Journal of Advances in Microbiology

Volume 23, Issue 10, Page 128-138, 2023; Article no.JAMB.107093
ISSN: 2456-7116

Isolation and Characterization of Bacteriophage against Drug-resistant *Staphylococcus aureus*

Sajib Kumar Saha [a], Md. Ashiqur Rahman [b*], Md. Sultan Mahmud [a], Md. Taijul Islam [a], Md. Nazmul Islam [a], Sadia Islam [c], Sadia Nabilah [d], Shohanur Rahaman [a], Anika Zafreen [b], Md. Raihanul Islam [b] and Md. Sujon Ali [c]

[a] Jashore University of Science and Technology, Bangladesh.
[b] Novus Clinical Research Services Limited (NCRSL), Bangladesh.
[c] Bangladesh University of Health Sciences (BUHS), Bangladesh
[d] University of Dhaka, Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2023/v23i10763

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/107093

Original Research Article

Received: 20/07/2023
Accepted: 26/09/2023
Published: 12/10/2023

ABSTRACT

Staphylococcus aureus is one of the most successful pathogens due to its vast arsenal of virulence factors, including anchor proteins, secreted toxins and enzymes, polysaccharides, and immune system modulators. Bacteriophages are viruses that specifically target and infect bacterial cells. Bacteriophage is studied widely to improve the safety of foods and prevent food borne diseases of bacterial etiology, as well as to reduce the use of antibiotics in livestock. Besides, therapeutic applications of bacteriophage in human and the usage of phages in veterinary medicine and

**Corresponding author: E-mail: ararashiqur@gmail.com;*

agriculture are also highly assessed. Hence, the present study aimed to isolate and to characterize *Staphylococcus aureus* specific bacteriophage. Bacteriophages were isolated from various environmental water samples. After incubation growth occurred in MSA and NA plate. On *Staphylococcus aureus*, antibiotic discs showed some useful identifying zones. It was performed with the use of five antibiotics: Penicillin, Oxacillin, Tetracyclin, Gentamycin and Cefoxitin. Penicillin, Oxacillin are showing no zones, for that reason showed resistant pattern. Environmental phages were then selected based on the size and clarity of their plaques. Bacteriophage produce some clear zone against bacteria. The isolated bacteriophages were found to be able to lyse the *Staphylococcus aureus*. *S. aureus* was isolated on nutrient agar media and mannitol salt agar media and confirmed by biochemical test like catalase test, coagulase test and oxidase test. Drug-resistant *S. aureus* also form some specific zones in the nutrient agar plates. Then allowed to compare this two specific zones stability.

Keywords: Bacteriophages; drug-resistance; Staphylococcus aureus; penicillin; oxacillin.

1. INTRODUCTION

Bacteriophages are viruses that specifically target and infect bacterial cells. They are the most abundant living entities on earth. They are found predominantly in area where their hosts live, for example, sewage, soil, deep thermal vents and natural bodies of water. They play an important role in regulating the microbial balance in every ecosystem due to their high level of specificity, long-term survivability and ability to reproduce rapidly in suitable host [1]. It is generally believed that most bacteriophages are only capable of infecting a narrow range of bacteria that are closely related [2]. This is due to a combination of factors including specificity of phages host binding proteins, biochemical interactions during infection, presence of related prophages or particular plasmids (especially for phages adsorbing to pili) and bacterial phage-resistance mechanisms [3]. Although they carry all the genetic information for their replication in a susceptible host, they have no machinery to generate energy and no ribosomes to make proteins. Therefore, they replicate inside a host cell with the aid of host biosynthetic machinery [4]. Because many bacteriophages kill the bacterial cells they infect, phages provide a possible alternative to antibiotics. This use of bacteriophages, called phage therapy, was the first use proposed for phage soon after their discovery [5]. While displaced by antibiotics in much of the world, the increasing frequency of antibiotic- resistant bacteria has led to renewed interest in phage therapy [6,7]. Host range is used to describe a bacteriophage that can infect multiple species of bacteria [8,9,10]. But it is also used to describe a bacteriophage that can infect multiple strains of the same species of bacteria [11]. For example, bacteriophage is able to infect species of Escherichiacoli, Citrobacter freundii, Shigella sonnei, Enterobacter, and Erwinia [12]; staphylococcal phage infects 95% of 782 strains of *Staphylococcus aureus* and 43% of other Staphylococcus species tested; and bacteriophage infects 60% of 28 *S. aureus* isolates [11].

Staphylococcus aureus is a Gram-positive coccal bacterium that is a member of the Firmicutes and is frequently found in the human respiratory tract on the skin. It is a common cause of skin infections (e.g. boils), respiratory disease such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins and the expression of cell surface protein that bind and inactivate antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. Staphylococcus was first identified in 1880 in Aberdeen, United Kingdom, by the surgeon Sir Alexander Ogston in pus form a surgical abscess in a knee joint. This name was later appended to *Staphylococcus aureus* by Rosenbach who was credited by the official system of nomenclature at the time. In 1884, Rosenbach described the two pigmented colony types of Staphylococci and proposed the appropriate nomenclature: *Staphylococcus aureus* (yellow) and Staphylococcus albus (white). The latter species is now named Staphylococcus epidermidis. Taxonomically, the genus Staphylococcus is in the Bacterial family Staphylococcaceae, which includes three lesser known genera, Gamella. Macrococcus and Salinicoccus. The best known of its nearby phylogenetic relatives are the members of the genus Bacillus in the family Bacillaceae, which is on the same level as the family Staphylococcaceae. The Listeriaceae are also a nearby family. *S. aureus* is a spherical

microbe and a member of the bacteria domain. The bacterium can be found naturally on the skin and in the mucus membranes of humans most importantly. The bacteria are spread most commonly through human contact be it hand- to-hand, from a wound secretion or mucus. *S. aureus* is a Gram-positive coccus that occurs in grape-like clusters. It is a eubacterium that is found on the surface of the human skin and mucus membranes. It is also found in other areas of human contact such as air, dust and food products [13,14].

S. aureus is a member of the group of Gram-positive bacteria called pyogenic (pus-producing) cocci (Sinsbury and Singleton, 2001). These cause various surpurative scalded skin syndrome [14]. As earlier said the organism is an opportunistic pathogen. Most strains become infectious usually when the skin or mucus membranes is punctured by variety of objects such as needles, blades, surgical devices etc [14,15]. The emergence of antibiotic-resistant bacterial pathogens is an increasing health hazard [16]. Production of extended-spectrum of β-lactamase (ESBL) and other resistance mechanism lead to the development of multi-drug resistance (MDR) by bacterial pathogens, due to the extensive usage of broad-spectrum antibiotics in hospitalized patients [17]. Phage therapy could serve as a prophylactic and therapeutic alternative treatment against strains that cause pathogenic infections. Phage therapy is already evolved and developed concept, but there is a need to regenerate this concept to fight against the antibiotic resistant bacterial infections [18].

Hence, the objectives of this study mainly tried to focused on isolation and characterization of *Staphylococcus aureus* specific bacteriophages that have potential to be used for therapeutic application and drug-resistance of *S. aureus.*

2. MATERIALS AND METHODS

2.1 Sampling Sites

Water samples were collected from Sadar Hospital, Jessore and Jessore University of Science and Technology area including drains, ponds.

2.2 Collection of Samples

Samples were collected, a minimum of 500 ml, aseptically in a sterile glass container (sampling bottle). The water at collection site was mixed thoroughly and the sediments were collected together with the overlying water.

2.3 Cultivation and Isolation of Organisms

Petri plates were used for cultivation of microorganisms. Each plate contained 20 ml of microbiological media. Nutrient agar and Mannitol Salt agar were used and each was poured in 6 plates. Each plate was marked with their identification number. Using a saline soaked sterile cotton bud, each sample collection from sites was rubbed and then streaked in the designated area of the petri plates. The plates were then brought back to the laboratory and incubated in 37°C for 24 hours and observed for growth. Distinct colonies with individual colony characteristics were isolated.

2.4 Bacteriophage Isolation

Spot Test: Prepare Nutrient Agar and Mannitol Salt Agar plate, which are poured with 20ml media. Plate are divided into few parts with the help of identifying marker. Take enrich culture to create a matt by the help cotton bud. When matt is solidifying, then add 10 µl filtrate sample in every part of the NA plate (Drop by drop). Incubate overnight at 37°C for 24h or until lysis zones appeared.

Plaque Assay: 100 µl of bacteria was transferred into a sterile 15 ml conical tube. The tubes were pre-incubated at 37°C for 20 min to allow phage adsorption onto bacterium. After pre-incubation, the tubes were added with 100 µl of filtrate sample and 2.5 ml of nutrient (45°C) soft agar (0.6%). The tubes were gently mixed by inversion for a few times and the contents were poured on Petri dishes containing NA. The plates were left at room temperature for 10-15 min until the soft agar has solidified and the Petri dishes were incubated at 37°C for 24 h. The following day, the plates were checked and observed for the plaque formation. The plaques formed in each plate were enumerated.

Phage Stock Preparation: All isolated phage plaque is inoculating in nutrient broth with the help of inoculating loop. The test tube shaking for 2-4 h at 37°C. After the end of shaking, some solutions are take in an Eppendorf tube and allow to centrifuge for discarding supernatant. Take the pellet. Prepare 200 µl glycerol broth and 800 µl nutrient broth and vortex it with mixing pellet and freezing.

2.5 General Enrichment

Bacteria enrichment method was performed on the collected samples. By preparing NB solution allowed to enrichment process of *staphylococcus aureus*. Then shaken into the shaker machine for 2-4 h at 37°C in static condition to allow specific bacterial enrichment. After the end of shaking process, enriched bacteria were centrifuged at 10,000 rpm for 10 min to remove large particle or cell debris. Supernatant are collected. The supernatant of the water sample was filtered slowly through the 0.22 µM syringe filter to a 1.5 mL micro centrifuge tube. Filtrate are stored at 4°C.

2.6 Biochemical Test

Catalase Test: A clear slide is taken and 1 drop of hydrogen peroxide is added. 2. A single colony from NA plate is taken and added on slide. 3. Then wait for 1-2 minutes. 4. Observe bubble formation on the slide.

Coagulase Test: Isolates stocked in glycerol broth were revived in NA plates and used for catalase test. 2. A single colony from the revival culture was taken with a sterile loop and an emulsion was made on the surface of a sterile glass slide. 3. A drop of sterile saline was used to make the emulsion. 4. After the preparation of the emulsion, a single drop of Human Blood Serum was placed on it. 5. The total preparation was incubated at 37°C for 1 h.

Oxidase Test: Take a Whitman filter paper soaked with the reagent. 2. Pick the colony to be tested with the inoculating loop and smear in the filter paper. 3. Same steps above were performed for another selected colony. 4. Observe inoculated area of paper for a color change to deep blue or purple within 10-30 seconds.

2.7 Drug-resistance of *S. aureus*

Nutrient broth (8 ml) was prepared and poured in a test tube for each of the isolates and autoclaved. A single colony from the pure culture of the isolate was inoculated in the broth and incubated for 3 h at 37°C. After incubation, 1 ml culture of the inoculated nutrient broth was transferred to 9 ml saline solution. A sterile cotton bud was soaked in the solution and spread on a nutrient agar plate by rubbing the cotton bud thoroughly in a horizontal and vertical way throughout the surface of the plate. Different types (5) of antibiotic disks were placed on the surface of the inoculated Mueller-Hinton agar plates and incubated for 24 h at 37°C.

3. RESULTS

3.1 Sample Collection Sites

Sample A1 (drain water) was collected from the Sadar Hospital, Jessore that was polluted by solid waste disposal which was flowing. The water sample collected was clear, with little sediment present. The sample B1 (ponds water) was collected from the Jessore University of Science and Technology. The water appeared to be clean and clear from sediments.

3.2 Morphological Characteristics

After incubation growth occurred in MSA and NA plate. Colonies were of different types. Colonies from the NA plates and MSA plates were selected for further identification and isolated with the loop.

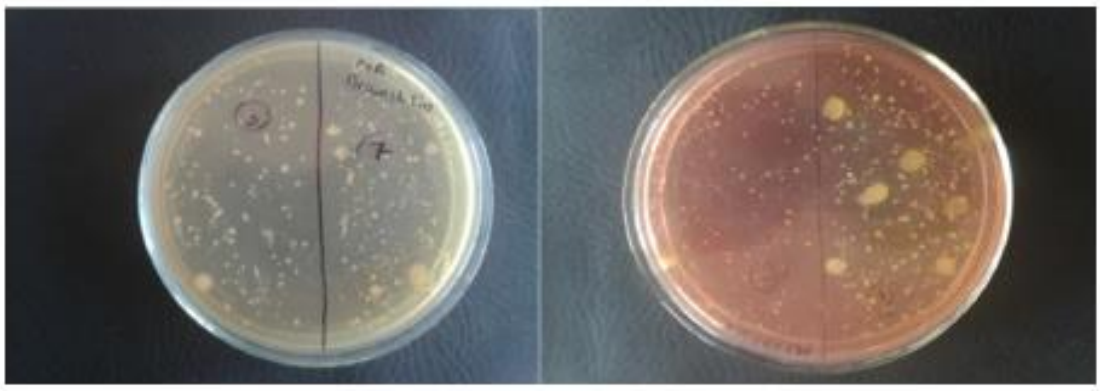

Fig. 1. MSA and NA plates showing growth after incubation

3.3 Phage Isolation

Plates showed positive for the isolation of bacteriophages. Round, clear and transparent plaques were observed in plates, indicating that the samples collected from A1, B1 sites contained bacteriophages that were infectious against *Staphylococcus aureus*. The phages produced clear zones with well-defined edges in bacterial lawn, showing that the isolated phages have lytic effect against *Staphylococcus aureus*. These two phages were selected for further characterization on the basis of differences in plaque size and morphology. Fig. 2. shows the plaques formation for water samples A1, B1. Table 2. shows the size and morphology of the isolated plaques.

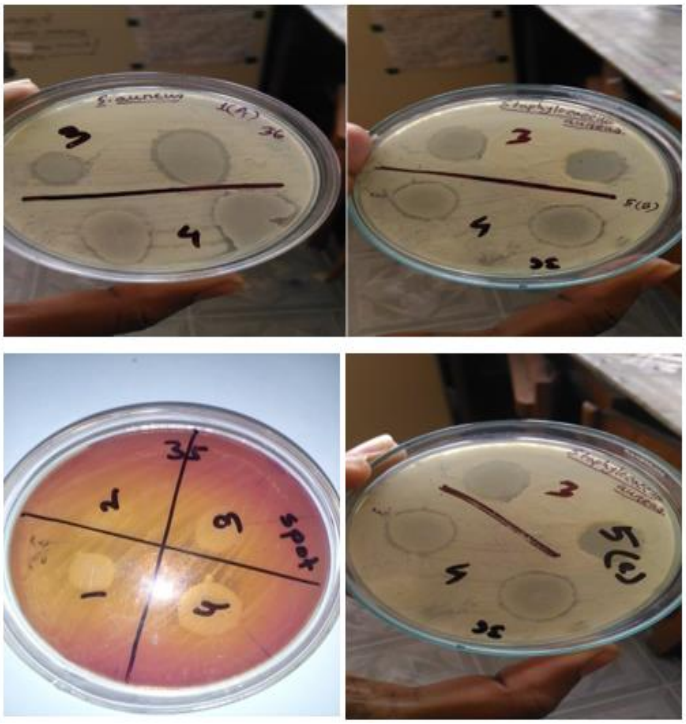

Fig. 2. Plaques formation for water samples

Table 1. Size of the isolated plaques from samples

Sampling Site	Plaque Size	Plaque Morphology
Phage -A1	20 mm	Clear zone
Phage -B1	15 mm	Clear zone

Table 2. Bacterial different isolates shows different sizes of phage

Staphylococcus aureus isolates	Phage-A1, Plaque Size (mm)	Phage-B1, Plaque Size (mm)
1 (a)	12	13
1 (b)	20	No zone
2 (a)	17	13
2 (b)	15	20
3 (a)	12	18
3 (b)	17	No zone
4 (a)	16	17
4 (b)	14	20
5 (a)	18	14
5 (b)	14	12

3.4 Observation and Result of Biochemical Tests

a. Catalase Test: A single colony from NA agar medium is taken and added into a slide containing hydrogen peroxide, then waited 1-2 minutes. Bubbles were formed which indicated presence of *Staphylococcus aureus*.

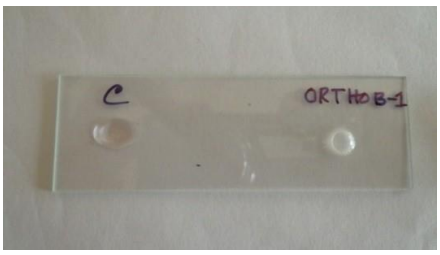

Fig. 3. Catalase test

b. Coagulase Test: Coagulase test was performed on the isolates to determine the presence of *S. aureus* which indicating that it is the prevalent microorganism

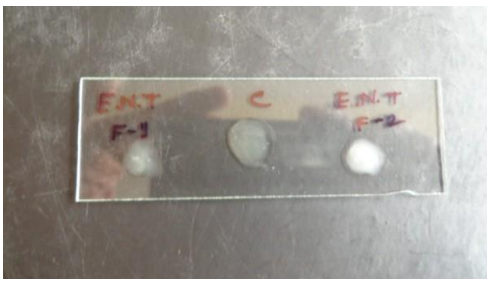

Fig. 4. Coagulase test

c. **Oxidase Test:** A piece of heavy metal free-clean filter paper was placed on a clean Petri dish; 3 drops of freshly prepared Oxidase reagent were added on the filter paper. With a burn loop, a colony of the test organism was pick and smeared on the filter paper. No color change is observed which is a negative result and indicative of the presence of *Staphylococcus aureus.*

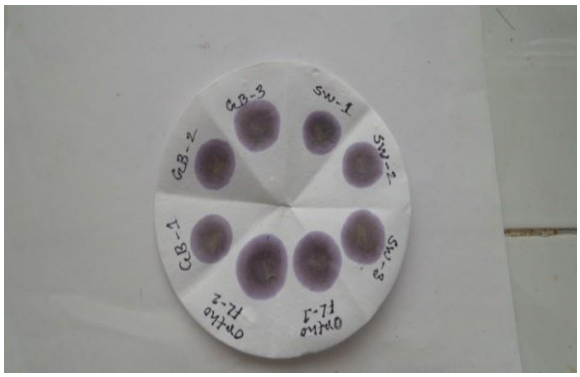

Fig. 5. Oxidase Test

3.5 Drug-resistance of *S. aureus*

On *Staphylococcus aureus*, antibiotic discs showed some useful identifying zones. It was performed with the use of five antibiotics: Penicillin, Oxacillin, Tetracyclin, Gentamycin and Cefoxitin. Penicillin, Oxacillin are showing no zones, for that reason showed resistant pattern. 4 isolates were resistant to Gentamycin, 6 being sensitive. 10 isolates were sensitive to Tetracyclin and Cefoxitin because they showed clear zone.

Table 3. Result of confirmatory biochemical tests of *S. aureus*

Catalase test	Coagulase test	Oxidase test	Organism
Positive	Positive	Negative	*Staphylococcus aureus*

Table 4. Antimicrobial agents with their Disc concentration

Antimicrobial Agents	Symbol	Disc Concentration
Penicillin	P	10 µg
Oxacillin	OX	1 µg
Gentamycin	CN	10 µg
Tetracyclin	TE	30 µg
Cefoxitin	FOX	30 µg

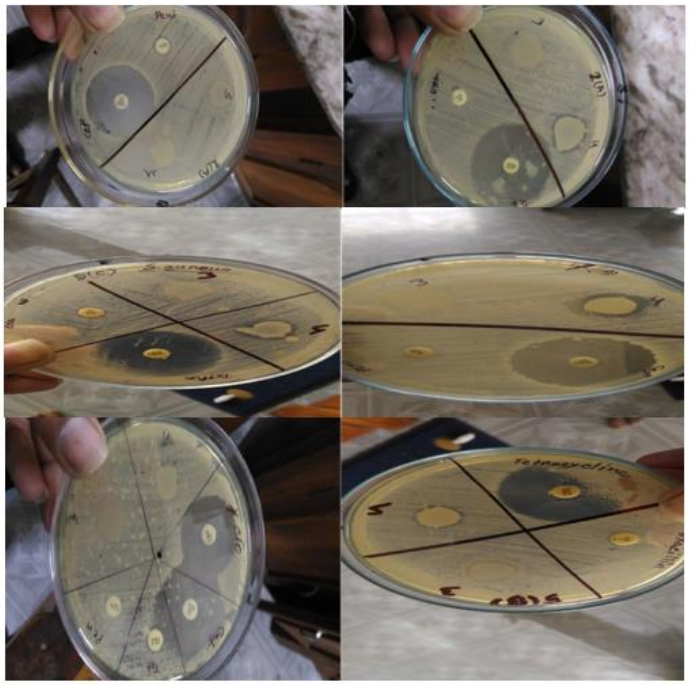

Fig. 6. Zones analysis of different antibiotic

Table 5. Results of Drug-resistance *S. aureus*

***Staphylococcus aureus* isolates**	**Penicillin (mm)**	**Oxacilln(mm)**	**Gentamycin (mm)**	**Tetracyclin (mm)**	**Cefoxitn (mm)**
1 (a)	No zone	No zone	24 (S)	20 (S)	22 (S)
1 (b)	No zone	No zone	6 (R)	18 (S)	26 (S)
2 (a)	No zone	No zone	23 (S)	20 (S)	24 (S)
2 (b)	No zone	No zone	6 (R)	26 (S)	24 (S)
3 (a)	No zone	No zone	20 (S)	16 (S)	20 (S)
3 (b)	No zone	No zone	6 (R)	27 (S)	21 (S)
4 (a)	No zone	No zone	21 (S)	17 (S)	25 (S)
4 (b)	No zone	No zone	25 (S)	20 (S)	24 (S)
5 (a)	No zone	No zone	26 (S)	24 (S)	28 (S)
5 (b)	No zone	No zone	6 (R)	24 (S)	25 (S)

Table 6. Comparisons between bacteriophage zones and drug-resistant zones of different strain

Isolates	**Phage-A1 (mm)**	**Phage-B1 (mm)**	**Penicillin (mm)**	**Oxacillin (mm)**	**Gentamycin (mm)**	**Tetracyclin (mm)**	**Cefoxitin (mm)**
1 (A)	12	13	No zone	No zone	24 (S)	20 (S)	22 (S)
1 (B)	20	No zone	No zone	No zone	6 (R)	18 (S)	26 (S)
2 (A)	17	13	No zone	No zone	23 (S)	20 (S)	24 (S)
2 (B)	15	20	No zone	No zone	6 (R)	26 (S)	24 (S)
3 (A)	12	18	No zone	No zone	20 (S)	16 (S)	20 (S)
3 (B)	18	No zone	No zone	No zone	6 (R)	27 (S)	21 (S)
4 (A)	16	17	No zone	No zone	21 (S)	17 (S)	25 (S)
4 (B)	14	20	No zone	No zone	25 (S)	20 (S)	24 (S)
5 (A)	18	14	No zone	No zone	26 (S)	24 (S)	28 (S)
5 (B)	14	12	No zone	No zone	6 (R)	24 (S)	25 (S)

4. DISCUSSION

Bacteriophages constitute a group of viruses that can specifically infect and lyses bacteria. Phages could prove to be superior to antibiotics, since they are persisting, inactive and non-pathogenic outside their bacterial hosts. Compared with other conventional therapeutic approaches, phage therapy could be more effective in treating emerging resistant pathogenic bacterial strains [19]. It is easily discovered and can kill the biofilm forming bacteria also have low inherent toxicities. Phages during the lytic course are capable of increasing in number specifically where hosts are located. It's generally will not affect beneficial bacteria, side-effects are uncommon in phages and do not affect eukaryotic cells [20]. Various typing schemes have been based upon either the production of or sensitivity to a range of different bacteriocins. Staphylococcal bacteriocins are lethal to strains belonging to the same or related species and act by binding to surface receptors followed interaction with an intracellular target. It has a broad activity spectrum against many Gram-positive (e.g. corynebacteria, listeriae, streptococci and bacilli) and gram-negative bacteria (e.g. *Neisseria gonorrhoeae* and *Escherichia coli*) [21,22].

The water samples were collected together with the sediment because virus present in higher number in bulk sediment compared to pore water [23]. Once viruses are adsorbed to sediment, they are immobilized, which may lead to accumulation and concentration of viruses in the sediments [24]. Therefore, in order to isolate bacteriophage successfully, sediments which include both the particle-adsorbed virus and virus in pore water were collected in this study. The water samples taken from sample collection sites A1 and B1 contain abundant amount of algae. The algae have symbiotic relationship with the bacteria. Photosynthetic algae convert large amount of carbon dioxide into oxygen. The high concentration of dissolved oxygen promotes the growth and survival of bacteria. Other than that, large amount of algae also provides an adsorption site for phage which protects the phage from inactivating factor in environment such as sunlight. As a result, higher phage count can be obtained from sample collection sites A1 and B1. Bacteriophages infective to the *Staphylococcus aureus* can be isolated from the water samples. The most probable reason is using the *S. aureus* as enrichment cultures for phage isolation. Therefore, it is easier to isolate the phages for certain bacterial strains that are being introduced in enrichment cultures [25-27]. Based on the study, plaque morphology obtained in plaque assay, all the phage isolates produced clear zones, indicating all bacteria in the plaque zone are being lysed. Some phages did not give plaques which were uniform in size. The heterogeneity of plaque size exhibited into the plate. The aim of the study was to isolate and characterize bacteriophages that have lytic activity against *S. aureus*. Phages were isolated from sample collection sites A1, B1. The water samples A1 were collected from drain and B1 were collected from pond. The water in drain and pond remains stagnant, therefore, higher number of phages will be concentrated in this region. A1 and B1 sites have abundant source of nutrient required for growth of *Staphylococcus aureus* which provides the necessary host for the phage to grow.

Acinetobacter nosocomialis, often known as *A. nosocomialis*, is a gram-negative, nonfermentative member of the Acinetobacter calcoaceticus-baumannii complex. Studies conducted recently have revealed an elevated clinical prevalence of *A. nosocomialis*. However, creating novel antibacterial drugs is essential given the rising trend of antibiotic resistance. There is currently little study on bacteriophage therapy for *A. nosocomialis* [28]. A well-known bacterium called *Staphylococcus aureus* is regularly found in medical facilities and food processing facilities. Effective control measures are needed to assure microbiological safety because of its propensity to acquire antibiotic resistance. Four lytic *S. aureus* phages (SapYZU10, SapYZU11, SapYZU12, and SapYZU13) were found in Yangzhou, China, sewage samples in one investigation. They were assessed for their biological properties and bactericidal activity against *S. aureus* isolates in milk and fresh pork in vitro. Under challenging circumstances (-80–70°C, pH 3.0–12.0), their activity remained largely steady [29]. Opportunistic pathogen *Acinetobacter baumannii* is frequently linked to infections acquired in hospitals. Acinetobacter strains that are rapidly becoming multi- and pan-drug resistant present a growing concern in hospitals. One approach for treating infections brought on by *A. baumannii* is phage therapy. In one investigation, the phages fBenAci001, fBenAci002, and fBenAci003 that infected clinical *A. baumannii* strains from Finnish patients were recovered from hospital wastewater in Benin. These phages are related to those of the family Autographiviridae of the genus Friunavirus, according to phylogenetic study. The separated phages are in compliance

with the guidelines for phages used in phage therapy. Their limited host range, which may limit their therapeutic potential, was discovered [30].

Drug- resistant *S. aureus* was also form some specific zones in the nutrient agar plates. *Staphylococcus aureus* is versatile and can grow in different environments able to utilize the mannitol salt agar and nutrient agar. The biochemical tests results are also in line with the reports of other authors on characteristics of *Staphylococcus aureus*. After that, mostly tried to show the Comparisons between bacteriophage zones and drug-resistant zones of bacterial different strains.

5. CONCLUSION

In conclusion, all of the bacteriophage isolates collected from sampling area were able to infect *Staphylococcus aureus*. All the isolated phages are produced round, clear plaques. It has shown that sampling sites A1 and B1 provide favorable condition for the growth of phage. Based on the number of phages isolated, higher number of bacterial population was present in A1 and B1. Phage isolation and characterization in this study was suggested to be a potential candidate for strong lytic capability. Nevertheless, more identification and characterization tests should be done to confirm this hypothesis.

The strains isolated from the sampling area showed resistant and sensitivity against a large number of antibiotics. Penicillin and Oxacillin was relatively resistant against the isolates. Very little amount of sensitivity was found in the case of Gentamycin. All these results signify to only one decision and that is the resistance capacity of microorganisms against antibiotic materials are increasing day by day. When antibiotics were introduced for the first time, very low dose of a type of antibiotic was enough to kill the microorganism's sensitive against that antibiotic. But as days are passing by, they are being more and more resistant and marshaling new ways of evading the actions of antibiotics. This situation is created by human itself. The study was done with a little number of isolates from a limited number of places. This study was performed with the intention of identifying the comparison between bacteriophage zone formation and drug-resistant zone of *S. aureus*.

DISCLAIMER

This paper is an extended version of a preprint /repository/ Thesis document of the same author. The preprint /repository/ Thesis document is available in this link: http://eprints.utar.edu.my/904/1/BM-2013-0903712-1.pdf

[As per journal policy, preprint /repository article can be published as a journal article, provided it is not published in any other journal]

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wong CL, Sieo CC, Tan WS, Abdullah N, Hair-Bejo M, Abu J, Ho YW. Evaluation of a lytic bacteriophage, Φ st1, for biocontrol of Salmonella enterica serovar Typhimurium in chickens. International Journal of Food Microbiology. 2014 Feb 17; 172:92-101.
2. Ackermann HW. Bacteriophages: biology and applications.
3. Abedon ST. Size does matter–distinguishing bacteriophages by genome length (and 'breadth'). Microbiology Australia. 2011;32(2):95-6.
4. Carlton RM. Phage therapy: Past history and future prospects. Archivum immunologiae et therapiae experimentalis-English Edition. 1999 Sep; 47:267-74.
5. Summers WC. The strange history of phage therapy. Bacteriophage. 2012 Apr 1;2(2):130-3.
6. Brüssow H. Editorial commentary: Phage therapy: quo Vadis. Clinical infectious diseases. 2014 Feb 15;58(4):535-6.
7. Kutter EM, Kuhl SJ, Abedon ST. Re-establishing a place for phage therapy in western medicine. Future microbiology. 2015 May;10(5):685-8.
8. Greene J, Goldberg RB. Isolation and preliminary characterization of lytic and lysogenic phages with wide host range within the streptomycetes. Microbiology. 1985 Sep;131(09):2459-65.
9. Uchiyama J, Rashel M, Maeda Y, Takemura I, Sugihara S, Akechi K, Muraoka A, Wakiguchi H, Matsuzaki S. Isolation and characterization of a novel Enterococcus faecalis bacteriophage φEF24C as a therapeutic candidate. FEMS Microbiology letters. 2008 Jan 1;278(2):200-6.
10. Khan Mirzaei M, Nilsson AS. Isolation of phages for phage therapy: a comparison of

spot tests and efficiency of plating analyses for determination of host range and efficacy. PLoS one. 2015 Mar 11;10(3): e0118557.
11. Gupta R, Prasad Y. Efficacy of polyvalent bacteriophage P-27/HP to control multidrug resistant *Staphylococcus aureus* associated with human infections. Current microbiology. 2011 Jan; 62:255-60.
12. Akhverdyan VZ, Gak ER, Tokmakova IL, Stoynova NV, Yomantas YA, Mashko SV. Application of the bacteriophage Mu-driven system for the integration/amplification of target genes in the chromosomes of engineered Gram-negative bacteria—mini review. Applied microbiology and biotechnology. 2011 Aug; 91:857-71.
13. Ogbulie JN, Okpokwasili GC. Haematological and histological responses of Clarias gariepinus and Heterobranchus bidorsalis to some bacterial diseases in River State, Nigeria.
14. Allamin IA, Shettima H, Abdullahi HM, Bukar UA, Faruk AU, Ruwa AM. Distribution of Heterotrophic and Nitrogen fixing Rhizobacteria in Irrigation Sites of Lake Alau Borno State Northeastern Nigeria. Journal of Environmental Bioremediation and Toxicology. 2021 Dec 31;4(2):27-30.
15. Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge university press; 2005.
16. Sulakvelidze A. Phage therapy: an attractive option for dealing with antibiotic-resistant bacterial infections. Drug discovery today. 2005;12(10):807-9.
17. Shukla I, Tiwari R, Agrawal M. Prevalence of extended spectrum β-lactamase producing Klebsiella pneumoniae in a tertiary care hospital. Indian journal of medical microbiology. 2004 Apr 1;22(2):87-91.
18. Wernicki A, Nowaczek A, Urban-Chmiel R. Bacteriophage therapy to combat bacterial infections in poultry. Virology journal. 2017 Dec;14(1):1-3.
19. Monk AB, Rees CD, Barrow P, Hagens S, Harper DR. Bacteriophage applications: where are we now. Letters in applied microbiology. 2010 Oct 1;51(4):363-9.
20. Matsuzaki S, Rashel M, Uchiyama J, Sakurai S, Ujihara T, Kuroda M, Ikeuchi M, Tani T, Fujieda M, Wakiguchi H, Imai S. Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. Journal of infection and chemotherapy. 2005 Oct; 11:211-9.
21. Wiley BB, Rogolsky M. Molecular and serological differentiation of staphylococcal exfoliative toxin synthesized under chromosomal and plasmid control. Infection and Immunity. 1977 Nov;18(2):487-94.
22. Arendse E, Fawole OA, Opara UL. Effects of postharvest handling and storage on physiological attributes and quality of pomegranate fruit (Punica granatum L.): A review. International Journal of Postharvest Technology and Innovation. 2015;5(1):13-31.
23. Drake LA, Choi KH, Haskell AE, Dobbs FC. Vertical profiles of virus-like particles and bacteria in the water column and sediments of Chesapeake Bay, USA. Aquatic Microbial Ecology. 1998 Oct 15;16(1):17-25.
24. Oun A, Yin Z, Munir M, Xagoraraki I. Microbial pollution characterization of water and sediment at two beaches in Saginaw Bay, Michigan. Journal of Great Lakes Research. 2017 Jun 1;43(3): 64-72.
25. Stenholm AR, Dalsgaard I, Middelboe M. Isolation and characterization of bacteriophages infecting the fish pathogen Flavobacterium psychrophilum. Applied and environmental microbiology. 2008 Jul 1;74(13):4070-8.
26. Rahman MA, Islam S, Rahaman S, Hossen MA, Sakib KR, Rimu AJ. Microbiological Study of Conventional Drinks in Mirpur Area, North Dhaka City of Bangladesh. Int. J. Path. Res. 2023;13(1):10-5.
27. Sakib KR, Islam S, Rahaman S, Ferdous K, Rahman MA, Hossen MA, Islam MR, Rimu AJ. Water-Supply Potability Status of Bangladesh University of Health Sciences (BUHS).
28. Lam HY, Lai MJ, Wu WJ, Chin YH, Chao HJ, Chen LK, Peng SY, Chang KC. Isolation and characterization of bacteriophages with activities against multi-drug-resistant Acinetobacter nosocomialis causing bloodstream infection in vivo. Journal of Microbiology, Immunology and Infection. 2023 Aug 5.
29. Wen H, Yuan L, Ye JH, Li YJ, Yang ZQ, Zhou WY. Isolation and characterization of a broad-spectrum phage SapYZU11 and its potential application for biological control of *Staphylococcus aureus*. Quality

Assurance and Safety of Crops & Foods. 2023 Apr 1;15(2):32-48.

30. Kolsi A, Haukka K, Dougnon V, Agbankpè AJ, Fabiyi K, Virta M, Skurnik M, Kantele A, Kiljunen S. Isolation and characterization of three novel *Acinetobacter baumannii* phages from Beninese hospital wastewater. Archives of Virology. 2023 Sep;168(9): 228.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/107093