Journal of
Medical Microbiology & Diagnosis

Mohammed-Ali and Jamalludeen, J Med Microb Diagn 2015, 5:1
DOI: 10.4172/2161-0703.1000213

Research Article Open Access

Isolation and Characterization of Bacteriophage against Methicillin Resistant *Staphylococcus aureus*

Mariem N Mohammed-Ali[1]* and Nidham M. Jamalludeen[1]

Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq

Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major human pathogen responsible for several life threatening conditions. MRSA have the ability to acquire resistance to several antimicrobial agents and phage therapy is one potential option to treat this pathogen. The aim of the study was to isolate and characterize bacteriophages effective against a wide range of methicillin-resistant *Staphylococcus aureus* (MRSA). A mixture of ten MRSA isolates was used for the isolation of phage from wastewater treatment plants. Three phages were selected for further characterization. All three phages belong to the Siphoviridae family and have long non-contractile flexible tails. The three phages showed a wide host range against *S. aureus*. Phages ϕSA1 and ϕSA2 were resistant to a pH range from 4-10 while ϕSA3 has a pH range from 3-11. DNA from all three phages was resistant to digestion by endonuclease enzymes such as EcoRI and AccI. There was a high degree of mosaicism among the three virulent phages and with their ancestor phages of Siphoviridae due to their non-uniform access to the common genetic pool by horizontal gene transfer and recombination. Since some of the staphylococcal toxins are phage encoded, the presence of genes for such toxins was tested by performing polymerase chain reaction and all three phages lacked genes for any of the staphylococcal toxins, including staphylococcal enterotoxins (sea, seb, sec and see), exfoliating toxins (eta and etb) and the toxic shock syndrome toxin (tst), therefore these bacteriophage are suitable candidates for future use in phage therapy against MRSA.

Keywords: *Staphylococcus aureus*; Phage therapy; MRSA phage; Siphoviridae; Anti-restriction mechanism; Mosaicism

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a worldwide pathogen that is responsible for a variety of diseases ranging from soft tissue and skin infections to life threatening conditions such as pneumonia, bacteremia and sepsis [1]. MRSA is one of the major human pathogens that may cause community and hospital acquired infections [2]. These organisms are frequently resistant to most of the commonly used antimicrobial agents, including β-lactam antibiotics [3]. The emergence and spread of strains resistant to oxacillin, methicillin and even vancomycin has made therapy of these multi drug resistant bacteria a global challenge [4]. MRSA has a wide variety of virulence factors that include structural and secreted factors [5]. These factors include superantigens, cytolytic toxins, exoenzymes and miscellaneous proteins [6]. Superantigens are a group of powerful immuno-stimulatory proteins implicated in a variety of human diseases including gastroenteritis and toxic shock syndrome [7].

One possible approach to treatment of methicillin resistant *S. aureus* is phage therapy, defined as the application of phage to selectively reduce or eliminate susceptible pathogens from specific environments [8]. Phage therapy may be a suitable alternative to antibiotic treatment due to the high specificity of and effectiveness against multi drug resistant bacteria [9,10]. The use of bacteriophages in clinical medicine was first introduced by Félix d'Herelle [11]. Many therapeutic phages have been isolated against MRSA, most of which belong to the Myoviridae family such as the well-known phage K and MR-10 [12,13]. Since all tailed phages are believed to share common ancestors, a high frequency of chimeric and mosaic structures can be observed among different tailed phage families due to their access to a common genetic pool [14,15]. Such mosaicism has resulted in a high degree of similarity among phages in both nucleic acids and proteins [16]. The main aims of this study were to isolate a set of bacteriophages effective against a wide range of MRSA isolates and characterize these phages according to their morphological features, host range, endonuclease enzyme digestion pattern, molecular identification, the presence of undesirable toxin encoding genes.

Materials and Methods

Culture media and chemicals

The following media and chemicals were used for the study: brain heart infusion agar (Salucea, Netherlands), brain heart infusion broth (HIMEDIA, India), blood agar (HIMEDIA, India), mannitol salt agar (OXOID, England), Mueller Hinton agar (HIMEDIA, India), Agar agar (HIMEDIA, India), agarose (Bio Basic, Canada), peptone (DIFCO, USA), beef extract (OXOID, England), yeast extract (Sigma-Aldrich, Switzerland), sodium chloride (Sigma-Aldrich, Switzerland), sodium hydroxide (Sigma-Aldrich, Switzerland), potassium dihydrogen phosphate (Sigma-Aldrich, Switzerland), Gelatin (BDH, England), barium chloride (Hopkins and Williams Limited, England), Gram stain (HIMEDIA, India), catalase test reagent (HIMEDIA, India), coagulase plasma (HIMEDIA, India), API Staph (bioMérieux, France), EcoRI (promega, USA), AccI (BioLabs, New England), sulfuric acid (BDH, England), hydrochloric acid (BDH, England), tris-(hydroxymethyl)-aminomethane (pH 7.5) (Riedel-deHaën, Germany), Chloroform (Sigma-Aldrich, Switzerland), glycerol (Chem-supply, Australia), absolute ethanol (BDH, England), nuclease free water (Promega, USA),

***Corresponding author:** Mariem N. Mohammed-Ali, Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq, Tel: 964 1886 8520; E-mail: mary.wadi91@gmail.com

Received October 30, 2015; **Accepted** December 28, 2015; **Published** December 30, 2015

Citation: Mohammed-Ali MN, Jamalludeen NM (2015) Isolation and Characterization of Bacteriophage against Methicillin Resistant *Staphylococcus aureus*. J Med Microb Diagn 5: 213. doi:10.4172/2161-0703.1000213