ORIGINAL ARTICLE

Could bacteriophages isolated from the sewage be the solution to methicillin-resistant *Staphylococcus aureus*?

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ABSTRACT

Introductions: The emergence of multidrug-resistant bacteria such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) complicates the treatment of the simplest infection. Although glycopeptides such as vancomycin still proves to be effective in treating MRSA infections, the emergence of vancomycin-resistant strains limits the long term use of this antibiotic. Bacteriophages are ubiquitous bacterial viruses which is capable of infecting and killing bacteria including its antibiotic-resistant strains. Bactericidal bacteriophages use mechanisms that is distinct from antibiotics and is not affected by the antibiotic-resistant phenotypes.

Objectives: The study was undertaken to evaluate the possibility to isolate bacteriolytic bacteriophages against *S.aureus* from raw sewage water and examine their efficacy as antimicrobial agents *in vitro*.

Methods: Bacteriophages were isolated from the raw sewage using the agar overlay method. Isolated bacteriophages were plaque purified to obtain homogenous bacteriophage isolates. The host range of the bacteriophages was determined using the spot test assay against the 25 MRSA and 36 MSSA isolates obtained from the Sarawak General Hospital. *Staphylococcus saprophyticus, Staphylococcus sciuri* and *Staphylococcus xylosus* were included as non-SA controls. The identity of the bacteriophages was identified via Transmission Electron Microscopy and genomic size analysis. Their stability at different pH and temperature were elucidated.

Results: A total of 10 lytic bacteriophages infecting S.aureus were isolated and two of them namely ΦNUSA-1 and ΦNUSA-10 from the family of *Myoviridae* and *Siphoviridae* respectively exhibited exceptionally broad host range against >80% of MRSA and MSSA tested. Both bacteriophages were specific to *S.aureus* and stable at both physiologic pH and temperature.

Conclusion: This study demonstrated the abundance of S.aureus specific bacteriophages in raw sewage. Their high virulence against both MSSA and MRSA is an excellent antimicrobial characteristic which can be exploited for bacteriophage therapy against MRSA.

KEY WORDS:
Staphylococus aureus, bacteriophages, Methicillin-resistant

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium linked to a multitude of diseases in both humans and animals.[1] S.aureus is known to cause mild diseases to life-threatening diseases. *S.aureus* is known to be present as a commensal in human and animals such as livestock, wildlife and domestic animals with human-to-animal, animal-to-animal transmission or vice versa and these have been reported and validated via molecular genotyping.[2-10]

Staphylococcal infections can be treated with beta-lactam antibiotics until the emergence of the Methicillin-resistant *S.aureus* (MRSA) in 1960, less than a year after the introduction of the second-generation beta-lactam antibiotic.[11] Since 1999, nine antibiotics against MRSA have been approved namely linezolid, daptomycin, tigecycline, ceftobiprole, telavancin, ceftaroline, dalbavancin, oritavancin and tedizolid but only oxazolidinones belongs to a new class of antibiotics.[12,13] Human is at risk to fall back into the pre-antibiotics era without the discovery of new classes of antibiotics.

Currently, the antibiotics of choice for MRSA infection are glycopeptides such as vancomycin but the emergence rate of vancomycin-resistant and vancomycin-intermediate S.aureus (VRSA and VISA) has been increasing in various parts of the world.[14] Neither VRSA nor VISA has been reported in Malaysia to date but Vancomycin 'MIC creep' phenomenon has been observed since the last decade[15] suggesting the continuous selective pressure for the emergence of Vancomycin-resistant strains. Nevertheless, the nephrotoxic nature of glycopeptides and other adverse side effects limit its use in clinical settings.[1,16]

As a solution to MRSA, we envisaged to revisit the potential use of bacteriophages, viruses that infect and kill susceptible bacterial host including the multidrug-resistant variants. Bacteriophages are known to be ubiquitous in nature, especially where the host can be found but some bacteriophages are globally distributed while the rest be geographically unique.[17-19] In the absence of commercially

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