Isolation and characterization of six gamma-irradiated bacteriophages specific for MRSA and VRSA isolated from skin infections

Eman Rashad Ahmed Mahmoud\textsuperscript{a}, Hala Ahmed Hussein Ahmed\textsuperscript{a}, Amal Saeid Mohamad Abo-senna\textsuperscript{a}, Omnia Karem M. Riad\textsuperscript{b} and Maha Mohamad Abd Al – Rahman Abo- Shadi\textsuperscript{a}\textsuperscript{a}

\textsuperscript{a}Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt; \textsuperscript{b}Department of Radiation Microbiology, National Center for Radiation Research and Technology, Egyptian Atomic, Energy Authority, Cairo, Egypt; \textsuperscript{c}Department of Botany and Microbiology, Faculty of Science, Al-Azhar University


ewblock **ABSTRACT**

Although skin infections are usually uncomplicated, it may indicate systemic disease or lead to life-threatening systemic infections. \textit{Staphylococcus aureus} is the most common cause of skin infections. Treatment is complicated by continuous emergence of resistant \textit{S. aureus} especially methicillin-resistant \textit{Staphylococcus aureus} (MRSA) and vancomycin-resistant \textit{S. aureus} (VRSA). Therefore, searching for new alternatives is essential to combat this escalating antibiotic resistance. Bacteriophage therapy is a promising solution in the post antibiotic area. Bacteriophage therapy confers safety and higher rate of success. In the present study, 10 bacteriophages (belonging to the family Siphoviridae and Myoviridae) were isolated with lytic activity against MRSA \& VRSA isolated from skin infections. The strongest six of ten phages were characterized morphologically by a JOEL_JEM_1010 electron microscope, physically through evaluating their lytic activity against wide ranges of temperature (35–95°C), PH (3–10) and radiation doses (0.5–2.5kGy) and biologically by evaluating their host ranges and phage longevity (1–120 day) in vitro. Finally, the anti-Staphylococcal effect of the isolated bacteriophages was tested and revealed that they have alone activity (10, 12) and synergistic activity with vancomycin and erythromycin by increasing their inhibition zone diameters (16, 22) mm, respectively.

\section{1. Introduction}

Skin infections could affect any age-group, sex, and race. Although skin infections are usually uncomplicated, but it may indicate systemic disease or lead to life threatening systemic infection (Witkowski & Parish, 1982). Skin and soft tissue infections range from the uncomplicated impetigo to the lethal necrotizing fasciitis (Clebak & Malone, 2018). Skin and soft tissue infections are characterized by microbial invasion of the skin layers and underlying soft tissues (Esposito et al., 2016). \textit{S. aureus} and beta-hemolytic Streptococci are the most common causes of skin infections (Kaye et al., 2019; Witkowski & Parish, 1982). Antibiotic therapy may be dispensed as the optimal treatment for purulent infections is drainage and incision. If required, the duration of antibiotics treatment ranges from 5 to 10 days of treatment in most uncomplicated bacterial skin infections (Sukumaran & Senanayake, 2016). For successful treatment, it is important to select the proper antibiotic based on disease severity, susceptibility patterns, clinical response to therapy and cost (Ellis & Lewis, 2005).

Treatment is complicated by the continuous emergence of resistant \textit{S. aureus} especially MRSA (Sukumaran & Senanayake, 2016). Although the drug of choice for MRSA is vancomycin, VRSA is reported and in a continuous spread (McGuinness et al., 2017). Therefore, searching for new alternatives is essential to combat the escalating antibiotic resistance.

Bacteriophage therapy is a promising solution in the post antibiotic area. They are viruses that infect and replicate only in bacterial cells. Bacteriophages are the most abundant microorganism on earth, varying in morphology, size and genomic organization (Simmonds & Aiewsakun, 2018). Bacteriophage therapy confers safety and higher rate of success. This promising successful therapy is due to specificity for selected bacteria. It can infect only one species, serotype or strain providing great feature, as it affects only the target pathogen without destruction of the normal flora. Nowadays, phages are being used successfully in humans and animals in targeted therapies for slow-healing infections (Wernicki et al., 2017).

Once a bacteriophage attaches to its host, it follows one of the two replication strategies: lytic or lysogenic. When the bacteriophage infects a host cell, it releases its genetic material into the host cell cytoplasm. In the lytic replication cycle, bacteriophage utilizes the host ribosomes for synthesis of its proteins, and then the host cell dies by lysis, releasing new bacteriophages to infect another host. In the lysogenic replication cycle, the phage genome is integrated into the bacterial
2.3.1. Isolation

The phage genome is passed to the daughter cells without killing and termed prophages. Change of the environmental conditions may convert the prophages to the lytic replication cycle (Doore & Fane, 2016). Kim et al. (2018) found that phage endolysins (lysins) are bacteriophage-encoded peptidoglycan hydrolases that lyse Gram-positive bacterial cell walls from within the bacterial cells in order to release progeny phages after replication in the bacteriophage life cycle.

Bacteriophages can be isolated from urban sewage (Maszewska & Różalski, 2019), the success of phage therapy is highly dependent on an isolated phage possessing a wide host range (Peng et al., 2019).

The aim of the current study was to isolate bacteriophages specific for MRSA & VRSA isolated from skin infections and characterize them by host-specific range, temperature, pH, and gamma radiation deactivation. Also, the effect of the isolated bacteriophages individually and with antibiotic discs was also evaluated.

2. Materials and methods

2.1. Identification of S. aureus isolates

Twenty-four Staphylococcal isolates from skin infections (77 total isolates) were collected from Al-Hod Almarsod Hospital for Dermatology (n = 62) and from different private clinics (n = 15) in Cairo (Egypt), isolated and identified according to (Chakraborty Pramanik & Roy, 2012).

2.2. Antimicrobial susceptibility testing

Isolated S. aureus was tested for susceptibility to amikacin, amoxicillin/clavulanic acid, ampicillin, cefoxitin, chloramphenicol, erythromycin, gentamicin, levofloxacin, oxacillin, rifampycin, trimethoprim/sulfamethoxazole, and vancomycin. Kirby–Baur disk diffusion method was used, procedure and interpretation were according to (CLSI, 2018).

2.3. Isolation, propagation and purification of S. aureus bacteriophages

2.3.1. Isolation of bacteriophages

Thirteen samples were collected from sewage, water, and soil as the following: six samples from sewage different locations (One sewage sample which contains all isolated bacteriophages specific for S. aureus was locally isolated from 1 sewage sample according to (Othman et al., 2015), collected from drainage systems in El-Yasmin 1, First assembly, New Cairo, Egypt), two from Nile water, two sand soil samples, and three mud soil samples to isolate bacteriophages specific for Staphylococcus aureus.

2.3.2. Preparation of crude phage suspension

Preparation of crude phage suspension was carried out according to (Othman et al., 2015). The crude lysate of the phages was obtained and assayed qualitatively and quantitatively.

2.3.3. Screening of the isolated bacteriophages for the lytic activity against MRSA isolates

2.3.3.1. Qualitative detection of bacteriophages (spot test assay). Staphylococcus aureus phages were assayed qualitatively by spot test technique (Adams, 1959). Formation of lytic clear zones indicates the presence of phages.

2.3.3.2. Quantitative detection of bacteriophages (plaque assay). The isolation of the phages was carried out according to (Othman et al., 2015) by the double-layer agar technique.

2.3.4. Preparation of high titer S. aureus phage stock (single plaque isolate)

Biologically stock phage lysate was obtained using the single plaque isolation method. The phage lysate was stored at 4°C (Goodridge et al., 2003).

2.4. Characterization of the isolated bacteriophages

2.4.1. Morphology of the isolated S. aureus phage particles

The purified phage suspension was prepared as described by (Kalatzis et al., 2016) and then examined using a JOEL_JEM_1010 electron microscope (electron microscope unit, a regional center for mycology and biotechnology, Al-Azhar University, Cairo, Egypt) which was made by Siemens & Halske company in German.

2.4.2. Physical properties

2.4.2.1. Thermal stability. Thermal inactivation point of S. aureus bacteriophages in vitro was carried out according to (Othman et al., 2015) with some modifications by exposing each six S. aureus bacteriophages to different temperature degrees 35, 45, 55, 65, 75, 85, and 95°C.

2.4.2.2. Stability of S. aureus bacteriophages to different pH. The infectivity of S. aureus bacteriophages to survive at different pH levels was evaluated by exposing each of phage suspensions to adjusted pH values ranged from 3 to 10 using 0.1 M HCL/NaOH over 1 hr at 37°C (Jamalludeen et al., 2007). Then, checked for survival qualitatively by spot test method.

2.4.2.3. Stability of S. aureus bacteriophages to Gamma radiation. The infectivity of S. aureus bacteriophages to survive at different gamma radiation doses
was evaluated (Jebri et al., 2013) with modifications by exposing each of phage suspension to adjusted gamma radiation doses (0.5, 1, 1.5, 2, and 2.5 kGy) using Indian – Cell Cesium 137Cs (dose rate 1kGy/145 sec.) in the National Center of Radiation Research and Technology, Egyptian Atomic Energy Authority, Egypt. Then, checked for survival qualitatively by spot test method.

2.4.3. Biological properties

2.4.3.1. Host range of isolated bacteriophage. Double layer plates were used for host range assay. The eight moderate antibiotic-resistant isolated S. aureus (isolate no.4 to 11) plus the three VRSA isolates (isolate no.1, 2, and 3) were used as indicator bacteria in individual plates. The surface of every plate was spotted with drops of each of the isolated S. aureus phage suspensions. After incubation for 24 h., plates were examined for clearance at the site where growth had been applied (Othman et al., 2015).

2.4.3.2. Phages longevity in vitro. The phage lysates were kept in screw-capped glass tubes at 4, 37, and –20°C for 120 days. These lysates were examined for surviving at days 1, 2, 3, 4, 5, 6, 7, 15, 30, 60, 90, and 120 by spot test (Fortier & Moineau, 2009).

2.5. Molecular identification of the most resistant MRSA isolate

Sanger’s method of sequencing was used to know the nucleotide sequence of the tested isolate (GST, Germany). All molecular identification steps were done in Sigma Company (6th October, Egypt).

2.6. Combination of phage and antibiotic

2.6.1. Propagation of phages

The molecularly identified S. aureus strain was cultured on nutrient broth at 37°C for 20 h., then centrifuged at 3,000 × g for 20 min at 4°C. The harvested cells were washed twice with phosphate-buffered saline (PBS, pH 7.2) and diluted to approximately 2 × 105 CFU/mL for inoculation. The two most longevity phage isolates were propagated according to (Othman et al., 2015). After incubation for 24 h. at 37°C, the phages were enumerated. Phage titer was expressed as plaque-forming unit (PFU/mL).

2.6.2. Qualitative detection of bacteriophages (spot test assay)

S. aureus phages were assayed qualitatively by spot test technique (Adams, 1959). Formation of lytic clear zones indicates the presence of phages.

2.6.3. Disk diffusion susceptibility test

The synergistic effect of phage and antibiotic against S. aureus was determined using disk diffusion susceptibility test. Disks of erythromycin (15 μg) and vancomycin (30 μg) were placed on the Mueller-Hinton agar plates containing S. aureus (105CFU/mL) alone and with phage (106 PFU/mL). After 18-h incubation at 37°C, the diameter of the clear inhibition zone was measured (Jo et al., 2016).

3. Results

3.1. Isolation of S. aureus and their antimicrobial susceptibility pattern

From the 77 specimens; 24 (41.4%) isolates were Staphylococci, 15 (25.9%) of them were biochemically identified as S. aureus. S. aureus isolates were tested for their antimicrobial susceptibility. Results in Figure 1 indicated that all the isolates were un-susceptible to erythromycin (100%) followed by Trimethoprim/ sulfamethoxazole (80%), Chloramphenicol (66.7%) and Ampicillin (AM) (46.7%). Nine (60%) isolates were found to be methicillin-resistant S. aureus (MRSA). Out of the nine MRSA isolates, three isolates (20%) were

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**Figure 1.** Antimicrobial susceptibility pattern of isolated S. aureus.
resistant to all tested antibiotic used included vancomycin as shown in Figure 1. The three isolates were used in bacteriophages isolation.

3.2. Isolation, propagation and purification of Staphylococcus aureus bacteriophages

Only one sewage sample from 6 contained the target bacteriophage. Ten different phages were isolated from sewage sample while sand; soil and Nile River water samples were completely free from the S. aureus phage. Four bacteriophages from the 10 had very weak lytic activity on S. aureus isolates.

3.3. Screening of the isolated bacteriophages for the lytic activity against MRSA isolates

The lytic activity of the strongest six phages isolates was tested against the most three antibiotic MRSA and VRSA isolates (each two phages against their proper isolate). They were resistant to the 12 of the tested antibiotics included vancomycin. The six bacteriophages lyzed the target bacteria which were assayed quantitatively according to size of the clear zone done by each phage. Table 1 shows the plaque diameters of the isolated MRSA-specific bacteriophages.

Bacteriophages of p1, p6 were the strongest phages, as they caused plaques 5 mm in diameter (P1 circled by 2 mm hallow zone) followed by phages no p2 and P 4 (4 mm diameter circle), and p3 (3 mm diameter circle) and finally phages number p5 had 2 mm diameter circle (Table 1 and Figure 2).

| S. aureus no. | Phage no. | Plaque diameter | Halo diameter |
|--------------|-----------|----------------|--------------|
| No.1         | p1        | 5 mm           | 2 mm         |
| No.1         | p2        | 4 mm           | -            |
| No.2         | p3        | 3 mm           | -            |
| No. 2        | p4        | 4 mm           | -            |
| No.3         | p5        | 2 mm           | -            |
| No.3         | p6        | 5 mm           | -            |

Each of these phages has been characterized according to the biological, physical, and chemical properties. Results in Table 1 showed that S. aureus isolate was the most affected isolate by its proper phages.

3.4. Characterization of the isolated bacteriophages

3.4.1. Scanning electron microscopic examination for the isolated S. aureus phages

Electron microscopy of the four lytic MRSA bacteriophages revealed that all of them belong to family Siphoviridae except p1 belong to Myoviridae family as shown in Figure 3. Table 2 describes p1, p3, p4 and P6.

3.4.2. Stability of the bacteriophages to different pH, temperature and gamma radiation

Regarding the thermal stability, P1, P4 and P6 were more thermally stable than P2, P3 and P5 as shown in Table 3. P1, P4, P5 and P6 phages retained their infectivity at wide pH conditions ranging from 3 to 10, 8, 9
3.4. Biological properties

3.4.3. Determining the host specificity of the isolated phages. Eight moderate antibiotic-resistant S. aureus isolates plus three VRSA isolates were chosen to determine the host specificity for the six isolated bacteriophages. The S. aureus phages had host range 18%–81.8% as shown in Table 4.

3.4.3.2. Phages longevity in vitro. The longevity test for the six phages against the three MRSA and VRSA isolates was done. Table 5 shows that all the isolated bacteriophages, could survive for 120 days at 4, 37 and −20°C, except p2 and p3 they remained active for 90 days of storage at the same conditions.

3.5. Molecular identification

The most affected isolate by phages MRSA isolate was selected for combination treatment between phage and antibiotics. This isolate was molecularly identified by Sigma Company as S. aureus. Figure 4 shows the sequencing of partial 16S rRNA gen for the isolate. The obtained nucleotide sequences were compared with the data on gene bank, it matches the complete sequence of S. aureus strain S33 R. This Sigma company’ results of the sequencing showed congruence with isolation S. aureus of amplified product of 16S rRNA gene appeared 99% compatibility.

3.6. Combination of each antibiotic Vancomycin/Erythromycin with bacteriophages Against MRSA isolate

The antibiotic susceptibility test was done in the presence of the two MRSA bacteriophages p1 and p6 against the chosen MRSA and VRSA isolated strain. In this experiment two antibiotics (vancomycin and erythromycin) were chosen for this test: erythromycin which all the S. aureus isolates was resistance to it, while vancomycin had the highest rate of susceptibility. The results are shown in Table 6.

4. Discussion

The search for alternative therapeutic agents, including phages. Bacteriophages were discovered to be an antibacterial agent.

In this study, all the isolated bacteriophages were isolated from sewage. Figure 2. Ribeiro et al. (2018) could successfully isolate bacteriophages from sewage. They revealed that to the sewage rich contents of bacteria and organic matters.

Six from the isolated bacteriophages were specific for MRSA and. MRSA isolates were previously isolated from some skin infections and represented 60% of the

![Figure 3. Electron microscopy of MRSA bacteriophages.](image_url)
Table 4. Host specificity of the isolated bacteriophages.

| S. aureus | Phage No. | Storage Temp | No.1 | No.2 | No.3 | No.4 | No.5 | No.6 | No.7 | No.8 | No.9 | No.10 | No.11 | Total | Total% |
|-----------|-----------|--------------|------|------|------|------|------|------|------|------|------|-------|-------|-------|-------|
| P.1       | +         | +            | +    | +    | +    | +    | +    | +    | +    | -    | +    | +     | 9      | 81.8% |
| P.2       | -         | -            | -    | -    | -    | -    | +    | +    | +    | -    | +    | -     | 3      | 27%   |
| P.3       | -         | -            | +    | -    | -    | -    | +    | -    | -    | +    | -    | -     | 2      | 18%   |
| P.4       | -         | -            | +    | -    | +    | -    | +    | -    | -    | +    | +    | +     | 6      | 54.5% |
| P.5       | -         | +            | +    | +    | -    | +    | -    | +    | -    | +    | +    | 6     | 54.5% |
| P.6       | +         | +            | +    | +    | +    | -    | +    | -    | +    | +    | +    | +     | 9      | 81.8% |

*MRSA isolates.
(+ indicates bacterial lysis (clear zone). - indicates no clear zone.

Table 5. Determination of the stability of Staphylococcus bacteriophage particles, at different temperature degrees for 120 days by spot test.

| S. aureus No. | Phage No. | Storage Temp | Days of Storage |
|---------------|-----------|--------------|-----------------|
|               |           |              | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 15   | 30   | 60   | 90   | 120  |
| 1             | p1        | 4°C          | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
|               | p2        | 37°C, -20°C  | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| 2             | p3        | 4°C, -20°C   | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
|               | p4        | 37°C, -20°C  | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| 3             | p5        | 4°C, -20°C   | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
|               | p6        | 37°C, -20°C  | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |

(+ indicates bacterial lysis (clear zone). - indicates no clear zone.

total isolated S. aureus. (Figure 1). This results agree with Kook, R., Becker, K., Cookson, B., van Gemert-Pijnen, J. E., Harbarth, S., Kluytmans, J., and Stuvelens, M. J., (2010) who found that 13 and 74% of worldwide S. aureus infections are MRSA Also Bessa, L. J., Fazzi, P., Di Giulio, M., and Cellini, L. 2015 isolated 28 species from 217 wounds, they found the most common bacterial species detected was methicillin-resistant represented 21, 8% of the isolates. While Kumar et al. (2015) who found that 66% of patients with skin and soft tissue infections (SSTIs) were colonized with S. aureus. Miller et al. (2012) reported that 80% of skin infections were due to S. aureus, The same authors added the targeting searching for phages specific for MRSA is due to its serious threads and continuous rapid spread of those resistant strains.

The images in this recent study taken by the electron microscopy revealed that the six isolated lytic MRSA bacteriophages belong to the family Siphoviridae as shown in Figure 3(b–d), p6(Figure 3(d)) indicates the base plates of the tail, except p1 belong to Myoviridae family (Figure 3(a)) Likewise the present study, another study isolated phages belong to the family Siphoviridae from sewage (Topka et al., 2018) while Czajkowski et al. (2015) isolated The ΦPD10.3 and ΦPD23.1 phages had morphology similar to other members of the Myoviridae family.

In the current study the isolated phages lytic ability was stable at 75°C while 50% of them couldn’t survive at 85°C (Table 3) unlike several studies that reported complete inactivation of phages at 70°C and above. Maszewska and Różalski (2019) reported that S. aureus bacteriophage inactivated at 85°C while Shende et al. (2017) found that viability of S. aureus phages was unaffected at 70°C within 2–3 min. Wang et al. (2016) isolated MRSA bacteriophage which its lytic ability was stable at 45°C and lose its activity at 65°C. Krasowska et al. (2015) reported similar finding for Bacillus phages they were resistant to high temperatures (80°C).

The sensitivity of bacteriophages lytic activity to the pH change was also detected, two of the isolated phages retain their lytic viability at pH from 4 to 7 also isolated four phages were stable at wide range of pH from 3 to 8, 9 and 10 (Table 3). Ibrahim et al. (2017) observed stable lytic activities of S. aureus phage at pH 6–8. While Wang et al. (2016) isolated a phage specific for MRSA, could survive at a pH ranging from 6 to 10. Krasowska et al. (2015) isolated B. subtilis phages resistant to the acidic (4.0) and alkaline (9.0 and 10.0) pH. On the contrary to our finding, several studies
Query 10 AGCTATACGGGATAA-ATTTTGAGCGGAAATGCAAATGAGGCTTCTGCTGT 68

Sbjct 166 AGCTATACGGGATAAATTTTGAGCGGAAATGCAAATGAGGCTTCTGCTGT 225

Query 129 AGATGCTATACGGGATATCTAGGCTCATTAGCTGTGTAAGTGATGAACTGCCTAACAGGCGA 285

Sbjct 226 AGATGCTATACGGGATATCTAGGCTCATTAGCTGTGTAAGTGATGAACTGCCTAACAGGCGA 188

Sbjct 286 AGATGCTATACGGGATAAATTTTGAGCGGAAATGCAAATGAGGCTTCTGCTGT 345

Query 189 CTCCTACGGGAGCGACATGGAAGAATCTCTCCGAAATGCGGAAACGTCGAGAACGAC 248

Sbjct 346 CTCCTACGGGAGCGACATGGAAGAATCTCTCCGAAATGCGGAAACGTCGAGAACGAC 405

Sbjct 406 CTCCTACCAGGATATCTACGCTGTAAGTGATGAACTGCCTAACAGGCGA 308

Query 309 TGTAAAGAATCTTGACATTTGACGATCCTAATCAGAAGAACGCGCTAATCAGTGC 368

Sbjct 466 TGTAAAGAATCTTGACATTTGACGATCCTAATCAGAAGAACGCGCTAATCAGTGC 523

Query 369 CACAAGGGCAATATATCGTGCAAGTTACGCTAACAGGCGCTAACAGGCG 428

Sbjct 526 CACAAGGGCAATATATCGTGCAAGTTACGCTAACAGGCGCTAACAGGCG 585

Query 429 CCTAGTACGGTTTATATAAGTGTGTAAGACCAGCGTAAATGGCTGAAAGGCG 488

Sbjct 586 CCTAGTACGGTTTATATAAGTGTGTAAGACCAGCGTAAATGGCTGAAAGGCG 688

Sbjct 646 CACAAGGGCAATATATCGTGCAAGTTACGCTAACAGGCGCTAACAGGCG 705

Query 549 CACAAGGGCAATATATCGTGCAAGTTACGCTAACAGGCGCTAACAGGCG 608

Sbjct 706 CACAAGGGCAATATATCGTGCAAGTTACGCTAACAGGCGCTAACAGGCG 868

Sbjct 766 CACAAGGGCAATATATCGTGCAAGTTACGCTAACAGGCGCTAACAGGCG 825

Query 669 CACAAGGGCAATATATCGTGCAAGTTACGCTAACAGGCGCTAACAGGCG 836

Table 4. Staphylococcus aureus strain 533 R 16S ribosomal RNA, complete sequence.

Figure 4. Staphylococcus aureus strain 533 R 16S ribosomal RNA, complete sequence.

Figure 5. Dendrogram showing genetic relatedness of isolated S. aureus (strain 533 R 16S RNA gene) to other Staphylococci.

Table 6. Combination treatment between bacteriophage and antibiotics against S. aureus.

| Inhibition zone diameter (mm) by disk diffusion test | Phage 1 S. aureus 20 | Phage 2 S. aureus 30 |
|-----------------------------------------------------|---------------------|---------------------|
| Phage only                                          | 10                  | 12                  |
| Erythromycin only                                   | 12                  | 12                  |
| Erythromycin+ Phage                                 | 15                  | 16                  |
| Vancomycin only                                     | 16                  | 16                  |
| Vancomycin+ Phage                                   | 22                  | 20                  |

observed maximal phage viability from pH 5 to 9 and completely inactivated at pH 3 and 11 (Ruchi et al., 2010; Shukla & Hirpurkar, 2011).

In order to examine the stability of phages isolated in the current study to select the ideal phage that can survive under stressful conditions, the isolated phages were subjected to different doses of gamma radiation. All the isolated phages could survive up to 2 kGy of gamma irradiation. Only 50% of them could survive after exposure to the irradiation dose of 2.5 kGy (Table 3). Harewood et al. (1994) reported that inactivation of bacteriophages depends on the nature of the matrix.
Sommer et al. (2001) reported that the dose requirement for a 2 log reduction of bacteriophage was about 17 kGy.

Jebri et al. (2013) compared between animal viruses, the authors found the most D10 values of Gamma radiation in the scientific literature range from 1 to 10 kGy, the most frequent values reported ranging between 1 and 5 kGy. In this investigation the S. aureus phages had host range 18%– 81.8% (Table 4) which was in conformity with study conducted by Synott et al. (2009) and Ajuebor et al. (2018) isolated Staphylococcal phages with wide host ranges from 43.5% to 78.3%. The most resistant isolate was molecularly identified and it matches the complete sequence of S. aureus strain 533 R (Figures 4, 5). Saleh et al. (2018) identified S. aureus sample molecularly and its sequences had shown 99% compatibility which was as similar as our results.

Finally, in the recent study antibiotic susceptibility test (erythromycin & vancomycin) was done in the presence of the two MRSA bacteriophages p1 and p6 against the chosen MRSA isolated strain. The synergistic effect of phages and antibiotics increased MRSA sensitivity toward the two antibiotics (Table 6, Figure 6). Sussman (2016) published that the combination of antibiotics and bacteriophage therapy is a more effective means of combatting MRSA than the utilization of each individual treatment on its own. Kumaran et al. (2018) reported the ability of the SATA-8505 phage to augment the effect of vancomycin, antibiotic, against biofilm-associated S. aureus cells. Kim et al. (2018) found that the phage endolysins SAL200 enhances the antibacterial activity of standard-of-care (SOC) antibiotics (Nafcillin and Vancomycin) against S. aureus both in vitro and in vivo.

5. Conclusion

In order to overcome the growing increase in antimicrobial resistance especially in skin infections MRSA, phages were isolated from sewage. The bacteriophages were belonging to the family Siphoviridae and Myoviridae. They had wide host ranges, wide range of temperature, pH and still active at 2.5 kGy doses of gamma radiation. Phages when combined with each antibiotics vancomycin and erythromycin obligated the isolated MRSA strain to be sensitive. This combination treatment could be applied successfully on multidrug-resistant MRSA and VRSA infections.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Maha Mohamad Abd Al – Rahman Abo- Shadi  http://orcid.org/0000-0002-9804-6298

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