ORIGINAL RESEARCH PAPER

Biochemistry

Volume - 7 | Issue - 2 | February - 2017 | ISSN - 2249-555X | IF : 3.919 | IC Value : 79.96

Induction of lysogenic phages from nosocomial infection causing Methicillin Resistant Staphylococcus Aureus (MRSA) in Saudi Arabia

KEYWORDS: Bacteriophages, MRSA, RFLP, nosocomial infections

Archana P.Iyer
Department of Biochemistry, King Abdulaziz University, Jeddah, Saudi Arabia.

Mohamed Nabil Alama
Consultant cardiologist, King Abdulaziz University hospital, Jeddah, Saudi Arabia.

ABSTRACT *Methicillin Resistant Staphylococcus Aureus (MRSA) are a major cause of nosocomial infections worldwide. MRSA are often multidrug resistant and hence are difficult to control. Bacteriophages are attractive as therapeutic agents because they are safe for humans and highly specific and lethal to the bacteria they target. Phage therapy is currently practiced routinely and successfully in some countries. The aim of this study was to screen MRSA strains obtained from health care workers from various units of the hospitals for presence of bacteriophages. The MRSA strains were induced with mitomycin C to obtain the bacteriophages. The phage lysates were then used for host range detection. Amplified titres of the phages were used to extract genomic DNA and subjected to Restriction Fragment Length Polymorphism (RFLP). We found two different phages which we named EBU1 and EBU2 to be effective in lysing the MRSA strains. Further characterization of the phages will help in designing phage therapy strategies.*

Introduction

Nosocomial infections are hospital acquired and caused by bacteria and or other microorganisms. The common definition of nosocomial infection is, an infection which occurs within 48 hours after hospitalization, or after 3 days from discharging, or 30 days from an operation. According to studies, the nosocomial infections are found mainly in intensive care units (ICU) compared other units of the hospital[1]. They may be endogenous, arising from an infectious agent present within a patient's body, or exogenous, transmitted from other sources within the hospital. MRSA cause superficial skin lesions such as boils and more serious infections such as pneumonia, phlebitis, meningitis, mastitis, and urinary tract infections, as well as deep-seated infections[2]. There have been increased reports of MRSA causing severe nosocomial infections in Saudi Arabia in the recent years and control measures are being strictly followed to prevent dissemination of the pathogen [3]. Moreover, many of these strains are highly resistant to a wide range of antibiotics making it very difficult to curb such pathogens. There is relatively little information on the diversity of strains causing infection. It is important to have knowledge of the most common strains associated with human infections and their sources in each episodes and environment in order to improve our understanding of the epidemiology of this pathogen and solve the problems.

Bacteriophages are natural killers of specific bacteria. Bacteriophages are highly specific to their hosts and hence can be used selectively to kill the pathogens Interest in phage therapy has especially grown over the past decade due to the rapid emergence of antibiotic resistance in bacterial pathogens[4]. Phage therapy is currently practiced routinely and successfully in countries such as Poland and Russia[5]. The recent approval of commercial phage preparations by the United States Food and Drug Administration to prevent bacterial contamination of meat and poultry [6] may pave the way for the global use of phage therapy to control bacteria in human infections. The development of phages for therapy has been hampered by concerns over the potential for immune response, rapid toxin release by the lytic action of phages, and difficulty of dose determination in clinical situations [7]. Phages multiply logarithmically in infected bacterial cells, and the release of progeny phage occurs by lysis of the infected cell at the end of the infection cycle, which involves the holin-endolysin system. An undesirable side effect of this phenomenon from a therapeutic perspective is the development of immunogenic reactions due to large uncontrolled amounts of phages in circulation [8]. Such concerns must be addressed before phage therapy can be widely accepted [9].

Many MRSA strains possess lysogenic prophages which on induction and propagation can be used to kill the respective hosts. The principal objective of this study was to induce clinical MRSA isolates from hospitals in Jeddah Saudi Arabia and screen for presence of lytic phages that can be used to control these infectious pathogens.

Materials and methods:

Sample collection: Samples were collected from hospital workers including doctors, nurses, and technicians. The healthcare workers, a total of 100 were from different hospitals that had various sectors such as outpatient departments, intensive care units, burn units, pediatric units, and maternity units. Samples from healthcare workers from only three units – the outpatient departments, intensive care units, and burn units – were collected. Of the 100 healthcare workers, 65 were men and 35 were women, all of different nationalities. All volunteers signed a consent form and ethical approval was obtained for the study. Swabs were taken from both the anterior nares using sterile swabs moistened with saline.

Isolation and identification of MRSA

Each swab was immediately placed in an enrichment broth, processed in the microbiology laboratory on the same day of sampling, and incubated at 35.8ºC overnight. Each 10 µL of incubated enrichment broth was inoculated in mannitol salt agar (HiMedia Labs, Mumbai, India), which is selective for S. aureus, and incubated at 35.8ºC between 24 and 48 hours; yellow colonies were selected and confirmed to be S. aureus following catalase, coagulase, and DNAse tests (10), and were finally confirmed by PCR using specific primers for mec A gene: FP 5 AAAATCGATGGTAAAGGTTGGC 3' and RP 5 AGTTCTGCAGTACCGGATTTGC 3'. Conditions for PCR were as followed by Iyer et al., 2014. Testing for methicillin resistance was also performed as detailed by Iyer et al., 2014. MRSA ATCC strain 700699 was used as a positive control in all the experiments.

Induction of phages from clinical MRSA strains

Phages were induced from early log phase (A580 c. 0.1) cultures grown at 37°C in Trypticase Soy Broth (TSB; BBL) by adding mitomycin C 2 pg/ml. Phage was titrated by spotting 10 ul of serially-diluted filtered lysate on to agar overlays containing the clinical isolates of MRSA. Phages induced in 3-ml cultures were used in the determination of host range. Phage from 300-ml cultures were purified on CsCl gradients and the DNA extracted [11].

Phage propagation and host range of induced phages

Phages induced from 3-ml cultures were filtered (0.22 pm membrane; Millipore) before infection of 300- ml cultures of S. aureus propagating strains. Infections were performed at a multiplicity of infection of c. 0.1 as described previously [12]. Dilutions of lysates from mitomycin C-treated cultures were plated on to ten strains of MRSA clinical isolates obtained from the healthcare workers.

Volume - 7 | Issue - 2 | February - 2017 | ISSN - 2249-555X | IF : 3.919 | IC Value : 79.96

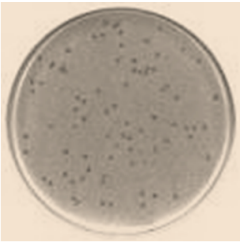
Restriction digestion of phage DNA

DNA was digested for two hours at 37°C with enzymes EcoRI and HindIII (New England biolabs). Digested DNA was electrophoresed at room temperature for two hours at 80 V in 1% agarose gel containing ethidium bromide 0.5 pg/ml. Gels were stained further with ethidium bromide 2 pg/ml and observed under an ultraviolet transilluminator. Lambda DNA EcoRI/HindIII DNA marker was used to estimate fragment sizes

Results

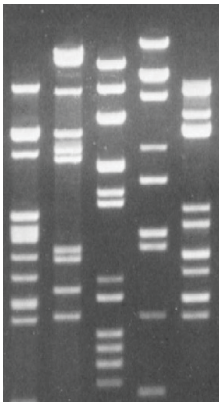
Of the ten MRSA isolates (designated as MRSA1 to MRSA10) induced with mitomycin C, only two of them yielded phages which we designated EBU1 and EBU2 (Figure 1).

Figure 1: Plaques of induced phages on MRSA clinical isolates



EBU 1 amplified to low titres of 104 to 105 pfu/ml on MRSA strains 3,5,9 and 10 while it showed high titres of up to 109 pfu/ml of MRSA strains 1,2,4,6,7 and 8. On the other hand, EBU2 phage amplified to high titres of 109 pfu/ml on MRSA strains 1,2,3,4,6,8 and 10 and showed low titres of 104 pfu/ml on strains 5,7 and 9. Interestingly, phage EBU2 amplified to high titres of 109 pfu/ml on the control MRSA ATCC strain 700699, while phage EBU1 attained only 103 pfu/ml on this strain. RFLP of phages EBU1 and 2 with EcoRI and HindIII enzymes showed different patterns indicating that they were different phages as shown in Figure 2. These preliminary studies indicate that EBU2 is a potent phage capable of lysing MRSA more effectively.

Figure 2: Restriction digestion of phage DNA



Lane 1 : DNA marker
Lane 2: EBU1 DNA digested with EcoRI
Lane 3: EBU1 DNA digested with HindIII
Lane 4: EBU2 DNA digested with EcoRI
Lane 5: EBU2 DNA digested with HindIII

Discussion

Multidrug-resistant bacteria particularly MRSA is well known as a worldwide problem. Since the rate of development of novel antimicrobial agents has been slowed down during the last years, there is an urgent need for the exploration of alternative solutions for the treatment of resistant bacterial infections. Phage therapy seems to be the ideal choice under these circumstances. According to Gorski et al., 2009 [11], bacteriophages are the latest innovative therapy against multidrug resistant bacteria. Our study is a preliminary study in which we have attempted to induce phages from clinical MRSA isolates from local hospitals in Jeddah. We have been successful in inducing phages from only two strains with mitomycin C. The recovery of two phages with the ability to lyse all the ten strains and propagate to high titres is very encouraging to extend this study to a large number of clinical isolates. Many studies around the world have been carried out where phages have been found effective against MRSA strains. A study by Sahin et al., 2013 [12], found that a phage designated as f LizAnk isolated from MRSA exhibited strong antibacterial activity against MRSA strains and no cytotoxic effect was detected against mammalian cells and hence it might be safely used alone or in a phage cocktail to treat skin infection caused by MRSA.

Recently, another approach has been developed known as phage cocktail treatment wherein a mixture of different phages can be used more effectively to lyse pathogenic multidrug resistant bacteria. A study by Gu et a., 2012 [13] on pathogenic *Klebsiella* control using phage cocktail showed that phage cocktail was more effective in reducing bacterial mutation frequency and in the rescue of murine bacteremia than monophage suggesting that phage cocktail has great therapeutic potential for multidrug-resistant bacteria infection.

In our study we found that phage EBU2 is capable of lysing the ten MRSA clinical isolates. This preliminary screening has given us encouraging results and we propose to extend this screening and induction of phages to a larger extent of clinical isolates. This would help us not only in successfully isolating potent phages but would also lead to preparation of phage cocktails that could have increased host range and more potent lysis effect on these pathogens.

References

1. Iyer A, Kumosani T, Azhar E, Barbour E and Harakeh S. (2014). High incidence rate of methicillin-resistant Staphylococcus aureus (MRSA) among healthcare workers in Saudi Arabia. J.Infect.Dev.Ctries. 8(3): 372-378
2. Inweregbu K, Dave J, Pittard A (2013) Nosocomial infections. Continuing Education in Anaesthesia Critical Care & Pain 5: 14-17.
3. Iyer AP, Baghallab I, Albaik M, Kumosani T (2014) Nosocomial Infections in Saudi Arabia Caused by Methicillin Resistance Staphylococcus aureus (MRSA). Clin Microbial 3: 146.
4. Soothill JS, Hawkins C, Anggard EA, Harper DR (2004). Therapeutic use of bacteriophages. Lancet Infectious Diseases, 4:544-545
5. Lang L(2006). FDA approves use of bacteriophages to be added to meat and poultry products. Gastroenterology, 131:1370-1372.
6. William Summers C (2001). Bacteriophage therapy. Annu Rev Microbiol, 55:437-451.
7. Young RJ (2002). Bacteriophage holins: deadly diversity. Mol Microbiol Biotechnol, 4:21-36.
8. Projan S (2004). Phage-inspired antibiotics? Nat Biotechnol, 22:185-91.
9. Padmanabhan S, Sriram B, Sagar P, Shashikala V, Ramachandran J: Insertional inactivation of the T4 lysozyme gene: Model for absolute lysis-defectives in phage therapy. (2004) ASM Conference on the New Phage Biology: the 'Phage Summit':1-5 Key Biscayne, Florida, USA .
10. Gorski A, Miedzybrodzki R, Borysowski J. (2009). Bacteriophage therapy for the treatment of infections. Curr Opin Investig Drugs 10(8): 766-74.
11. Sahin F, Karasartova D, Ozsan TM, Gerçeker D, Kıyan M. (2013). Identification of a novel lytic bacteriophage obtained from clinical MRSA isolates and evaluation of its antibacterial activity. Mikrobiyol Bul. 47(1):27-34.
12. Stewart PR, Waldron HG, Lee JS, Matthews PR (1985) Molecular relationships among serogroup B bacteriophages of Staphylococcus aureues. J.Virol. 55:111-116.
13. Gu J, Liu X, Li Y (2012). A method for generation phage cocktail with great therapeutic potential. PLoS One, 7(3): e31698.