Available online on www.ijpqa.com
International Journal of Pharmaceutical Quality Assurance 2019; 10(1); 84-89
doi: 10.25258/ijpqa.10.1.14
ISSN 0975 9506

Research Article

Isolation of Lytic *Acinetobacter baumannii* Phage vB_Acib_C_A10 from Iraq pond waters and Comparing Its Antibacterial Effect with Cefotaxime Antibiotic

Zahraa Falah Azeez*, Wathiq Abbas Hatite Al-Daraghi

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.

Received: 5th Jan, 19; Revised: 10th Feb, 19, Accepted: 25st Feb, 19; Available Online: 25th Mar, 2019

ABSTRACT

Bacteriophages are viruses that attack bacteria and lead to their lysis in an efficient and highly specific manner. These phages could be an ideal option for microbial control. These natural enemies of bacteria were used as therapeutic agents before the advent of antibiotics. Currently, with the rapid spread of multidrug resistant bacteria, phage therapy can be an effective alternative treatment for antibiotic resistant bacteria. This study evaluated the effectiveness of bacteriophages in removing Cefotaxime-resistant clinical *Acinetobacter baumannii* strains (CTX_RAB) in vitro. Our *A. baumannii* strains were isolated and identified by standard and genetic methods. The antibiogram resistant was ascertained using phenotypic and genotypic method for cefotaxime antibiotics. The bacteriophages were isolated from environmental water samples. They were exposed to the host bacteria by the double-layer agar technique (DLA) to observe plaques. Cross reaction of the phages on test *A.baumannii* strains was performed to determine broader-spectrum phages. We successfully isolated Bacteriophage vB_Acib_C_A10 (ϕ Acib_A10) active against clinical strains of CTX_RAB by enrichichment from activated pond water samlies using representatives of those strains. Purified bacteriophage suspensions obtained were tested on a range of clinical isolates that included representatives of multiple strains of each of the international clonal lineages, as well as minor and sporadic strains. An effective bacteriophage was isolated for each strain. Examination by transmission electron microscopy revealed bacteriophage of the Corticoviridae family. The cross-reaction showed phages which affect more than six *A.baumannii* strains. They can be a good choice for clinical therapeutic use. Conclusions: According to the results, six strains were resistant to all concentration of cefotaxime antibiotics. However, for each of these resistant bacteria one bacteriophage was isolated from environmental samples, which showed the effectiveness of Effective bacteriophages to remove clinically resistant *A. baumannii* in vitro.

Keywords: Phage (vB_Acib_C_A10), Cefotaxime Antibiotic, *Acinetobacter baumannii*, Burn infection, Iraq.

INTRODUCTION

Therapeutics bacteriophage has been considered as a promising alternative to antibiotics since they present several benefits over chemotherapy for microbial control[1]. Phage therapy refers to the utilization of bacteriophages (phages or viruses infecting bacteria) to treat bacterial diseases. These include their high host specificity indicating that they are harmless to the natural macrobiotic[2]. Given the increasing number of drug-resistant bacterial infections, especially within hospital settings, the exploration of alternatives to conventional antibiotics has become an important research objective[3]. Bacteriophages are very abundant. Yet, the discovery of broadly effective antibiotics led to the demise of the development of phage therapy in western countries and only as the antibiotics are starting to fail there has been a serious attempt to restore the old tool[4]. However, the second coming of phage therapy faces challenges regarding to the strict regulatory guidelines and the development of effective therapeutic practices (Yet, phage therapy can provide an evolutionarily sustainable alternative to conventional antibiotics, should we be able to adjust our regulations and procedures to meet the special requirements of phage based medicine[5]. It is important to note that phages infect bacterial hosts very selectively. Often, the narrow host-range is considered as an advantage over traditional antibiotics since phage treatment can focus accurately on the pathogen without harming commensal bacterial flora[6].

On the other hand, the epidemic potential and the clinical severity of *Acinetobacter baumannii* infections are primarily related to the ability to survive and spread within hospital environment and to develop resistance to a variety of antimicrobial agents, including broad-spectrum beta-lactams, fluoroquinolones, aminoglycosides, and carbapenems that has become a major nosocomial pathogen due to its multidrug resistance[7]. *A.baumannii* strains have been isolated which are resistant to almost all antibiotics, including a high prevalence of resistance to β- lactamase which has been reported worldwide since the 1990's[8]. Today generally, with dissemination of multi-drug resistant (MDR)

*Author for Correspondence: azz.zarha@yahoo.com

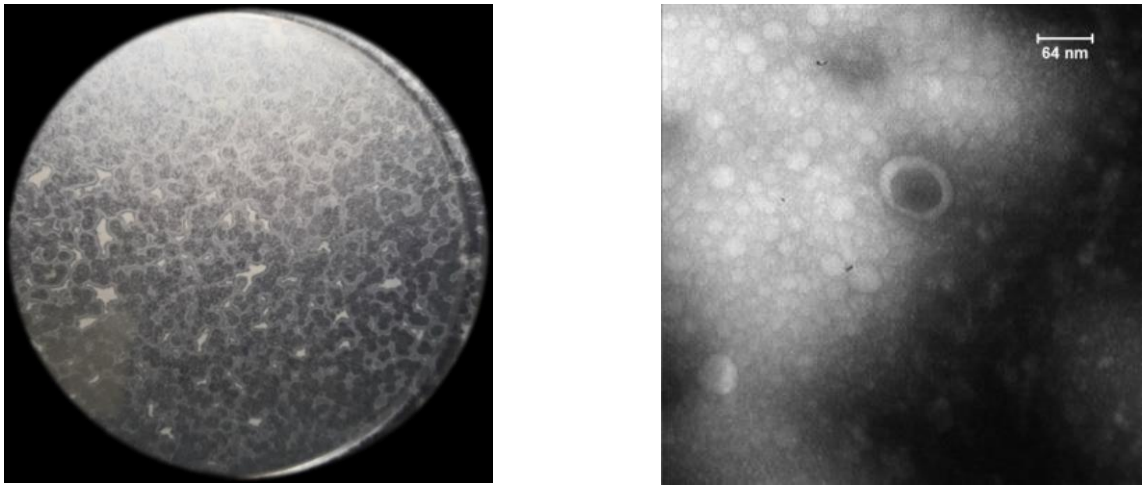

Figure 1: Plaque Formation from left and electron micrographs of *A. baumannii* phage ϕ Acib_A10 from right. Phage suspension was loaded onto a copper grid, stained with 2% uranyl acetate and observed with transmission electron microscopy. The phage particles (the arrows indicated) showed a hexagonal head about 59 nm in diameter that lacks a neck and tail.

bacteria we need to find new remedies to overcome MDR pathogens[9]. Developing new antibiotics with new modes of action is critical in the battle against antibiotic-resistant bacteria, yet this solution has had a slow and expensive pathway over the past years[10,11]. To the best of our knowledge, the probability of finding therapeutically useful phages against different resistant pathogens on-demand has not been studied per se despite the fact that it is likely to be the limiting factor in attempts to update premade cocktails or to generate on-demand personalized therapies[12]. As an example, hospital acquired wound infections have been suggested to be especially suitable target for phage therapy as the causative agents are generally resistant to various antibiotics. The primary aim of this study was to evaluate effective alternative antibiotics in treatment of cefotaxime-resistant *A. baumannii* strains isolated from different units at burn and plastic surgery Centre (BPSC) include burn ICU (BICU) patients in Iraq, during 2017-2018. Antibiotic resistance is an emerging global health crisis, resulting from the continuous use of antibiotics in healthcare, farming industry, However, the phage used in that study, vB_Acib_C_A10 (ϕ Acib_A10) was only morphologically characterized. Therefore, a successful phage-based treatment can be dependent on the practicality of being able to simultaneously and rapidly isolate new durable phages against very different pathogens.

MATERIAL AND METHODS

Study design

This prospective study was conducted of Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq, with the burn and plastic surgery Centre (BPSC) include Burn Care Unit in Burn Specialist Hospital of Al Diwaniyah city, Iraq, from January 2017 to September 2018. This study was approved by the Ethical committee of our institution.

Bacteria strains

Eleven non-duplicate *A. baumannii* isolates obtained from performed in the microbiology laboratory from wounds and abscesses swabs, derived from different wards (burn units, paediatric burn, and plastic units) and BICU patients designated as (Ab1 to Ab11) were identified in the BPSC (Al_Diwaniyah city, Iraq) between 2017 and 2018 were selected Table1. Initially, *Acinetobacter* were characterized by phenotypic method[16]. All were verified as *Acinetobacter* by 16S rRNA gene sequencing and later amplified by PCR[17]. Clinical third-generation Cephalosporins–resistant *A.baumannii* phenotypic and genotypic via carrying $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} genes isolation within our previous study[18].

Isolation and enrichment of bacteriophage

Primary effluent samples were collected from the pond water samlies 100 samples of Al-Diwaniyah, Iraq, coded (Pw), and then approximately 50ml aliquot from these samples was centrifuged at 5000 RPM for 10 min to remove large particulate matter, the supernatants were collected in separate tubes and sterilized with a 0.45- m filter (Millipore). The filtered effluent was concentrated to 150 ml by the use of a low-binding affinity 30-kDa concentration filter. A 50 µl aliquot of the concentrate was mixed with 220µl of an overnight liquid culture (20 µl of eleven bacterial of isolates in this study, with concentration 1.5×10^8CFU) and 5 ml of BH Broth (brain, heart broth) was inoculated at 37°C, shaking for24 hours in each tube. After 48h, the least turbid tube was selected for purification, the culture was centrifuged, and the supernatant was used for the detection of lytic bacteriophages by a double-layer method. Phage enrichment and purification were performed as described by Kitti *et al.*[25]. The purification of phage a total 200µl of 10^8 PFU/ml Ab_1 was mixed with 3 ml of BHI 0.6 % agar (50℃), and this mixture was poured onto 2 % solid agar to make double-layer agar plates. 5 min later, after solidification, we spotted a 5µl aliquot of supernatant stock solution on each plate with 17 different clinical *A. baumannii* strains. 12 h later, we observed whether lysis

Table 1: The minimum inhibitory concentrations (MICs) distributions, MIC values and antimicrobial susceptibilities of eleven cefotaxime-resistant *A. baumannii* isolates.

No. Isolates tested (A.N)	≤0.06	0.125	0.25	4	8	16	32	64	≥128	MIC Range	%S	%I	%R
Ab_1-MH071350				4	12	2	18	8	61	≤0.06 - ≥64	45	40	15
Ab_2-MH071349			22	4	12	19	28	8	140	0.125-≥128	30	4	66
Ab_3-MH071348		1		28	87	130	18	12	4	0.25- ≥128	2.5	11	86
Ab_4-MH071347				2	8	65	158	45	4	4- ≥128	0	0.5	99.5
Ab_5-MH071346				12	35	80	120	30	4	2- ≥128	-	-	-
Ab_6-MH071345				2			6	58		≥128	17	0	73
Ab_7-MH071344			5	12	2	16	12	30	150	0.5- ≥128	0	0.5	99.5
Ab_8-MH071343			23	15	1					0.125- 8	-	-	-
Ab_9-MH071342				11	30	66	120	38	4	2- ≥128	-	-	-
Ab_{10}-MH071341			5	12	2	16	12	30	150	0.5- ≥ 128	18	2	80
Ab_{11}-MH071340				66	78	28				≤0.06- 16	65	25	9.8

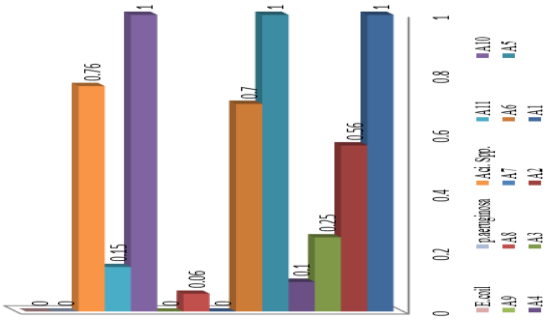

Figure 2: Total number of susceptible strains infected by the phages.

plaques had emerged[19]. The plates were gently swirled and left for 10min at room temperature in order to fix upper agar and then inverted and incubated at 37°C overnight. Then the plaque was cut by a scalpel and put in a tube containing BHI broth and stored in 4 °Cuntil next steps. A total of 99 individual plaques were picked from several plates and stored in 1 ml of phage buffer (10 mM Tris-HCl [pH 7.4], 10 mM MgSO4, 0.01% [wt/vol] gelatin) at 4°C. Phage concentration and plaque morphology was determined by serial dilution of the phage lysate, 1 ml serial diluted phage and 0,1 ml indicator bacteria was added to 4.5 ml 46°C 0.7% soft-agar and poured onto the 1, 5% bottom agar on Petri dishes. After 30 min the plate was incubated at 37°C. The results were counted after 18–24 h. Plaque Purification of bacteriophage was repeated 15–20 times[20]. To increase the titer of each phage isolate briefly,100 µl of the primary phage stock in SM buffer was mixed with 150 µl of overnight liquid culture and 4 ml of top agar with 10 mM-$CaCl_2$ (MRS-Ca^{++} when specified) using the double-layer agar plate method as described by Adams[21]. After overnight incubation at room temperature, suspended in 3ml of BEG-8000 containing chloroform 0.4%, and then shaken vigorously for 2 min. The lysates were centrifuged for 20 min at 6000 RPM to remove the remnants of soft agar. The supernatants were collected and stored over chloroform at 4°C. Dilution series ranging from 10^1 to 10^{12} were generated for each phage stock, and 10^l of each was spotted on a top agar overlay containing 200 µl of overnight culture. Plaques were counted to determine the overall titer of the stock. This process was repeated to generate titers 10^9 PFU/ml[21].

Morphology of bacteriophages

Phage ϕ Acib_A10 which showed high lytic activity were used to determine the morphology. Phage suspension (10^{12} PFU/ml) was dropped onto the surface of a formvar-coated grid and negatively stained using 0.5% uranyl acetate for 3-5 min. After drying, the preparations were viewed with a Philips CM120 at 80 kV and documented using an SIS (Olympus Soft Imaging Solutions) Mega View III digital camera in a transmission electron microscope (LE/Iran).

Phage host sensitivity typing

In order to eliminate potentially redundant and sibling phages ϕ Acib_A10, each isolate was assayed for its ability to produce plaques on more than one bacterial species and genus was determined. The bacterial strains include eleven clinical (CTX_RAB) isolates from Burn Specialist Hospital, in addition to *Acinetobacter* spp., *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC 25922, isolates were obtained from hospitals in AL- Diwaniyah, Iraq. By spotting technique[22] was determined the host range of phage ϕ Acib_A10, in brief 1 ml of log phase suspension of host strains was spreader separately on sterile nutrient agar then a 0.2 ml aliquot of phage lysate (8×10^9 PFU/ml) was spot inoculated at the center of each plate. Then the plates were incubated at 37°C and examined after 24 h. A clear zone in the bacterial lawn was recorded as host susceptible. A host strain plaquing "fingerprint" was generated for each phage. A hierarchical cluster analysis was performed on the fingerprints, and the results were visualized by generating a dendrogram using the phylogenetic analysis programs Gene Cluster 3.0 and Java TreeView[23].

Comparing Bacteriophage effect with Ceftriaxone antibiotic

For comparing effect of bacteriophage on bacteria with antibiotic, MIC (Minimum Inhibitory Concentration of lytic activity) and MBC (Minimum Bacterial Concentration of *A. baumannii*) methods were considered and followed as described by Jennifer M Andrews[24].

Table 2. Average ϕ Acib A10 Titration

Dilution	Plaque No	Stock titer per ml plaque no.× invert dilution/0.1	Dilution titer plaque no.× DF ** /0.1 *
10^{-2}	TMTC***	--	--
10^{-3}	TMTC	--	--
10^{-4}	TMTC	--	--
10^{-5}	300	3×10^{8}	3×10^{4}
10^{6}	295	2.95×10^{9}	2.95×10^{4}
10^{7}	190	1.9×10^{10}	1.9×10^{4}
10^{-8}	99	9.9×10^{10}	9.9×10^{3}
10^{-9}	62	6.2×10^{11}	6.2×10^{3}
10^{-10}	12	1.2×10^{12}	1.2×10^{3}

*Volume of diluted virus added: 0.1 ml
** Dilution Factor (DF) =10
*** Too much to count and
--- Bacteria did not grow

Briefly, for preparation of antibiotic stock solution, the below formula was used:

$$\frac{1000}{P} \times C \times V = W$$

P: Potency given by the manufacturer (µg/mg)
C: Concentration of solution (multiples of 1000) (mg/L)
V: Volume required (ml)
W: Weight of antibiotic in mg to be dissolved in volume V (ml).

Preparation of antibiotic dilution range: Dilution ranges were prepared from: 0.06 - 128 mg/L.: 0.06, 0.125, 0.25, 4, 8, 16, 32, 64 and 128 µl into each the container labelled 1 to 9 respectively. The samples then incubated in 37℃ for 24h to determine MIC results. To determine the MBC result, after obtaining MIC, a sample of tubes, which were without turbidity was cultured in, plates containing EMB culture media. The first sample, which bacteria did not grow in it, was considered as MBC result.

Preparation of Phage serial dilution

For preparation of phage serial dilution, the method was similar to the MIC method with some differences: Eight sample and 7 different dilutions were prepared. The first tube contained original phage solution and the rest of the tubes were dilutions. In the first tube 1ml of isolated bacteriophage was added to 9ml of Muller Hinton Agar so the dilution factor would be 10^{-1} and the other samples 10^{2}, 10^{-3} and… 10^{-8} respectively. Then 1ml of *A. baumannii* (Ab_1) was added into first tube and after mixing by vortex 1ml of the solution was added to other tubed respectively. Number of Viruses in the sample can be counted by:

$$\frac{Number\ of\ phages\ in\ 1\ ml}{ditution\ factor \times phages\ solutor\ volume} \quad "number\ of\ plaques"$$

The process was being repeated to all clinical *A.baumannii* isolated used in this study (Ab2 to Ab11) respectively.

RESULTS

Eleven clinical *A.baumannii* strains were used as host indicators for the isolation of lytic phage. Finally, in total we isolated four *A.baumannii*-specific phages from percentage 67% of treatment pond water samples and their host ranges were determined with lytic. Three of these were temperate phages induced from human *A. baumannii* isolates. The remaining was virulent phage that showed virulent to most cefotaxime-resistant strains, was designated as ϕ Acib_A10. We also found that the big, clear plaques of ϕ Acib_A10 were 2 mm larger in diameter than those of the other phages, and then was propagated for purification and characterization. Fig1. Following plaque purification, high titer phage stocks were produced and titered by limiting dilution Table 2.

An electron micrograph of single phage particle revealed that the phage has a hexagonal head 45 nm in diameter and lacks a tail Fig. 1. The ϕ Acib_A10 phage particle were concentrated in a visible band at a density of 1.5 g ml in a CsCl gradient. These morphological features suggest that the Phage ϕ Acib_A10 belongs to Corticoviridae virus family, was similar in morphology and size to phage wkm18p[31] and PM2[32] according to the taxonomic database of ICTVdB[13].

In the lytic activity of ϕ Acib_A10 tests and their host ranges was examined by inoculating it's into each of *A. baumannii* and other strains used in this study (Approximately 56×10^{8} CFU ml) was showed the widest range of hosts, including cefotaxime-susceptible and other MDR strains. As shown in Fig. 2.

Obtaining MIC and MBC test results**:** After 24h incubation, the tube numbers were shown less turbid so the Minimum Inhibitory Concentration (MIC) for antibiotic which means this amount of antibiotic inhibited growth of bacteria in the sample. For obtaining MBC result, a sample of tubes, most which were with turbidity, was cultured in EMB culture media. After 24 hours, incubation in 37℃ tubes number effect on some isolates and at some concentrations and not all of the isolates that have shown the effect of 8, 16, 32 and 128 have effect no isolates Ab3, Ab5, Ab5, Ab8 and Ab9 bacteria growth. So the MBC result was the same as MIC result. The MIC and MC results can be seen in Table (1).

Obtain amount of bacteriophage which prevent growth of bacteria: In bacteriophage serial dilution method, the dilution had a minimum amount of bacteriophages, which prevent growth of bacteria which was 3×10^{4}. The results can be seen in Table (2).

DISCUSSION

In this study, we present the characterization of phage ϕ Acib_A10, a lytic phage infecting the CTX_RAB clinical isolate Ab1. In addition, in the bacteriophage dilution no bacteria have grown after 24 hr. which has shown best results compared to MBC test. We conclude from our results that treatment with phage ϕ Acib_A10 saints has an effective effect in Consequences of the mechanisms of the bacteria that prevent the inhibitory effects of the antibiotics in the treatment of animals is widespread[26]. There is now an ever-greater need for the development of new drugs that show anti–*Acinetobacter* activity.

Outbreaks caused by multidrug-resistant *A. baumannii* have been reported in all parts of the world with ever increasing frequency[33]. In particular, it is the development of Cephalosporin-resistance that has left clinicians with few viable alternatives[34]. Cefotaxime is often the only antimicrobial showing measurable activity, but because of toxicity and low serum concentrations, itis not always effective *Enterobacteriaceae, Pseudomonas aeruginosa* and *A. baumannii* the use of this compound[35]. Over the last decade there have been only a few novel anti-Gram-negative drugs developed, including ceftolozane/tazobactam and ceftazidime/avibactam, which have only poor activity against A. baumannii[36]. The antibiotic capacity that causes the resistant of bacteria is an important problem for public health. Antibiotic residues can be found in the environment for long periods of time after treatment[27]. In this study, Positive effect of Bacteriophages in killing bacteria cells have compared with effects of antibiotic and it was shown that bacteriophages are as effective as antibiotics except they do not make any resistance and do not make a dangerous situation for human health.

Raghu *et al*., have discussed about several roles that bacteriophages play in the environment, biofilm control and water treatment[29]. There are a lot reports suggested that the presence of bacteriophages in environments could be useful in pond water treatment especially in procedure of activated sludge[30]. In addition, it has suggested that phages can be used as biological tracers of pathogenic bacteria in water treatment[23]. Zumstein *et al*. studied the interactions of bacterial populations and bacteriophages in anaerobic wastewater treatment using laboratory anaerobic digesters. They suggested that bacteriophages could be effective on the dominance of bacterial strains during the process[15]. Periasamy and Sundaram have reported the potential of bacteriophages for removal of bacterial pathogens including *E. coli* in hospital wastewater. They showed that the specific phages of *E. coli* could destroy the bacterial host after 14 hours of incubation[14]. Meanwhile, using bacteriophages which have shown same results as antibiotics can be a substitution of them in cases that antibiotic makes resistance and can reduce the investment costs of treating bacterial deceases than producing antibiotics, which are so hard to find and need high level technologies. In addition, they can be used directly in waste water treatment plants to reduce bacteria jams so they are potentially useful for environmental sciences too in the environment for long periods of time after treatment[27,28].

CONCLUSION

This article emphasizes the value of phage ϕ Acib_A10 as effective alternatives to cefotaxime in resistant cases.

ACKNOWLEDGMENT

We thank staff from Burn Care Unit in Burn Specialist Hospital of Al Diwaniyah city, Iraq for providing suport. We are grateful to Mrs. Magda Attia Alwan for her material support for the project.

REFERENCES

1. Muhammad I. Q., Tahira M., Ardas M. 2018. Phage therapy: progress in pharmacokinetics. Brazilian Journal of Pharmaceutical Sciences doi.org/10.1590/s2175-97902018000117093
2. Abedon, S. T. 2017. Bacteriophage clinical use as antibacterial “drugs”: utility and precedent. Microbiol. Spectr. 5: BAD-0003-2016. doi: 10.1128/microbiolspec.BAD-0003-2016
3. Abedon ST. 2015. Phage therapy of pulmonary infections. Bacteriophage. 18(5):e1020260.
4. S Mattila, P Ruotsalainen, M Jalasvuori. 2015. On-Demand Isolation of Bacteriophages against Drug-Resistant Bacteria for Personalized Phage Therapy. Journal ofFront Microbiol. 13(6):1271.
5. R Peltomaa, et. al. 2015. Application of bacteriophages in sensor development.Analytical and Bioanalytical Chemistry. 1-24
6. Kitti T, et. al. 2014. Characterization and detection of endolysin gene from three Acinetobacter baumannii Bacteriophages Isolated from Sewage Water. Indian J. Microbiol. 54(4):383-8.
7. Garnacho-Montero J, Amaya-Villar R 2010. Multiresistant *Acinetobacter baumannii* infections: epidemiology and management. Curr Opin Infect Dis 23: 332–339
8. European Association for the Study of the Liver. 2010. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. J Hepatol 53: 397-417 [PMID: 20633946].
9. Ariza X, et. al. 2012. Risk factors for resistance to ceftriaxone and its impact on mortality in community, healthcare and nosocomial spontaneous bacterial peritonitis. J Hepatol, 56: 825-832 [PMID: 22173153]
10. Adams MD, et al. 2009. Resistance to colistin in *Acinetobacter baumannii* associated with mutations in the PmrAB two-component system. Antimicrob Agents Chemother, 53:3628-34.
11. Park SY, et. al. 2013. Risk factors for mortality in patients with Acinetobacter baumannii bacteremia. Infect. Chemother. 45(3): 325-30.
12. Thawatchai K., et. al. 2015. Efficacy of Acinetobacter baumannii bacteriophage cocktail on Acinetobacter baumannii growth. African Journal of Microbiology Research. 9(42), 2159-2165.
13. ICTVdB Management 2006. In: ICTVdB - The Universal Virus Database, version 4. Buchen-Osmond C., editor. New York: Columbia University. Available: http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/. Accessed 2012 Sep 14.
14. Periasamy, A Sundaram. 2013. A novel approach for pathogen reduction in wastewater treatment. Journal of Environmental Health Science and Engineering; 11 (1): 12.
15. Beaudoin R.N. et al. 2007. Isolation of a Bacteriophage from sewage sludge and characterization of its bacterial host cell, River academic journal, 3 (1).

16. Verhaegen J., et al. "Basic Laboratory Procedures in Clinical Bacteriology, 2nd Edition". 2003. WHO Geneva 91-93
17. Thompson JD, Higgins DG, Gibson TJ 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic acids research 22: 4673-4680.
18. Zahraa F. A. & Wathiq A.D. 2018. Molecular Phylogenetic Study in 16s rRNA Gene Among *Acinetobacter Baumannii* Isolates Characteristic Producing To ESBLs Genes In Burn Infection. Sci.Int. (Lahore), 30(4), 579-585.
19. Kitti T, et. al. 2014. Characterization and detection of endolysin gene from three Acinetobacter baumannii Bacteriophages Isolated from Sewage Water. Indian J. Microbiol. 54(4):383-8.
20. Lin, N. T., et. al. 2010. Isolation and characterization of phi AB2: a novel bacteriophage of Acinetobacter baumannii. Res. Microbiol. 161, 308–314. doi: 10.1016/j.resmic.2010.03.007.
21. Adams, M. H. 1959. Methods of study of bacterial viruses, p. 447–448. In Bacteriophages. Interscience Publishers, London, United Kingdom.
22. Douglas J. 1975. " Bacteriophages". Chapman and Hallpublishers, 4th ed., London. pp.20-46
23. BeheshtiMaal K. et. al. 2012. Characterization of Two Lytic Bacteriophages of Streptococcus sobrinus Isolated from Caspian Sea. Asian J Biology Sci.5: 138–4
24. M Andrews Jennifer. 2001. J. Antimicrob. Chemother. 48(1): 5-16.
25. Kitti T, et. al. 2014. Characterization and detection of endolysin gene from three *Acinetobacter baumannii* Bacteriophages Isolated from Sewage Water. Indian J. Microbiol. 54(4):383-8.
26. Raghu H.V. et. al. 2012. Beneficial face of bacteriophages: Applications in food processing. International Journal of Qual Res.; 6 (2): 101–8.
27. Abdulla H. et al. 2007. Bacteriophages in Engineered Wetland for Domestic Wastewater Treatment. Research Journal Microbiology; 2(12): 889–99.
28. Huff W.E. et al. 2004. Therapeutic efficacy of bacteriophage and Baytril (enrofloxacin) individually and in combination to treat colibacillosis in broilers. PoultSci 83: 1944-1947.
29. Guangtao H., et. al. 2013. Characterization and Genome Sequencing of Phage Abp1, a new phiKMV-Like Virus Infecting Multidrug-Resistant *Acinetobacter baumannii.* Curr Microbiol 66:535–543 DOI 10.1007/s00284-013-0308-7.
30. Cai Y, et. al. 2012. Colistin resistance of Acinetobacter baumannii: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother 67:1607-15.
31. Gwan-Han S., et. al. 2012. Isolation and Characterization of wkm18p, a Novel Lytic Phage with Therapeutic Potential against Extensively Drug Resistant *Acinetobacter baumannii.* Volume 7, Issue 10:e46537
32. Kivela¨ HM, et. al. 2002. Bacteriophage PM2 has a protein capsid surrounding a spherical proteinaceous lipid core. PLOS. J Virol 76: 8169–8178.
33. Tomaschek F, et. al. 2016. Head-to-head comparison of two multi-locus sequence typing (MLST) schemes for characterization of *Acinetobacter baumannii* outbreak and sporadic isolates. PLoS ONE 11:e0153014.
34. Garnacho-Montero J, et al. 2015. Task force on management and prevention of *Acinetobacter baumannii* infections in the ICU. Intensive Care Med 41:2057–75
35. Dickstein Y, et al. 2016. Multicentre open-label randomised controlled trial to compare colistin alone with colistin plus meropenem for the treatment of severe infections caused by carbapenem-resistant Gram-negative infections (AIDA): a study protocol. BMJ Open 6:e009956.
36. Testa R, et al. 2015. In vitro activity of ceftazidime, ceftaroline and aztreonam alone and in combination with avibactam against European Gram-negative and Gram-positive clinical isolates. Int J Antimicrob Agents 45:641–6.