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AN *IN-VITRO* PHAGE THERAPY - IN OPPOSITION TO FOOD POISON ENGENDERED *STAPHYLOCOCCUS AUREUS VIA* PRECISE BACTERIOPHAGE FROM TIRUNELVELI DISTRICT, TAMIL NADU

V. Prasanth Kumar, Divya S. Raj, M. Natarajan and S. Umamaheswari *

Department of Biotechnology, Manonmaniam Sundaranar University, Tirunelveli - 627012, Tamil Nadu, India.

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Correspondence to Author:
Dr. S. Umamaheswari

Professor & Head,
Department of Biotechnology,
Manonmaniam Sundaranar University, Tirunelveli - 627012,
Tamil Nadu, India.

E-mail: umamsu@gmail.com

ABSTRACT: *Staphylococcus aureus*, the facultative anaerobic and opportunistic Bacterium has been characterized as the pathogen with diversified molecular adaptability leading to the emergence of MDR. Advanced use of alternate antibiotics is increasing the scenario of the emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) to Oxacillin Resistant *Staphylococcus aureus* (ORSA) after that generating associated health infections. Even though *Staphylococcus aureus* is being considered as one of the important pathogens for its combination of virulence including toxin-mediated multidrug resistance and invasiveness, staphylococcus food poisoning is of high on interest as this infection rate is increasing day by day. Bacteriophage therapy has been of interest due to the absence of secondary resistance and targeted attack and also the lack of recolonization of the pathogen. The present study aims to isolate and purify *Staphylococcus aureus*-specific bacteriophages from sewage samples and thereby expunging food poison, causing *Staphylococcus aureus.* On this aspect *Staphylococcus aureus* causing food poison were isolated from a stool sample of patients at hospitals, and the specific Bacteriophage was isolated from the sewage in Tirunelveli District, India. Enrichment technique improved the stability of the phages. Spot and plaque assay experiments were done to examine the specificity of the isolated phage. Techniques employed result in providing purified specific bacteriophage for *Staphylococcus aureus*.

INTRODUCTION: Emergence of Multidrug-resistant (MDR) and persistent phenotypes of Staphylococcus owe to its molecular adaptive and evolutionary strategies [1]. This brings them to the limited group of successful pandemic clonal species which has mainly evolved through mutation.

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Apart from the core gene which mainly contributes to bacterial metabolic functions, the presence of Mobile Genetic Elements (MGEs) that normally encode for virulence and resistance determinants is the reason behind the divergent evolutionary levels [2, 3].

This includes the gene for beta-lactam antibiotic resistance gene paving the way for the emergence of Multidrug-resistant *Staphylococcus aureus* (MRSA) [4]. *Staphylococcus* is a Gram-positive opportunistic pathogen, which is the main reason for septicemia infections, soft tissue inflammations, and toxin associated toxicity in eukaryotes. The enterotoxins (SE) encoded by MGEs cause food

poisoning in spite of its commensal presence in humans being, an etiological transporter of foodborne diseases. Infection acquired from hospital scenario outrages community-acquired ones [5]. There are more than 50 species and sub-species of *Staphylococci* of which *S. aureus* is often associated with pathogenicity in humans. *Staphylococcus aureus* is distinguished from the other species by its ability to clot blood plasma [6].

Even though Staphylococcal food poisoning is not a fatal infection, it causes depletion in health. As toxin is salt tolerant and heats stable, cooking fails in the evacuation of the bacterium. The depletion in the identification of new antibiotics and the emergence of MRSA through molecular adaption demands the phage therapeutic approach to control the incidence of Staphylococcal infections [7]. Phage therapy or bacteriophage employed biocontrol measures have renewed the current scenario of the emergence of MDR. Bacteriophage therapy has been proven to be effective alone or in combination. This fact is supported by several clinical and animal trials proving that Staphylococcal phages effectively clear Staphylococcal infections, particularly Multi-Drug Resistant (MDR) [8, 9]. Where antibiotics and vaccines fail to provide a safer biological environment, bacteriophage has proven its safety through host specific strategies [10]. The advantage of phage over antibiotics listed includes its localized activity towards specific bacterium, absence of reproduction as the target bacteria get expunged and also it lacks secondary resistance which all are quite often in case of antibiotics.

MATERIALS AND METHODS:

Bacterium: *Staphylococcus aureus* were isolated from stool samples of inpatients and outpatients at Tirunelveli Medical College for one year. Stool samples were carried on Mannitol salt broth to the laboratory. Strains were grown at 37 ºC in Mannitol Salt broth (MSB) for 48 h. Solid media contained 1.5% (wt/vol) bacteriological agar **Fig. 1**. Pure cultures were maintained on Mannitol Salt Agar medium. Biochemical **Table 1** and molecular characterization was done to confirm the species level.

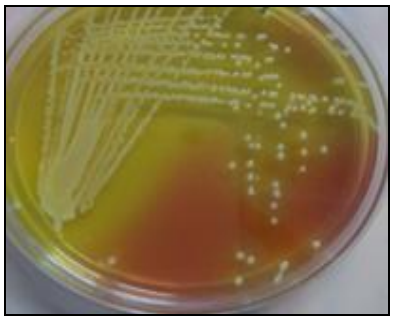

FIG. 1: ISOLATED SINGLE COLONIES OF *STAPHYLOCOCCUS AUREUS*

TABLE 1: STAINING AND BIOCHEMICAL CHARACTERS OF THE ISOLATED CULTURES

S. no.	Test	Result
1	Gram Staining	Gram positive cocci in cluster
2	Catalase	Positive
3	Oxidase	Negative
4	Coagulase	Positive
5	Mannitol Fermentation	Positive

Antibiotic Susceptibility: Susceptibility of all isolates of *Staphylococcus aureus* from the previous step were determined against fourteen different antibiotics *viz*., Vancomycin (30 mcg), Methicillin (5 mcg), Ampicillin (25 mcg), Cefotaxime (30 mcg), Amikacin (30 mg), Erythromycin (50 mg), Nitrofurantoin (300 mg), Kanamycin (30 mg), Oxacillin (1 mcg), Tetracycline (30 mcg). Norfloxacin (10 mcg), Penicillin (10 unit), Clindamycin (2 mg), Ciprofloxacin (5 mcg). The experiment was performed on bacterium incorporated Muller Hinton Agar **Table 2**.

TABLE 2: ANTIBIOTIC SUSCEPTIBILITY TEST FOR *S. AUREUS.* MEASUREMENTS IN MILLIMETRE

Antibiotics	PC6689	PC6700	PC6701	PC6702	PC6703	PC6704	PC6707	PC6708
Vancomycin	12±0.23	12±0.19	17±0.16	11±0.07	12±0.19	7±0.07	10±0.18	10±0.10
Methicillin	14±0.10	11±0.09	15±0.26	12±0.23	12±0.22	NIL	10±0.10	10±0.21
Ampicillin	16±0.29	9±0.20	22±0.21	15±0.11	17±0.31	5±0.11	6±0.21	6±0.22
Cefrotoxime	20±0.11	12±0.27	22±0.18	20±0.30	18±0.27	8±0.12	10±0.11	11±0.18
Amikacin	12±0.07	15±0.17	15±0.09	12±0.23	14±0.19	12±0.23	12±0.09	10±0.13
Erythromycin	15±0.21	12±0.31	16±0.16	15±0.03	17±0.21	NIL	15±0.12	12±0.16
Gentamycin	12±0.19	12±0.21	12±0.19	11±0.17	12±0.10	5±0.07	8±0.23	8±0.28
Nitrofurintoin	11±0.27	11±0.08	12±0.21	11±0.19	10±0.15	10±0.12	10±0.31	11±0.17
Kanamycin	11±0.21	7±0.10	18±0.32	11±0.21	12±0.28	9±0.17	12±0.10	13±0.08

Oxacillin	15±0.33	10±0.32	NIL	15±0.18	13±0.13	NIL	12±0.21	10±0.23
Tetracyclin	15±0.23	11±0.27	15±0.08	15±0.12	15±0.21	NIL	15±0.12	16±0.15
Narfloxacin	18±0.31	10±0.19	17±0.15	18±0.19	17±0.30	9±0.18	13±0.10	9±0.11
Penicillin	21±0.21	10±0.31	23±0.05	16±0.23	18±0.28	10±0.27	6±0.26	7±0.04
Clindamycin	12±0.32	16±0.19	20±0.02	17±0.12	16±0.14	NIL	16±0.18	19±0.13
Ciprofloxacin	17±0.09	6±0.024	17±0.10	20±0.07	17±0.11	10±0.23	11±0.08	5±0.05

Bacteriophage Isolation: Sewage samples from 30 different areas of Tirunelveli were collected as per the standard procedure. Samples were transported at 4 ºC and used within 24 h of collection for further use **Table 3** Isolation of Phage was performed through two steps:
a) Direct Isolation
(b) Enrichment Method.

(A) Direct Isolation Method: Around 8 ml of the collected water sample was fortified with 2 ml of chloroform, allowed to stand for 2 h to settle solid particles which were later subjected to removal. Water was centrifuged for 7 min at 10,000 rpm. The supernatant collected as cell lysate was stored at 4 °C for future use.

(B) Enrichment Method: In this method, 1 ml of the host bacterium was fortified into 8 ml of Nutrient broth and incubated overnight at 37 °C. After the incubation, the overnight culture was added with 1 ml of cell lysate and incubated at room temperature for overnight to which 2 ml of chloroform was added and centrifuged at 10,000 rpm for 10 min. The supernatant was transferred as the cell lysate and stored at 4 °C for future use. All the lysates were named as IIS and numbered fro 1-135 respectively.

TABLE 3: SEWAGE SOURCE AND PHYSICAL APPEARANCE WITH POTABALILITY

Sample	Sampling Area	Source	Color	Portability
S1	Kadayam	Sewage Water	Blackish	-
S2	Alwarkurichi	Sewage Water	Brownish	-
S3	Kurumbalaberi	Sewage Water	Yellowish	-
S4	Ambai	Sewage Water	Blackish	-
S5	Senkottai	Sewage Water	Blackish	-
S6	V.K. Puram	Sewage Water	Yellowish	-
S7	Palayamkottai	Sewage Water	Blackish	-
S8	Junction	Sewage Water	Brownish	-
S9	Ambur	Sewage Water	Yellowish	-
S10	Gopalasamudram	Sewage Water	Yellowish	-
S11	Pavoorchatram	Sewage Water	Brownish	-
S12	Town	Sewage Water	Blackish	-
S13	Papanasam	Sewage Water	Yellowish	-
S14	Uthumalai	Sewage Water	Colorless	-
S15	Ramayanpatti	Sewage Water	Blackish	-
S16	MSU	Sewage Water	Brownish	-
S17	Gandhinagar	Sewage Water	Yellowish	-
S18	Perumalpuram	Sewage Water	Brownish	-
S19	Sindupoondurai	Sewage Water	Blackish	-
S20	Pillayarkulam	Sewage Water	Yellowish	-
S21	Ravanasamudram	Sewage Water	Yellowish	-
S22	Abishekapatti	Sewage Water	Blackish	-
S23	Tenkasi	Sewage Water	Brownish	-
S24	Surandai	Sewage Water	Yellowish	-
S25	Therkumadathur	Sewage Water	Blackish	-
S26	Veeravanallur	Sewage Water	Brownish	-
S27	Pettai	Sewage Water	Blackish	-
S28	Pottalpudur	Sewage Water	Brownish	-
S29	Mukkudal	Sewage Water	Blackish	-
S30	Melagaram	Sewage Water	Yellowish	-

'-' indicates No Portability

Identification of Specific Bacteriophage: Two methods were employed to detect *Staphylococcus aureus*-specific phages.

(A) Plaque Assay: The cell lysate obtained from the enrichment method were used for plaque assay. The cell lysate (0.1 ml) was added to the overnight grown cells (0.2 ml) in Nutrient broth and mixed with 2.5 ml of aliquots of soft agar. The mixtures were subsequently overlaid on Nutrient Agar plates and incubated for 48 h at 37 °C. After the incubation period, the plates were characterized by the formation of plaques. A bacteriophage free sterile water with *Staphylococcus aureus* was employed as a negative control **Fig. 2**.

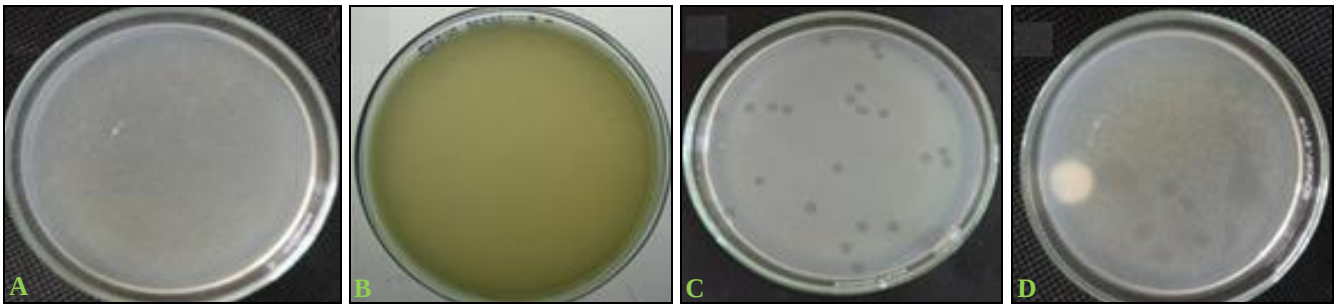

FIG. 2: PLAQUE ASSAY AGAINST THE ISOLATED BACTERIAL CULTURE (A) CONTROL PLATE (B) NEGATIVE CONTROL (C), POSITIVE CONTROL (D)

(B) Spot Assay: To reduce labor, time, and media, the spot assay method was carried out. Overnight culture of bacteria was mixed with Soft Agar to which a drop of phage stock solution was added and incubated at 37 °C for 48 h. After the incubation period, the plates were checked for the formation of plaques. Sterile phage free water as lysate with *Staphylococcus aureus* was maintained as control. The infected area was characterized under four categories *viz*., clear plaques, turbid plaques, faint plaques, or no plaques [11] **Fig. 3**.

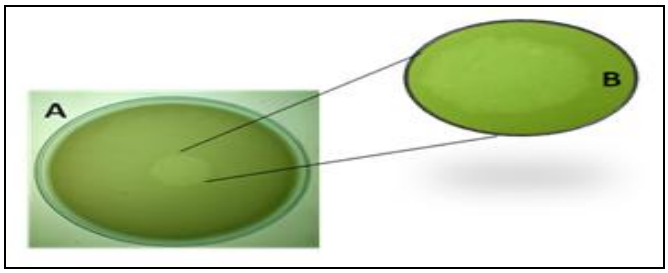

FIG. 3: SPOT ASSAY AGAINST THE ISOLATED *STAPHYLOCOCCUS AUREUS*. (A) CLEAR PLAQUE (B) ENLARGED VIEW OF PLAQUE

Concentrating and Purification of the Phage: The clear plaques formed in the plaque assay and spot assay method were subcultured by picking of plaques with a sterile inoculation loop and inoculated in fresh overnight *Staphylococcus aureus* broth culture and incubated for 24 h at 37 ± 2 °C. After 24 h, the incubated broth was mixed with chloroform (2 ml) and incubated for 4 h at 4 °C. The mixture was centrifuged for 5 min at 10,000 rpm to remove the bacterial cells and cell debris. After the centrifugation, the supernatant solution was stored with an addition of a few drops of chloroform at 4 °C.

For the plaque formation assay, the supernatant solution was spotted on the soft agar overlay with the host *Staphylococcus aureus* phage. The soft agar overlay plates were incubated for 48 h. The differentiated plaques were inoculated in the *Staphylococcus aureus* broth culture separately and incubated for 24 h **Fig. 4**.

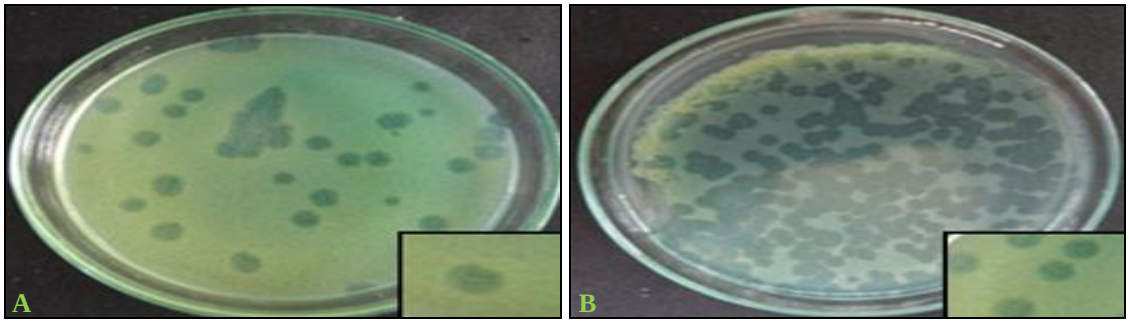

FIG. 4: PURIFICATION & CONCENTRATION OF *STAPHYLOCOCCUS AUREUS* SPECIFIC PHAGE

Host Range Determination: The isolated phages were inoculated with different types of *Staphylococcus aureus viz*., ATCC 19814, MRSA, and KU377336 (Microbial Laboratory, MSU). *E. coli* was maintained as negative control. Host Range was determined as per plaque assay techniques as indicated earlier **Table 4**.

TABLE 4: HOST RANGE ANALYSIS OF *S. AUREUS* PHAGE

S. no.	Bacterial Strains	Phage Sample (S8)
1	*Staphylococcus aureus* ATCC 19814	+
2	*Staphylococcus aureus* PC6701	+
3	*Staphylococcus aureus* PC6702	+
4	*Staphylococcus aureus* PC6703	+
5	*Staphylococcus aureus* KU377336 PC6704	+
6	*Staphylococcus aureus* PC6705	+
7	*Staphylococcus aureus* PC6706	-
8	*Staphylococcus aureus* PC6707	+
9	*Staphylococcus aureus* PC6708	+
10	*Escherichia coli*	-
11	*Pseudomonas* sp.	-
11	*Bacillus cereus*	-
12	*H. pylori* ATCC 26695	-

RESULTS AND DISCUSSION: To select specifically *S. aureus*, several preliminary characteristics were carried out according to standard procedures [12]. Nearly 100 stool samples were subjected to identification experiment. Based on the culture, Gram staining, and specific biochemical analysis including coagulase, catalase and oxidase tests, cultures from hospital samples were grouped to Staphylococcal genus. Preliminary identification was made by visualizing the color change of mannitol salt media from red to yellow, which is a characteristic strategy of *Staphylococcus* sp. Later bacterial samples were identified to be Gram-positive, cluster shape, catalase positive with the release of water showing the presence of catalase enzyme which is a characteristic feature of aerobes and facultative aerobes like Staph and also shown the absence of oxidase and presence of coagulase activity.

All bacteria with these characteristics were sorted and transferred to mannitol salt broth for incubation and further screening work. Around 25 out of 100 samples were positively found to harbor Staphylococcus. Twenty samples were further taken for molecular characteristics. The cultures were stored at 4 °C for further works.

The other sugar reduction tests were avoided to limit the time consumption and expenditure. Out of 25 Staphylococcus cultures, 8 were confirmed to be *Staphylococcus aureus* from the molecular analysis by 16sRNA sequencing. Around fifty years ago, MRSA strains were identified, which exhibited resistance to methicillin antibiotics and β-lactams. MRSA being the reason of limiting the effect of antibiotics and increasing the scenario of fatal STI (Staphylococcal infections), another group of resistant variety has also emerged which is resistant to the oxacillin antibiotic called oxacillin-resistant *Staphylococcus aureus* (ORSA). However, the percentage of ORSA is outraging MRSA, which is the after effect of alternative usage of this antibiotic to methicillin [13, 14].

The antibiogram assay was carried out not only in the view of identifying MRSA or ORSA but also to sort out *S. aureus* causing food poison from stool sample harbouring diverse range of other microbial community and the phage which is specific for *Staphylococcus aureus* not limiting to MRSA, MDR or ORSA as the genetic adaptability of this bacterium is remarkable and is subjected to a wide change [15]. The anti-susceptibility results emphasize the need for general isolation of phage to specific phage identification. On examination of the isolated 08 cultures of *Staphylococcus aureus* against fifteen different antibiotics, PC6704 rendered resistance towards more than 3 antibiotics *viz*., Methicillin, Oxacillin, Clindamycin, Tetracycline and Erythromycin which suggests the possibility of PC6704 to be an MRSA which was further confirmed by 16s rRNA sequencing as Methicillin-Resistant *Staphylococcus aureus* (Accession No: KU377336).

Apart from the MRSA strain, PC6701 also exhibited resistance to oxacillin whereas it was susceptible to Methicillin indicating the genetical heterogeneity of *S. aureus* adaptations being Oxacillin, a first generation antibiotic and Methicillin, a third generation antibiotic evolution showed limited susceptibility towards oxacillin. cefotaxime a third-generation antibiotic rendered sensitiveness to the isolated 08 bacterium cultures. The MRSA culture, PC6704 was found to be sensitive towards the first generation antibiotic, Penicillin, and resistant towards the third generation antibiotic, tetracycline.

This finding is similar to some of the studies done by Kenyatta [14], Iran [16], Eritrea [17]. This could be attributed to the continuous adaptability change of bacteria. ATCC strain was found to be sensitive towards all the antibiotics of different generations employed. The data retrieved through susceptibility experiment proves the constant genetic variations of *S. aureus* for their adaptation. However, the isolated strains were taken further to detect the host-specific phages.

Around 30 sewage samples were collected from different areas of the Tirunelveli city. They all were sorted based on their color from yellow to black even though the colour of the water does not have any access in the presence of phages. From the collected sewage water samples, *Staphylococcus aureus*-specific phage lysates were prepared as per the procedure mentioned, and the plaque assay was performed by double layer agar method. The lysates (L1-L30) yielded no visible plaques in all the triplicate plates.

Enrichment method was performed to empower the fewer concentration of phages in sewage, which could have been missed by the direct isolation method. Plaque assay and spot assay methods were employed to confirm the presence of phage from the previous steps. As plaque assay method consumes time and labor, spot assay for the detection of phages was employed with the samples harboring high load of phages. From the enriched lysates, the plaque assay was performed using double layer agar method to confirm the phages. Plaque formed were characterized into four clear, turbid, faint with no plaques. The occurrence of clear plaques exemplifies the purity and range of phages. The S8 cell lysate, which showed clear plaque formation was selected and subjected for further purification and concentration. Purification and concentration were carried out by several passages and sorting out plaques according to the clearance and size of formation for obtaining phages from plaques of equal size.

Several passages result in same plaques, which indicates the purity of phages. To confirm the host ranges of *S. aureus* phage specificity of the particular phage was performed against, ATCC 19814, MRSA and KU377336 & PC6701 – PC6708 *S. aureus* where *Escherichia coli, Pseudomonas* sp., *Bacillus cereus*, *H. pylori* ATCC 26695 were employed as negative controls. The selected phage exemplifies Host Specificity plaques towards all the test bacterium of *Staphylococcus aureus* series except PC6706.

CONCLUSION: Information from this research could be useful further to specifically characterize the host level determination and stableness of the purified phages. This would be useful in the diagnosis and targeted therapeutical applications that demand success over antibiotics without side effects limiting the molecular adaptability.

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CONFLICT OF INTEREST: Authors report there is no conflict of interest in the present study.

REFERENCES:

1. Kahl BC: Impact of *Staphylococcus aureus* on the pathogenesis of chronic cystic fibrosis lung disease. Int J Med Microbiol 2010; 300: 514-519.
2. Feil EJ, Cooper JE and Grundmann H: How Clonal Is *Staphylococcus aureus* ? J Bacteriol 2003; 185: 3307.
3. Alibayov B, Baba-Moussa L, Sina H, Zdenkova K and Demnerova K: Staphylococcus aureus mobile genetic elements. Mol Biol Rep 2014; 41: 5005-18.
4. Varela-Ortiz DF, Barboza-Corona JE and Gonzalez-Marrero J: Antibiotic susceptibility of *Staphylococcus aureus* isolated from subclinical bovine mastitis cases and *in-vitro* efficacy of bacteriophage. Vet Res Commun 2018; 42: 243-50.
5. Wakabayashi Y, Umeda K and Yonogi S: Staphylococcal food poisoning caused by Staphylococcus argenteus harboring staphylococcal enterotoxin genes. Int J Food Microbiol 2018; 265: 23-29.
6. Harris LG, Foster SJ and Richards RG: An introduction to *Staphylococcus aureus*, and techniques for identifying and quantifying *S. aureus* adhesins in relation to adhesion to biomaterials: review. Eur Cell Mater 2002; 4: 39-60.
7. Kazmierczak Z, Gorski A, Addendum DK, Kazmierczak Z, Gorski A and Dabrowska K: Facing antibiotic resistance: *Staphylococcus aureus* phages as a medical tool. Viruses 2014; 6: 2551-70.
8. Abatangelo V, Bacci NP and Boncompain CA: Broad-range lytic bacteriophages that kill *Staphylococcus aureus* local field strains. PLoS One 2017; 12: e0181671.
9. Capparelli R, Nocerino N and Lanzetta R: Bacteriophage-resistant *Staphylococcus aureus* mutant confers broad immunity against staphylococcal infection in mice. PLoS One 2010; 5: e11720.
10. Matsuzaki S, Rashel M and Uchiyama J: Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. J Infect Chemother 2005; 11: 211-19.

11. Iwano H, Inoue Y and Takasago T: Bacteriophage PhiSA012 has a broad host range against *Staphylococcus aureus* and effective lytic capacity in a mouse mastitis model. Biology (Basel) 2018; 7(1): E8.
12. Brown DF, Edwards DI and Hawkey PM: Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). J Antimicrob Chemother 2005; 56: 1000-18.
13. Al-Zoubi MS, Al-Tayyar IA, Hussein E, Jabali AA and Khudairat S: Antimicrobial susceptibility pattern of *S. aureus* isolated from clinical specimens in the Northern area of Jordan. Iran J Microbiol 2015; 7: 265-72.
14. Gitau W, Masika M, Musyoki M, Museve B and Mutwiri T: Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates from clinical specimens at Kenyatta National Hospital. BMC Res Notes 2018; 11: 226.
15. De-Backer S, Xavier BB and Vanjari L: Remarkable geographical variations between India and Europe in the carriage of the staphylococcal surface protein-encoding sasX/sesI and in the population structure of methicillin-resistant Staphylococcus aureus belonging to clonal complex 8. Clin Microbiol Infect 2018.
16. Khoramrooz SS, Dolatabad SA and Dolatabad FM: Detection of tetracycline resistance genes, aminoglycoside modifying enzymes, and coagulase gene typing of clinical isolates of Staphylococcus aureus in the Southwest of Iran. Iran J Basic Med Sci 2017; 20: 912-19.
17. Naik D and Teclu A: A study on antimicrobial susceptibility pattern in clinical isolates of *Staphylococcus aureus* in Eritrea. Pan Afr Med J 2009; 3: 1.

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