International Journal of Antimicrobial Agents 67 (2026) 107838

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Characterization and host range associating factors of *Staphylococcus aureus* bacteriophage PBSA08; Using Australian MRSA reference set

Shinwon Lee[a,b], Stephanie Lynch[b,c,d], Clare Fisher[b,c,d], Nouri Ben Zakour[b,c,d], Geoffrey W. Coombs[e], Shakeel Mowlaboccus[e], Heejoon Myung[f,g], Soon Ok Lee[a], Sohee Park[a], Ruby CY Lin[b,c,d,h], Jonathan R. Iredell[b,c,d,*]

[a] *Department of Internal Medicine, Pusan National University School of Medicine and Medical Research Institute, Pusan National University Hospital, Busan, Korea*
[b] *Centre for Infectious Diseases and Microbiology, Westmead Institute for Medical Research, Sydney, Australia*
[c] *Western Sydney Local Health District, Westmead Hospital, New South Wales, Australia*
[d] *Sydney ID/ Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, Australia*
[e] *School of Medical, Molecular and Forensic Sciences, Murdoch University, Murdoch, Western Australia, Australia*
[f] *Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Yong-In, Gyung-Gi Do, Korea*
[g] *LyseNTech Co. Ltd. Yong-In, Gyung-Gi Do, Korea*
[h] *School of Biomedical Sciences, University of New South Wales, Sydney, New South Wales, Australia*

ARTICLE INFO

Article history:
Received 17 December 2025
Accepted 29 April 2026

Editor: Dr Jim Gray

Keywords:
Phage therapy
Staphylococcus aureus

ABSTRACT

Objective: Antimicrobial resistance is a global health problem, with methicillin-resistant *Staphylococcus aureus* (MRSA) causing significant morbidity and mortality. As antibiotic options dwindle, (bacterio) phage therapy is re-emerging as a promising alternative.
Methods: We characterised a novel lytic *S. aureus* phage, PBSA08, and evaluated its host range using a reference panel of 39 MRSA isolates selected from the 2021 Australian Group on Antimicrobial Resistance repository. Phage susceptibility was tested by efficiency of plating (EOP) and growth kinetics assays (GKA). Factors associated with phage susceptibility were evaluated.
Results: Phage PBSA08, classified under the genus *Silviavirus*, revealed an icosahedral head and long tail structure using transmission electron microscopy. Adsorption assays indicated efficient bacterial binding, with a burst size of approximately 120 plaque forming units (PFU) per infected cell. EOP testing revealed 51.3% of the MRSA reference panel isolates were susceptible (EOP ≥10%). GKA results correlated strongly with EOP. In univariate analysis, phage PBSA08 susceptibility was associated with multilocus sequence type (ST), ciprofloxacin susceptibility, and the presence of the Panton-Valentine leucocidin-associated genes, with all ST30-IV and ST93-IV isolates susceptible to PBSA08 and all ST45-V isolates resistant to PBSA08. Firth logistic regression showed that ST30/93 isolates had significantly higher odds of susceptibility (aOR 68.9, 95% CI 2.0–2378.5; $P = 0.0191$), as did ciprofloxacin-susceptible strains (aOR 15.4, 95% CI 1.7–143.0; $P = 0.0160$).
Conclusions: PBSA08 demonstrates potential as a therapeutic phage, particularly against community-associated MRSA lineages such as ST93. Its inclusion in phage cocktails targeting prevalent MRSA clones warrants further investigation.

1. Introduction

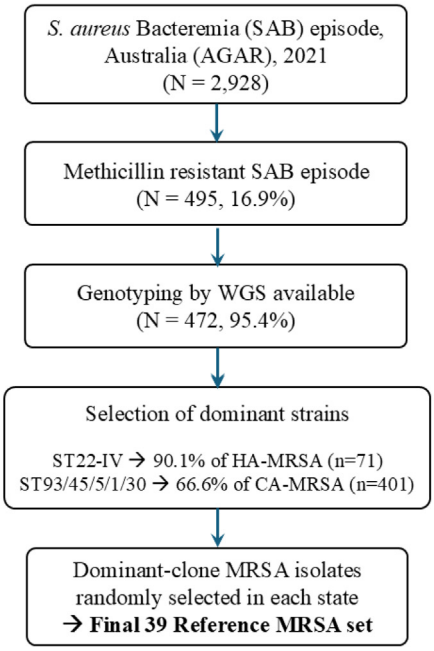
Antimicrobial resistance (AMR) is a rapidly growing worldwide concern, recognised by the World Health Organization (WHO) as one of the top 10 threats to human health [1].

Globally, methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most widespread multidrug-resistant (MDR) bacteria [2], and in 2024 the WHO ranked MRSA as a high priority pathogen for research and development of new antibiotics [3]. However, even with appropriate antibiotic administration, MRSA infections have a high mortality rate [4], which highlights the urgent need for alternative effective options to be developed.

In recent years, (bacterio)phage therapy has gained renewed attention as a potential treatment for many antimicrobial-resistant infections. Phage therapy uses viruses (phages) to kill bacteria and

* Corresponding author. Mailing address: Jonathan R. Iredell, Centre for Infectious Diseases and Microbiology, Westmead Institute for Medical Research, 176 Hawkesbury Rd, Westmead NSW 2145, Australia.
E-mail address: jonathan.iredell@sydney.edu.au (J.R. Iredell).

https://doi.org/10.1016/j.ijantimicag.2026.107838
0924-8579/© 2026 Published by Elsevier Ltd.



Reference MRSA set of Australia

	ST22-IV	ST93-IV	ST45-V	ST5-IIV	ST1-IV	ST30-IV	Total
ACT	1	1	1		1		4
NSW	1	1	1		1	1	5
NT	1	1		1	1		4
QLD	1	1	1		1	1	5
SA	1	1	1	1	1		5
TAS	1			1	1	1	4
VIC	1	1	1	1	1	1	6
WA	1	1	1	1	1	1	6
Total	8	7	6	5	8	7	39

ST, Sequence type; ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia)

Fig. 1. Flow diagram of MRSA reference panel construction from the 2021 AGAR SAB dataset. Dominant MRSA clones (ST22 for HA-MRSA; ST1, ST30, ST45, ST5, ST93 for CA-MRSA) were selected from each Australian state/territory to generate a 39-isolate reference set for phage susceptibility testing. Dark blue indicates phage-resistant isolates, whereas pale blue indicates phage-susceptible isolates. (ST, Sequence type; ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia)

treat bacterial infections, and offers a promising alternative or adjunct to antibiotics to treat MRSA infections [5–10].

Most anti-staphylococcal virulent phages previously described have a broad host range and belong to the *Caudoviricetes* class of viruses [11–15]. In this study, we have characterised a previously undescribed *S. aureus* phage, PBSA08, classified under the genus *Silviavirus*, subfamily *Twortvirinae*, within the family *Herelleviridae* [16]. We determined the therapeutic potential of PBSA08 by evaluating its activity against a collection of previously well-characterised MRSA bacteraemia isolates.

2. Materials and methods

2.1. Bacteriophages and bacterial isolates

The lytic *S. aureus Silviavirus* phage PBSA08 [17], (GenBank: OP856857.1), originally isolated from sewage, was provided by the Bacteriophage Bank of Korea.

The 39 MRSA included in the study were predominant clones identified in the 2021 Australian Group on Antimicrobial Resistance (AGAR)'s Australian *S. aureus* Surveillance Outcome Program [18]. The panel of MRSA included the healthcare-associated MRSA clone sequence type (ST) 22-IV (n = 8 isolates), and five community-associated MRSA clones, ST1-IV (n = 8), ST93-IV (n = 7), ST45-V (n = 6), ST5-IV (n = 5) and ST30-IV (n = 5) [18]. The MRSA were isolated from *S. aureus* bacteraemia cases from all jurisdictions across Australia (Fig. 1).

Unless otherwise stated, all isolates and phages were cultured overnight in Tryptic Soy Broth (TSB) (Becton Dickinson GmbH, Heidelberg, Germany).

2.2. Propagation of bacteriophage

Phage PBSA08 was propagated using *S. aureus* ATCC® 13301 as the production host, based on methods previously described [19]. Briefly, 0.5 mL of host culture was added to 50 mL TSB (containing 1 mM CaCl2 + 1 mM MgCl2) in a 250 mL flask. Once the bacterial culture reached an optical density (OD) of 0.2 at 600 nm (OD600), 0.05 mL of PBSA08 was added to achieve a multiplicity of infection (MOI) of 0.1. After overnight incubation (37°C, shaking at 180 rpm), the host and phage co-culture was centrifuged at 4,000 x g for 40 min at 4°C, and the supernatant was filtered using a 0.22 μm PES filter. The titer of filtered phage lysate was determined by the modified plaque assay [20].

2.3. Electron microscopy

The phage lysate was deposited onto carbon and formvar film-coated copper grids (Electron Microscopy Sciences, Hatfield, PA, USA) and negatively stained with 1.5% uranyl acetate (SPI Supplies, West Chester, PA, USA). Electron micrographs of the phages were produced using a transmission electron microscope (TEM) (JEM1200EXII; JEOL, Bath, United Kingdom) operated at 120 kV.

2.4. Adsorption rate and burst size measurements

The phage adsorption experiment was performed at 37°C with a phage inoculum at a MOI of 0.01 [21]. Free phage particles were quantified by measuring the plaque-forming units (PFU) of chloroform-treated samples within 5 min post inoculation [22]. The adsorption rate was calculated as described by Alves et al. [22]. The latent period and burst size of the phage were determined by one-step growth curves [22,23].

Mid-exponential phase cultures of the propagation host were harvested by centrifugation (7,000 × g, 10 min, 4°C) and resuspended in 5 mL of TSB to obtain an OD600 of 1.0. Phage PBSA08 was added to the suspension to obtain a multiplicity of infection (MOI) of 0.001 and allowed to adsorb at 4°C for 30 min. The mixture was then centrifuged, and the pellet was resuspended in 10 mL of fresh TSB. The phage and bacteria co-culture was incubated at 37°C with continuous shaking, and samples were collected

every 5 minutes and plated on the overlay agar to determine PFU [22,23].

2.5. Susceptibility of PBSA08 against reference isolates of S. aureus

The efficiency of plating (EOP), the relative activity of phage against specific strain/host strain, was measured using the double-layer agar methods [24]. The EOP was calculated by dividing the titer of the phage on the test strain by the titer of the same phage on its primary host strain. An EOP $\geq$10% was defined as phage susceptible [25,26]. This threshold was chosen to identify isolates with relatively preserved productive infection efficiency compared with the propagating host, while excluding isolates with only minimal or markedly reduced plaquing activity.

For the growth kinetics assay (GKA), the log phase growth of bacterial cultures was used, and the cell concentration was adjusted to approximately10^6 colony-forming units (CFU)/mL for each experiment. The bacterial suspensions were inoculated with phages at a concentration of approximately 10^6 PFU/mL, resulting in a MOI of 1.0. All bacterial reduction curves were generated using 96-well plates with a working volume of 150 μL. The OD600 was measured using a Molecular Devices Microplate Reader (SpectraMax ABS PLUS). OD600 readings were recorded immediately post inoculation and at 10-minute intervals thereafter for 18 hours. The cultures were incubated at 37°C with intermittent shaking at a medium speed.

Growth curves were generated by plotting OD600 values, adjusted for baseline, against time. The lytic activity of phage was assessed in two ways, and their performances were compared with endpoint measurements (final OD600 measurement following the 18-hour assay) and liquid assay score measurements (Equivalent to Area Under the Curve (AUC)) [27]. The liquid assay score was calculated by dividing the area between the positive control and the phage treatment curve by the total area under the positive control curve, then multiplying by 100. [27]. For the liquid assay score measurements, the AUC for each bacterial isolate was calculated by the equation: AUC = Σ [($OD600_i$ + $OD600_{i+1}$)/2]. The AUCs were normalised as percentages of the area under the curve of the positive control using methods outlined by Xie et al. [27].

2.6. Whole genome sequencing and comparative genomics

Illumina paired-end sequencing was performed on extracted bacterial genomic DNA according to the manufacturer's instructions. Quality control included FastQC v0.12.1 [28] read assessment, read trimming with Trimmomatic [29], Kraken2 v2.1.3 [30] taxonomic verification, and CheckM v1.2.2 [31] assembly metric evaluation (N50, contigs, genome size, completeness, contamination). Genomes were assembled using Unicycler v0.5.0 [32] and annotated using Bakta v1.11.4 [33], abritamr v1.0.17 [34] and PADLOC v2.0.0 [35]. Core genome alignment was generated using Panaroo v1.5.0 [36] in strict mode then used as input in iQtree v3.0.1 [37] for phylogenetic tree computation (-m TEST -bb 1000 -alrt 1000 -nt AUTO –scfl 100) and lastly visualised using the iTOL [38] web server.

Phylogenetic analysis of PBSA08 and 28 *Silviavirus* representatives was performed using the Virus Classification and Tree Building Online Resource (VICTOR) with the Genome-BLAST Distance Phylogeny (GBDP) method (Meier-Kolthoff and Göker, 2017). Six representative *silviavirus* genomes were selected for comparative analysis with PBSA08, aiming to maximise phylogenetic diversity while considering available genome metadata. All phage genomes were uniformly reannotated using Pharokka v1.8.0 [39] to ensure consistent gene calling and functional predictions, with subsequent visualization performed using clinker [40].

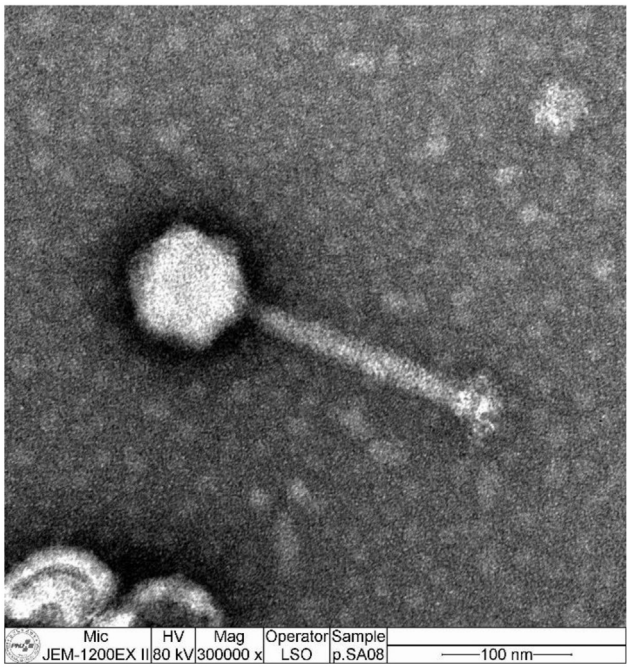


Fig. 2. Electron micrograph image of phage PBSA08 negatively stained with 1% uranyl acetate, showing an icosahedral head and a contractile long tail.

2.7. Comparison of characteristics between PBSA08 susceptible and non-susceptible MRSA

MRSA isolates were assigned to the PBSA08 susceptible group or non-susceptible group, according to their EOP test results (Susceptible >10% EOP). To evaluate associating factors of phage PBSA08 susceptibility, the PBSA08 susceptible and non-susceptible groups were compared using the demographics, clinical information and microbiological analysis data which were provided by the AGAR.

2.8. Statistical analysis

Statistical significance was determined by the Student *t*-test or Mann–Whitney Wilcoxon test, as appropriate, with alpha <0.05 using R (version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria). To evaluate the association between ST and phage susceptibility, univariable and multivariable logistic regression models were used. Given the presence of quasi-complete separation in some categorical variables, Firth's bias-reduced logistic regression was applied.

3. Results

3.1. Characterization of phage PBSA08

TEM analysis of phage PBSA08 revealed an icosahedral head and a long tail (100 to 145 nm), consistent with myovirus morphology [41]. Phylogenetic and comparative genome analyses placed PBSA08 within the genus *Silviavirus* (subfamily *Twortvirinae*, family *Herelleviridae*, class *Caudoviricetes*) (Fig. 2). The phage adsorption assay demonstrated 95% of PBSA08 adsorbed to its susceptible *S. aureus* host (ATCC® 13301) within 20 minutes (Fig. 3A). The one-step growth curve of PBSA08 indicated a latent period of 10 minutes and a burst size of 120 PFU per infected cell after 35 minutes (Fig. 3B). Phylogenetic and comparative genomic analysis confirmed PBSA08′s classification within genus *Silviavirus* (Supplementary Fig. 1).

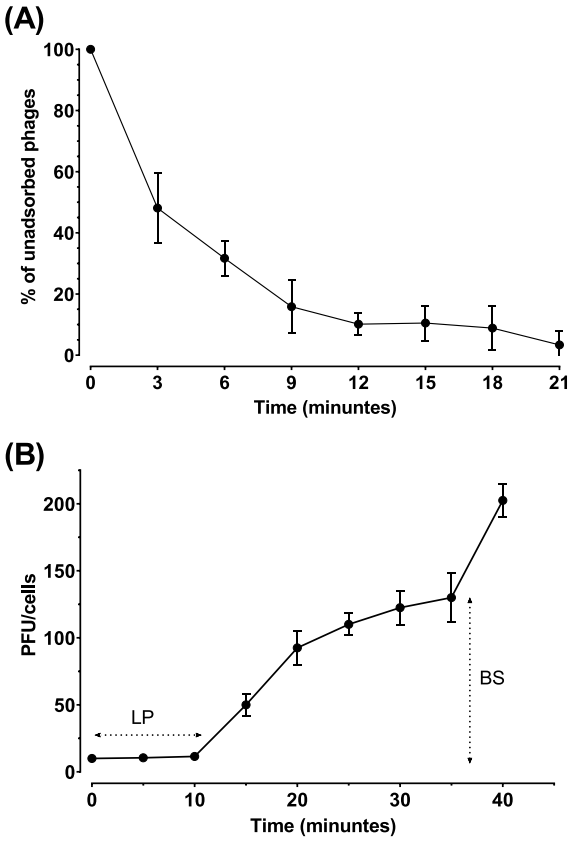


Fig. 3. Adsorption rate and burst size of the phage PBSA08; (A) adsorption assay and (B) one-step growth curve experiment demonstrating the latent (LP) periods and the burst size (BS).

3.2. Susceptibility and host range of PBSA08 against S. aureus

From the EOP test, PBSA08 showed lytic activity against the MRSA reference panel, with a median EOP of 40.0% (interquartile range [IQR], 0–76%). Based on the susceptibility threshold of EOP ≥10%, 20 of the 39 MRSA isolates (51.3%) were classified as susceptible (Supplementary Fig. 2).

PBSA08 exhibited relatively high EOPs against the ST93-IV (median EOP, 114%; IQR, 72–130%), ST30-IV (median EOP, 63%; IQR, 43–93%), and ST1-IV isolates (median EOP, 62.5%; IQR, 0–77.8%). In contrast, EOPs were markedly lower against the ST45-V, ST5-IV and ST22-IV isolates (all median 0%, IQRs 0–0% or 0–16.5%). Susceptibility rates from the EOP test were 100% for ST93-IV and ST30-IV, 62.5% for ST1-IV, and 0% for ST45-V, ST5-IV, and ST22-IV, indicating strain-specific variation in phage susceptibility.

In the growth kinetics assay, PBSA08 produced marked reductions in bacterial growth, with relative AUC values typically ranging between 5 and 30% of untreated controls for ST93-IV and ST30-IV isolates, and between 20 and 60% for most ST1-IV isolates. In contrast, isolates belonging to ST45-V, ST5-IV, and ST22-IV exhibited minimal reductions, with AUCs generally exceeding 80% of control levels. These quantitative trends mirrored the EOP results, demonstrating phage efficacy was strongly lineage-associated (Supplementary Fig. 2).

Regrowth of phage-treated bacteria, commonly observed during later GKA stages in Gram-negative bacteria, was not observed in the majority of *S. aureus* isolates. From the GKA test, end-point measurement correlated strongly with liquid assay scores among reference MRSA strains ($R^2 = 0.93$), indicating that approximately 93% of the variation in end-point reduction could be explained by the liquid assay scoring system (Fig. 4).

Comparison between the EOP and GKA assays demonstrated a high level of concordance. One isolate showed discrepant results, with high efficiency of plating but minimal growth inhibition in the GKA assay (Supplementary Fig. 3).

3.3. Factors associated with the host range of the phage PBSA08

We assigned 20 MRSA with EOP≥ 10% to the susceptible group and 19 MRSA strains with EOP <10% to the non-susceptible group. We compared the microbiological and clinical characteristics of the two groups. In univariate analysis, phage PBSA08 susceptibility was associated with specific STs, ciprofloxacin susceptibility, and carriage of the Panton-Valentine leucocidin (PVL)-associated genes. Notably, all ST93-IV and ST30-IV isolates were susceptible to phage PBSA08, whereas all ST45-V isolates were resistant (Supplementary Table 1). In multivariable analysis using Firth's bias-reduced logistic regression, ST30/93 isolates showed significantly higher odds of PBSA08 susceptibility compared to other STs (adjusted OR 68.9, 95% CI 2.0–2378.5; $P = 0.0191$). Ciprofloxacin-susceptible isolates showed higher odds of phage susceptibility (adjusted OR 15.4, 95% CI 1.7–143.0; $P = 0.0160$), although the large odds ratios and wide confidence intervals suggest limited precision of these estimates, likely reflecting the small sample size and sparse data structure. The presence of the PVL-associated genes was not significantly associated with phage susceptibility (adjusted OR 0.18, 95% CI 0.02–2.05; $P = 0.167$) (Table 1). Phylogenetic analysis of the MRSA reference set revealed clustering patterns corresponded with PBSA08 susceptibility profiles (Fig. 5). Notably, closely related isolates within ST93 and ST30 lineages uniformly demonstrated high susceptibility to PBSA08 (EOP ≥0.7), whilst ST45 isolates consistently showed resistance. While known bacterial defense systems are readily identifiable from genomic data, the distribution of these characterised systems, including restriction-modification and CRISPR-Cas, showed no clear correlation with phage susceptibility patterns across the phylogeny.

4. Discussion

S. aureus is responsible for a wide range of serious infections, including bacteraemia, infective endocarditis, osteoarticular infections, skin and soft tissue, pleuropulmonary, and device-related infections [42,43]. Despite the continuous, albeit slowed, development of novel antibiotics, invasive MRSA remains a major clinical challenge with persistently high mortality rates [4]. Consequently, there has been a renewed interest in using phages as adjuvant or alternative agents for treating MRSA infections. However, the high host-specificity of phages, while advantageous for personalised therapy, requires the collection and characterization of diverse phage libraries to ensure effective treatment options.

This study evaluated the characteristics of the novel *S. aureus* lytic phage PBSA08 and its host range using a reference panel of previously characterised MRSA bacteraemia isolates.

Our study demonstrated that phage PBSA08 showed lytic activity against a substantial proportion of the panel of 39 MRSA tested, with susceptibility varying markedly by clonal lineage. To assess susceptibility and host range, many previous studies have relied on spot tests [14,22,44–49]. However a single dilution spot test is not able to differentiate between initial lytic effects or a productive infection in host bacterial cells [50]. Since productive infection by phage is an important factor for effective phage therapy, spot testing alone cannot be considered a reliable indicator of phage susceptibility. In 2015 Khan Mirzaei et al. demonstrated the EOP test is a more reliable method for determining the host range of phages

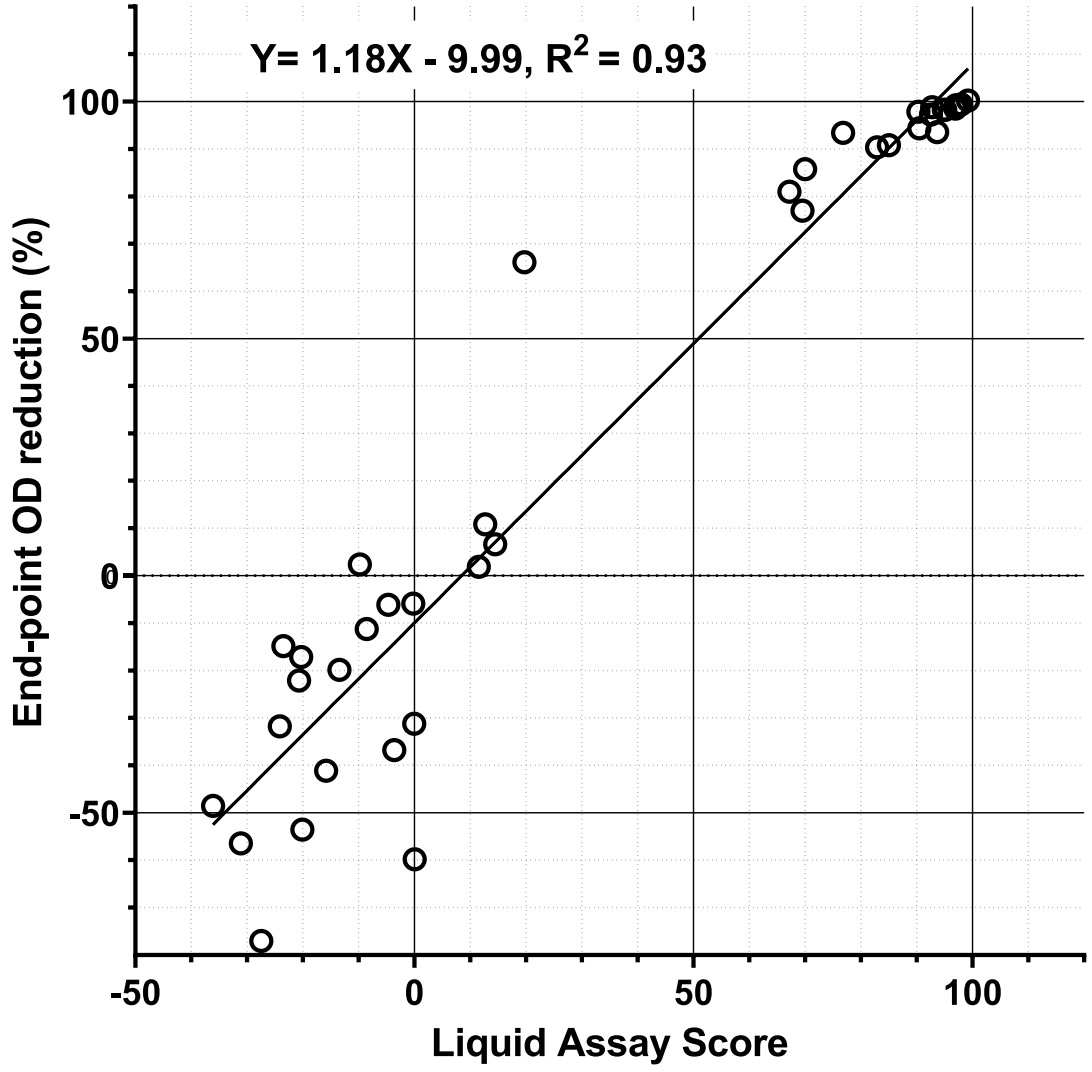


Fig. 4. Correlation between End-point measurement and liquid assay score in growth kinetics assay of phage PBSA08 lytic activity against MRSA reference isolates.

Table 1
Multivariable firth logistic regression model for factors associated with methicillin-resistant *Staphylococcus aureus* susceptibility to phage PBSA08.

Variable	Estimate	Std Error	OR (05%CI)	*P* value
MLST ST93/ST30 (versus others*)	4.119	1.708	61.51 (2.00–2378.53)	0.0191
Ciprofloxacin resistance	2.7367	1.1359	15.44 (1.67–143.03)	0.0160
PVL positivity	−1.7112	1.2388	0.18 (0.02–2.05)	0.1672

Intercept term is omitted from the table for clarity.
P-values were derived from Wald-type tests.
MLST, Multi-Locus Sequence Typing; PVL, Panton-Valentine Leucocidin.
* Including ST1, ST5, ST22, ST45.

[50]. In our EOP-based host range analysis, 51.3% of the MRSA isolates were susceptible to PBSA08. In this context, PBSA08 appears to have potential as a candidate component of therapeutic phage cocktails, particularly for isolates belonging to susceptible lineages.

We have shown the EOP test's performance in assessing phage host range and virulence correlated with the GKA test. Overnight cultures of Gram-negative bacteria including *Escherichia coli* and *Pseudomonas aeruginosa*, may lead to the emergence and regrowth of resistant phage mutants [27]. Determining phage host range based only on assays such as the EOP may increase the risk of false negatives [27]. Therefore, many argue that the susceptibility of phages can be more accurately assessed by observing growth kinetics through liquid culture-based assays, which offer the advantage of continuously monitoring bacterial growth over time compared to spot assays [25,51,52]. In our study, the end-point measurement and the AUC-based measurement in liquid culture did not show significant differences, and the results were consistent with the EOP test for the majority of MRSA tested. Our findings therefore suggest, at least for *S. aureus,* the EOP can reliability determine phage susceptibility and may serve as practical basis for host range assessment.

Phage susceptibilities or host ranges are commonly influenced by differences in bacterial surface receptors, restriction-modification systems or CRISPR-Cas systems that protect against

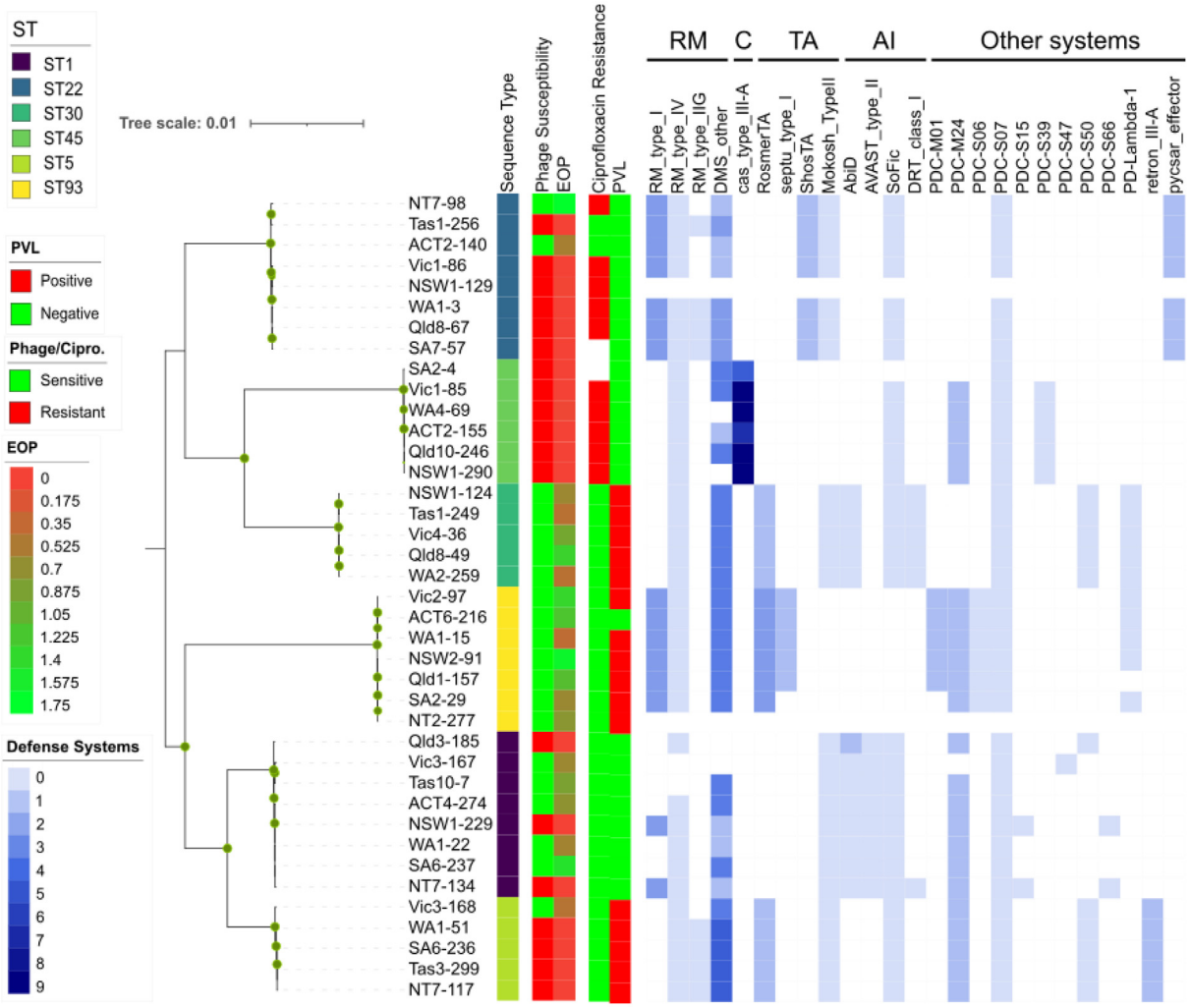


Fig. 5. Phylogenetic analysis of MRSA reference panel and correlation with PBSA08 susceptibility. Maximum-likelihood tree of 39 MRSA isolates (IQTree v3.0.1, Panaroo v1.5.0 core genome alignment). Scale: 0.01 nucleotide substitutions per site. Branches coloured by sequence type (ST). Heatmap shows: (1) Efficiency of plating (EOP) from 0 (white) to 1.75 (dark blue); (2) Phage susceptibility: green = susceptible (EOP $\geq$ 0.1), red = resistant (EOP <0.1); (3) Bacterial defense system gene counts (RM, Restriction-Modification, C, CRISPR-Cas, TA, Toxin-Antitoxin, AI, Abortive Infection), with blue intensity indicating gene count.

phage infection [46,53]. Prior studies have also shown specific *S. aureus* STs are associated with differential phage susceptibility profiles [11,46,54]. Our phylogenetic analysis revealed clear clustering of susceptibility by ST (Fig. 5); however, the distribution of identifiable bacterial defense systems showed no correlation with PBSA08 susceptibility patterns. While these computationally detectable defense mechanisms represent only a subset of possible resistance factors, their lack of association suggests that ST-specific surface modifications, cell-envelop-associated determinants, or other uncharacterised mechanisms may be more important determinants of PBSA08 host range. However, the present study was not designed to identify the mechanistic basis of this lineage-specific susceptibility. Future work should therefore include experimental receptor identification and comparative genomic analysis of bacterial surface structures, in addition to screening for defense systems.

In our study, susceptibility to phage PBSA08 was associated with certain STs, particularly ST93-IV and ST30-IV, which were uniformly susceptible, while ST45-V isolates were consistently resistant. Although ciprofloxacin resistance and PVL-associated gene carriage also appeared to be associated with PBSA08 resistance in univariate analysis, multivariable modelling suggested ST was the dominant corelate of susceptibility. However, the large odds ratios and wide confidence intervals indicate that the effect size estimates were unstable and should be interpreted cautiously. Given the strong overlap among ST, PVL, and antibiotic resistance profiles, the apparent ciprofloxacin association likely reflects lineage confounding rather than an independent biological relationship.

Occasionally reported in Europe and Asia, it is believed ST93-IV originated in Australia, where it is the dominant community-associated MRSA clone [55]. The activity of the Korean-isolated PBSA08 phage against ST93-IV highlights the value of international networking of phage libraries in expanding the repertoire of phages available for therapeutic matching [5,7,56]. These findings suggest that geographically distinct phages may still provide useful activity against clinically important clones from other regions. Such collaboration may improve host-range coverage and support the development of phage therapeutics with relevance across geographical and clonal boundaries [4,6,43].

Our study utilised a panel of isolates collected by AGAR, one of Australia's national antimicrobial resistance surveillance programs [18], to determine the host range of PBSA08. Several studies have reported the host range of phage using various *S. aureus* isolates and methods [12,44,46,57]. In this context, the activity of PBSA08 appears moderate rather than exceptionally broad as host range among therapeutic *S. aureus* phages has been reported to vary substantially across studies [12,44,46,57]. This variability likely reflects

differences in the bacterial isolate collections tested and the susceptibility methods used across studies. This suggests that PBSA08 may be better interpreted not as a stand-alone but as a potentially useful component of lineage-informed or personalised phage cocktails. However, direct comparison of host range between studies is difficult because the tested isolate collections and susceptibility methods are often heterogenous. Standardization is therefore essential for the networking and clinical use of phage, and our study highlights the value of national surveillance programs and standardised reference sets of bacteria that allow the potency and host range of different phages to be compared more directly.

Our study has limitations that should be considered when applying the results. First, the reference panel used in this study consisted only of MRSA isolates. However, it is important to note methicillin-susceptible *S. aureus* (MSSA) is currently the predominant cause of community-onset staphylococcal infections in Australia [58,59]. Therefore, our results may not fully represent the genetic diversity of clinical isolates and could limit the applicability of the findings regarding phage susceptibility and host range. Further studies building on this work should aim to develop a panel of isolates that include MRSA and MSSA in order to elucidate the phage susceptibility and host range across different *S. aureus* clones. Second, although we applied Firth's bias-reduced logistic regression to account for small-sample bias, the relatively limited number of isolates (n = 39) constrained our ability to identify factors independently associated with phage susceptibility. In particular, the small number of isolates in each ST required grouping and contributed to unstable effect size, limiting interpretation at the level of individual predictors. In addition, this study did not identify the mechanistic determinants underlying lineage-specific susceptibility to PBSA08. Third, the efficacy of PBSA08 in phage therapy still needs to be evaluated in clinical settings. Based on our findings, PBSA08 may be most appropriately advanced within a personalised phage therapy framework, such as inclusion in isolate-matched phage combinations, rather than as a single broadly active agent. Clinical evaluation, for example in a setting, such as the *Staphylococcus aureus* Network Adaptive Program (SNAP) trial [60], would help clarify the therapeutic relevance of PBSA08 and its interaction with bacterial and treatment-related factors. In summary, our study characterised a previously undescribed *S. aureus* phage, PBSA08, which demonstrated – *in vitro* lytic activity against a substantial proportion of a representative panel of Australian MRSA isolates. Importantly, the cross-geographic activity of PBSA08—isolated in Korea—against selected Australian MRSA clones underscores the potential value of international collaboration in sharing genomic epidemiology and phage susceptibility data to inform future phage matching across regions.

PBSA08 may represent a useful component of tailored phage combinations for personalised therapy, potentially in combination with antibiotics to enhance bacterial clearance. Future work should expand testing to include MSSA and a broader range of clinical isolates, further elucidate bacterial resistance mechanisms, and evaluate PBSA08 in clinical trials. Such efforts will be essential to translate these laboratory findings into effective therapeutic options against antibiotic-resistant *S. aureus* infections.

Acknowledgements: The authors thank the Phage Australia at Westmead team, specifically Carina Lauter, Biobanking Coordinator, and Dr Jackie Chan, Genomics Coordinator, for their invaluable assistance in sample and genomic pipeline management. They thank the hospitals and patients who contributed *Staphylococcus aureus* isolates for curation within the Australian Group on Antimicrobial Resistance (AGAR) repository. HM received personal support from the HUFS Research Fund (2025), which was not related to the conduct of this study.

Funding: This work was supported by a 2-Year Research Grant of Pusan National University.

Declaration of competing interests: Given their roles as Editors for IJAA, Geoffrey W Coombs, Shakeel Mowlaboccus and Ruby C Y Lin, had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to another journal Editor.

Ethical approval: Not required.

Authors' contributions: SWL, JI, SL contributed to the conception and design of the study. SWL, SL, CF, SM, SOL, SP performed the experiments and carried out the data analysis. NBZ carried out bioinformatics analyses. GWC, SM contributed methicillin-resistant *S. aureus* (MRSA) using the Australian Group on Antimicrobial Resistance (AGAR) repository. HM contributed phage from LyseNTech. JI, RL contributed phage therapy platform (Phage Australia, Westmead). All authors provided critical revision to the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2026.107838.

References

[1] Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, et al. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. The Lancet 2024;404:1199–226.
[2] De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, et al. Antimicrobial resistance in ESKAPE pathogens. Clin Microbiol Rev 2020;33:10–1128.
[3] WHO bacterial priority pathogens list. 2024. https://www.who.int/publications/i/item/9789240093461; access date: 21 January 2023.
[4] Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. Nat Rev Microbiol 2019;17:203–18.
[5] Ferry T, Kolenda C, Laurent F, Leboucher G, Merabischvilli M, Djebara S, et al. Personalized bacteriophage therapy to treat pandrug-resistant spinal *Pseudomonas aeruginosa* infection. Nat Commun 2022;13:4239.
[6] Gilbey T, Ho J, Cooley LA, Petrovic Fabijan A, Iredell JR. Adjunctive bacteriophage therapy for prosthetic valve endocarditis due to *Staphylococcus aureus*. Med J Aust 2019;211 142-3.e1.
[7] Khatami A, Lin RCY, Petrovic-Fabijan A, Alkalay-Oren S, Almuzam S, Britton PN, et al. Bacterial lysis, autophagy and innate immune responses during adjunctive phage therapy in a child. EMBO Mol Med 2021;13:e13936.
[8] Ramirez-Sanchez C, Gonzales F, Buckley M, Biswas B, Henry M, Deschenes MV, et al. Successful treatment of *Staphylococcus aureus* prosthetic joint infection with bacteriophage therapy. Viruses 2021;13:1182.
[9] Rubalskii E, Ruemke S, Salmoukas C, Boyle EC, Warnecke G, Tudorache I, et al. Bacteriophage therapy for critical infections related to cardiothoracic surgery. Antibiotics (Basel) 2020;9:232.
[10] Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, et al. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii* infection. Antimicrob Agents Chemother 2017;61:10–1128.
[11] Botka T, Pantůček R, Mašlaňová I, Benešík M, Petráš P, Růžičková V, et al. Lytic and genomic properties of spontaneous host-range kayvirus mutants prove their suitability for upgrading phage therapeutics against staphylococci. Sci Rep 2019;9:5475.
[12] Vandersteegen K, Mattheus W, Ceyssens PJ, Bilocq F, De Vos D, Pirnay JP, et al. Microbiological and molecular assessment of bacteriophage ISP for the control of *Staphylococcus aureus*. PLoS One 2011;6:e24418.
[13] Gutiérrez D, Vandenheuvel D, Martínez B, Rodríguez A, Lavigne R, García P. Two phages, phiIPLA-RODI and phiIPLA-C1C, Lyse mono- and dual-species staphylococcal biofilms. Appl Environ Microbiol 2015;81:3336–48.
[14] Hsieh SE, Lo HH, Chen ST, Lee MC, Tseng YH. Wide host range and strong lytic activity of *Staphylococcus aureus* lytic phage Stau2. Appl Environ Microbiol 2011;77:756–61.
[15] Sáez Moreno D, Visram Z, Mutti M, Restrepo-Córdoba M, Hartmann S, Kremers AI, et al. ε(2)-phages are naturally bred and have a vastly improved host range in *Staphylococcus aureus* over wild type phages. Pharmaceuticals (Basel) 2021;14:325.
[16] Vandersteegen K, Kropinski AM, Nash JH, Noben JP, Hermans K, Lavigne R. Romulus and Remus, two phage isolates representing a distinct clade within

the Twortlikevirus genus, display suitable properties for phage therapy applications. J Virol 2013;87:3237–47.
[17] Imm S, Bai J, Chang Y. Development of an antibacterial poly(Lactic Acid)/poly(ε-Caprolactone)/tributyl citrate film loaded with *Staphylococcus aureus* bacteriophages using a sodium alginate coating. Int J Mol Sci 2025;26:7793.
[18] Coombs GW, Daley DA, Shoby P, Mowlaboccus S. Australian Group on Antimicrobial Resistance (AGAR) Australian *Staphylococcus aureus* surveillance Outcome Program (ASSOP). Commun Dis Intell (2018) 2022;46.
[19] Gonzalez-Menendez E, Arroyo-Lopez FN, Martinez B, Garcia P, Garrido-Fernandez A, Rodriguez A. Optimizing propagation of *Staphylococcus aureus* infecting bacteriophage vB_SauM-phiIPLA-RODI on *Staphylococcus xylosus* using response surface methodology. Viruses 2018;10:153.
[20] Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages by double agar overlay plaque assay. In: Clokie MRJ, Kropinski AM, editors. Bacteriophages. Totowa, NJ, USA: Humana Press; 2009. p. 69–76.
[21] Rahman M, Kim S, Kim SM, Seol SY, Kim J. Characterization of induced *Staphylococcus aureus* bacteriophage SAP-26 and its anti-biofilm activity with rifampicin. Biofouling 2011;27:1087–93.
[22] Alves DR, Gaudion A, Bean JE, Perez Esteban P, Arnot TC, Harper DR, et al. Combined use of bacteriophage K and a novel bacteriophage to reduce *Staphylococcus aureus* biofilm formation. Appl Environ Microbiol 2014;80:6694–703.
[23] Pajunen M, Kiljunen S, Skurnik M. Bacteriophage phiYeO3-12, specific for Yersinia enterocolitica serotype O:3, is related to coliphages T3 and T7. J Bacteriol 2000;182:5114–20.
[24] Kutter E. Phage host range and efficiency of plating. Methods Mol Biol 2009;501:141–9.
[25] Yerushalmy O, Braunstein R, Alkalay-Oren S, Rimon A, Coppenhagn-Glazer S, Onallah H, et al. Towards standardization of phage susceptibility testing: the Israeli phage therapy center "clinical phage microbiology"-a pipeline proposal. Clin Infect Dis 2023;77:S337–SS51.
[26] Young MJ, Hall LML, Merabishvilli M, Pirnay JP, Clark JR, Jones JD. Phage therapy for diabetic foot infection: a case series. Clin Ther 2023;45:797–801.
[27] Xie Y, Wahab L, Gill JJ. Development and validation of a microtiter plate-based assay for determination of bacteriophage host range and virulence. Viruses 2018;10:189.
[28] Andrews S. Quality control tool for high throughput sequence data. 2010. https://www.scirp.org/reference/referencespapers?referenceid=4024153; access date: 23 January 2023.
[29] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.
[30] Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. Genome Biol 2019;20:257.
[31] Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 2015;25:1043–55.
[32] Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 2017;13:e1005595.
[33] Schwengers O, Jelonek L, Dieckmann MA, Beyvers S, Blom J, Goesmann A. Bakta: rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. Microb Genom 2021;7:685.
[34] Sherry NL, Horan KA, Ballard SA, Gonçalves da Silva A, Gorrie CL, Schultz MB, et al. An ISO-certified genomics workflow for identification and surveillance of antimicrobial resistance. Nat Commun 2023;14:60.
[35] Payne LJ, Todeschini TC, Wu Y, Perry BJ, Ronson CW, Fineran PC, et al. Identification and classification of antiviral defence systems in bacteria and archaea with PADLOC reveals new system types. Nucleic Acids Res 2021;49:10868–10878.
[36] Tonkin-Hill G, MacAlasdair N, Ruis C, Weimann A, Horesh G, Lees JA, et al. Producing polished prokaryotic pangenomes with the Panaroo pipeline. Genome Biol 2020;21:180.
[37] Thomas KF, Wong NLT, Huaiyan R, Hector B, Andrew JR, Edward S, et al. IQ-TREE 3: phylogenomic inference software using complex evolutionary models. Mol Biol Evol 2026;43(5):117.
[38] Letunic I, Bork P. Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res 2021;49:W293–W2w6.
[39] Bouras G, Nepal R, Houtak G, Psaltis AJ, Wormald PJ, Vreugde S. Pharokka: a fast scalable bacteriophage annotation tool. Bioinformatics 2023;39:776.
[40] Gilchrist CLM, Chooi YH. Clinker & clustermap.Js: automatic generation of gene cluster comparison figures. Bioinformatics 2021;37:2473–5.
[41] Ackermann HW. Phage classification and characterization. Methods Mol Biol 2009;501:127–40.
[42] Lowy FD. *Staphylococcus aureus* infections. N Engl J Med 1998;339:520–32.
[43] Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 2015;28:603–61.
[44] Feng T, Leptihn S, Dong K, Loh B, Zhang Y, Stefan MI, et al. JD419, a *Staphylococcus aureus* phage with a unique morphology and broad host range. Front Microbiol 2021;12:602902.
[45] Iwano H, Inoue Y, Takasago T, Kobayashi H, Furusawa T, Taniguchi K, et al. Bacteriophage ΦSA012 has a broad host range against *Staphylococcus aureus* and effective lytic capacity in a mouse mastitis model. Biology (Basel) 2018;7:8.
[46] Moller AG, Winston K, Ji S, Wang J, Hargita Davis MN, Solís-Lemus CR, et al. Genes influencing phage host range in *Staphylococcus aureus* on a species-wide scale. mSphere 2021;6:10–1128.
[47] O'Flaherty S, Ross RP, Meaney W, Fitzgerald GF, Elbreki MF, Coffey A. Potential of the polyvalent anti-*Staphylococcus* bacteriophage K for control of antibiotic-resistant staphylococci from hospitals. Appl Environ Microbiol 2005;71:1836–42.
[48] Synnott AJ, Kuang Y, Kurimoto M, Yamamichi K, Iwano H, Tanji Y. Isolation from sewage influent and characterization of novel *Staphylococcus aureus* bacteriophages with wide host ranges and potent lytic capabilities. Appl Environ Microbiol 2009;75:4483–90.
[49] Wen H, Zhou W, Wu Y, Li Y, Zhu G, Zhang Z, et al. Effective treatment of a broad-host-range lytic phage SapYZU15 in eliminating *Staphylococcus aureus* from subcutaneous infection. Microbiol Res 2023;276:127484.
[50] 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;10:e0118557.
[51] Daubie V, Chalhoub H, Blasdel B, Dahma H, Merabishvili M, Glonti T, et al. Determination of phage susceptibility as a clinical diagnostic tool: a routine perspective. Front Cell Infect Microbiol 2022;12:1000721.
[52] Gelman D, Yerushalmy O, Alkalay-Oren S, Rakov C, Ben-Porat S, Khalifa L, et al. Clinical phage microbiology: a suggested framework and recommendations for the in-vitro matching steps of phage therapy. Lancet Microbe 2021;2:e555–ee63.
[53] Egido JE, Costa AR, Aparicio-Maldonado C, Haas PJ, Brouns SJJ. Mechanisms and clinical importance of bacteriophage resistance. FEMS Microbiol Rev 2022;46:48.
[54] Cui Z, Feng T, Gu F, Li Q, Dong K, Zhang Y, et al. Characterization and complete genome of the virulent Myoviridae phage JD007 active against a variety of *Staphylococcus aureus* isolates from different hospitals in Shanghai, China. Virol J 2017;14:26.
[55] van Hal SJ, Steinig EJ, Andersson P, Holden MTG, Harris SR, Nimmo GR, et al. Global scale dissemination of ST93: a divergent *Staphylococcus aureus* epidemic lineage that has recently emerged from remote Northern Australia. Front Microbiol 2018;9:1453.
[56] Pirnay JP, Djebara S, Steurs G, Griselain J, Cochez C, De Soir S, et al. Personalized bacteriophage therapy outcomes for 100 consecutive cases: a multicentre, multinational, retrospective observational study. Nat Microbiol 2024;9:1434–53.
[57] Ajuebor J, Buttimer C, Arroyo-Moreno S, Chanishvili N, Gabriel EM, O'Mahony J, et al. Comparison of *Staphylococcus* phage K with close phage relatives commonly employed in phage therapeutics. Antibiotics (Basel) 2018;7:37.
[58] Coombs GW, Daly DA, Pearson JC, Nimmo GR, Collignon PJ, McLaws ML, et al. Community-onset *Staphylococcus aureus* Surveillance Programme annual report, 2012. Commun Dis Intell Q Rep 2014;38:E59–69.
[59] Macmorran E, Harch S, Athan E, Lane S, Tong S, Crawford L, et al. The rise of methicillin resistant *Staphylococcus aureus*: now the dominant cause of skin and soft tissue infection in Central Australia. Epidemiol Infect 2017;145:2817–26.
[60] Tong SYC, Mora J, Bowen AC, Cheng MP, Daneman N, Goodman AL, et al. The *Staphylococcus aureus* Network Adaptive Platform Trial Protocol: new tools for an old foe. Clin Infect Dis 2022;75:2027–34.