addition to straight-forward structural proteins, also the presence of enzymes such DNA polymerase, helicases, recombinases, exonucleases, and RNA ligase polymer. They are likely to be injected into the bacteria along with the genomic DNA to take over the host metabolism as soon as possible after infection.

Keywords: MRSA; Kayvirus; bacteriophage; genome; proteome; stability
https://doi.org/10.3390/v12020133

- ✓ **Oduor J.M.O,** Kadija E, Kiljunen S, Mureithi.W.M, Nyachieo. A, Skurnik M (2019). Genomic characterization of four novel *Staphylococcus* myoviruses. *Archives of Virology.*

Abstract
We report here the annotation of the complete genomes of four novel lytic Staphylococcus phages; Stab20, Stab21, Stab22 and Stab23. These phages have double-stranded DNA genomes ranging between 153,338 and 155,962 bp in size with terminal repeats of 10,814-12,304 bp. The genome analysis suggests that they represent new phage species within the genus *Kayvirus* in the subfamily *Twortvirinae* of the family *Herelleviridae*.
Key words: *Kayviruses; Staphylococcus aureus; Twortvirinae;*
https://doi.org/10.1007/s00705-019-04267-0

Appendix VIII: Conference presentations certificates.

i). AIBBC conference:

Joseph M. Ochieng Oduor, Ermir Kadija, Saija Kiljunen, Marianne W. Mureithi, Atunga Nyachieo, Mikael Skurnik. **Bioprospecting for novel phages with therapeutic significance against pathogenic *Staphylococcus* spp**. *4th African International Biomedical and Biotechnology Conference* (August 28 – 30, 2019) Nairobi - Mombasa, Kenya.

ii). BSI conference:

Joseph M. Ochieng Oduor', Ermir Kadija, Saija Kiljunen, Marianne W. Mureithi, Atunga Nyachieo, Mikael Skurnik. **Isolation and characterization of a novel *Staphylococcus aureus* bacteriophages**. (February 9 -10, 2019) *B*eyond *Sciences Conference- 4th International Remote Conference*.

CERTIFICATE OF EXCELLENCE

issued to

Joseph Michael Ochieng Odour

for selection as a top presenter at the
Beyond Sciences Initiative 4th International Remote Conference:
Science & Society

February 9-10th, 2019

Prof. Eleanor Fish

Beyond Sciences Initiative · TRINITY COLLEGE IN THE UNIVERSITY OF TORONTO · Immunology UNIVERSITY OF TORONTO · Be Mo Academic Consulting · BioLegend® · HARVARD INFECTIOUS DISEASES CONSORTIUM · Janice Wright LL.B.

iii). EMBO workshop\conference:

Joseph M. Ochieng Oduor, Ermir Kadija, Saija Kiljunen, Marianne W. Mureithi, Atunga Nyachieo, Mikael Skurnik. **Isolation and characterization of a novel *Staphylococcus aureus* bacteriophages.** EMBO workshop Virus of Microbes (July 9-13, 2018) Wroclaw, Poland.

EMBO Courses & Workshops

CERTIFICATE OF PARTICIPATION

This is to confirm that

Joseph Michael Ochieng' Oduor

attended the EMBO Workshop

Viruses of microbes 2018

from 09 – 13 July 2018 in Wrocław, Poland

Gerlind Wallon, PhD
EMBO Deputy Director

Date and location: 14 July 2018, Heidelberg

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EMBO excellence in life sciences

Appendix IX: Ethical approval letter.

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Ref: KNH-ERC/A/230

27th July, 2017

Joseph Michael Ochieng' Oduor
Principal Investigator (PhD candidate)
KAVI- Institute of Clinical Research
College of Health Sciences
University of Nairobi

Dear Joseph

Revised Research Proposal - Complete Genome Analysis of Lytic Phages and Identification of Hypothetical Phage Proteins Targeting Major Protein Complexes of *Staphylococcus Aureus* (P262/05/2017)

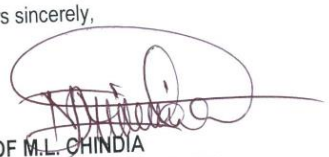
This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above proposal. The approval period is from 27th July, 2017 – 26th July 2018.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,



PROF M.L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Chair, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Director, KAVI-Institute of Clinical Research, UoN
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Dr.Atunga Nyachieo, Institute of Primate Research, UoN
Dr. Marianne W.Mureithi, KAVI-ICR, UoN