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Biocontrol of *Acinetobacter baumannii*-S-MH Using Bacteriophages Isolated from Sewage Water

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ABSTRACT

The aim of this study is to isolate and characterize bacteriophages, as an alternative solution against multidrug-resistant *Acinetobacter baumannii*-S-MH bacteria causing an outbreak in hospital environments and intensive care units (ICUs). Here, biochemically; isolating and characterizing *Acinetobacter baumannii*-S-MH by the automated Vitek 2 system. Isolated *Acinetobacter baumannii* -S-MH bacteria was tested against a collection of Antibiotics by Antibiotic sensitivity test. *Acinetobacter baumannii* showed resistance to most of the antibiotics tested. To overcome this problem, *Acinetobacter baumannii's* phage (VB_AciM-AM-M) was isolated, identified, and applied to control the growth of this opportunistic pathogen. Morphologically, phage was identified by TEM; showed that (VB_AciM-AM-M) phage is shaped Myoviridae morphotype. The one-step growth curve of the phage showed burst sizes of 290 PFU/cell with a latent period of 20 minutes for (VB_AciM-AM-M) phage. Complete inhibition of bacterial growth was achieved using phage with MOIs of 1, 3 and 5 after 1, 3, and 24 h. of incubation at 37°C. Hence, this study indicates that the isolated bacteriophages are promising biocontrol agents that could challenge antibiotic-resistant *Acinetobacter baumannii*-S-MH bacteria to announce new successful alternatives to antibiotics.

INTRODUCTION

Acinetobacter baumannii is a genus of coccbacilli, aerobic, nonmotile, gram Negative bacterium (Van Looveren *et al.,* 2004; Perez *et al.,* 2007; Peleg *et al.,* 2008; Martín-Aspas *et al.,* 2018) which make up a ratio of 2-10% of all G (-ve) hospital infections (Joly Guillou *et al.,* 2005). *Acinetobacter baumannii* is an opportunistic pathogen found in soil, water, animals (Baumann *et al.,* 1968; Towner *et al*., 2009); in veterinary medicine with infection and colonization of sick animals in veterinary clinics. It outbreaks also in hospital environments and Intensive Care Units (ICUs) (Zordan *et al.,* 2011*)* which cause mortality ranged from 7.86 to 23% and from 10% to 43% respectively (Falagas *et al.,* 2006).

Some of *A. baumannii* are isolated from nonhuman sources such as animals, lice, vegetables, aquaculture and soils (Eveillard *et al.,* 2013*). Acinetobacter baumannii* when confirmed by different phenotypic tests show that it is Gram(-ve) coccobacilli, catalase (+ve), oxidase (-ve), non-fastidious, non-fermentative (oxidative), gas (-ve), H_2S (-ve), hemolysis (-ve), growth at 44°C, nitrate reduction (-ve), urease (-ve), indole (-ve), methyl red (+ve), Voges-Proskauer (-ve), citrate (+ve), and utilization 10% lactose (+ve) (Malini *et al.,* 2009*). Acinetobacter baumannii* is recognized as an emerging opportunistic pathogen and can cause several different types of infection especially in immunocompromised individuals including Pneumonia, Urinary tract infection, bacteremia meningitis, wound; surgical infection, endocarditis (Bergogne-Berezin *et al.,* 1996; Garcia-Garmendia *et al.,* 2001; Chastre *et al.,* 2003; Villegas *et al.,* 2003; Wilson *et al.,* 2004; Joly-Guillou *et al.,* 2005; Fournier *et al.,* 2006; Leung *et al.,* 2006; Perez *et al.,* 2007; Maragakis *et al.,* 2008; Peleg *et al.,* 2008; Dexter *et al*., 2015).

Acinetobacter baumannii infection may have some symptoms including chest, muscle pain, breathing problems, nausea, cough, fevers, chills, rash, and sensitivity to bright light or not in case of colonizing open wound. factors increasing the risks for infection include immunosuppression, Having a weak immune system, diabetes, chronic lung disease or respiratory failure, prolonged hospitalization, previous antimicrobial therapy or previous sepsis in ICUs, using hospital ventilator, open wound treated in hospital, breathing tubes, or central venous cathere, direct contact with infected person's skin or objects, it can also spread through contaminated surfaces by one to one (García-Garmendia *et al.,* 2001; Mahgoub *et al.,* 2002; Robenshtok *et al*., 2006; Karageorgopoulos and Falagas, 2008; Maragakis *et al.,* 2008; Munoz-Price and Weinstein, 2008; Wong *et al*., 2017). Outbreaking of *A. baumannii* infection become worldwide (Perez *et al.,* 2007).

In U.S. military personnel in Iraq and Afghanistan; *A. baumannii* has been a deep problem due to its responsibility for a large number of infections (Peleg *et al.,* 2008). In 2009; studying in European ICU, reported that *Acinetobacter baumannii* is responsible for 19.1% of ventilator-associated pneumonia cases. The success of making *A. baumannii* is a hospital pathogen is it can be prolonged on different environmental surfaces and it is regarded as one of its characteristics (McDonald *et al.,* 1996; Eliopoulos *et al.,* 2008). Some studies reported that *A. baumannii* responsible for about 5 to 10% of infections in hospitals worldwide, it was responsible for 60% of pneumonia cases and 25% of bloodstream infections (Timsit *et al.,* 2014; Cornejo-Juárez *et al.,* 2015). The studies on Mortality rates for *A. baumannii* infections have been reported up to 64% (Anstey *et al*.,1992; Patamatamkul *et al.,* 2017).

Acinetobacter baumannii is recognized to be among the most pathogen that is difficult to treat or control due to its survival for extended periods in the environment; its ability to become rabidly resistant to most antimicrobial agents (Perez *et al.,* 2007; Peleg *et al.,* 2008). *Acinetobacter baumannii* is the most important challenging bacterial pathogen due to its special characteristics of antibiotic-resistant (Gonzalez-Villoria and Valverde-Garduno , 2016). Nowadays, *Acinetobacter baumannii* (MDR) makes therapeutic resolutions for nosocomial infections experimental even more difficult (Maragakis *et al.,* 2008). An available antibiotic for treating nosocomial infections caused by *Acinetobacter baumannii* is very limited due to *A. baumannii* being multidrug-resistant.

The studies on MDR *A. baumannii* from hospitals in Europe, North America, Argentina, Brazil, China, Taiwan, Hong Kong, Japan and Korea and from areas as remote as Tahiti in the South Pacific show that the MDR strain cause outbreak in all countries (Levin *et al.,* 1996; Houang *et al*., 2001; Landman *et al.,* 2002; Barbolla *et al.,* 2003;

Lee *et al.,* 2003; Quale *et al*., 2003; Da Silva *et al.,* 2004; Nishio *et al.,* 2004; van Dessel *et al.,* 2004; Van Looveren *et al.,* 2004; Yu *et al.,* 2004; Naas *et al.,* 2005; Liu *et al.,* 2006).

A. baumannii has virulence factors, which increase its ability pathogenicity and toxicity. These virulence factors, including OmpA, biofilm formation, phospholipase, iron acquisition, efflux pumps, surface motility, hemolytic factors and stress resistance (Antunes *et al.,* 2011; Ali HM *et al.,*2017). *A. baumannii* can form a biofilm which makes it easy to adhere to biotic or abiotic surfaces (Hall-Stoodley *et al*., 2004; Gaddy and Actis and, 2009). OmpA, can promote the restraint of the host immune system or bacterial attachment of bacteria to epithelial cells (Ali *et al.,* 2017).

Bacteriophage or phage, it is a virus that invades bacterial cells; its genome is either DNA or RNA (Brssow and Hendrix, 2002; Hatfull *et al.,* 2011; Doore *et al.,* 2016; Simmonds and Aiewsakun, 2018). Bacteriophages were discovered by F'élix d'H'érelle (D'Herelle *et al.,* 1931) at 20th century. Bacteriophages can be found in different environments where polluted with microorganisms. Optimum temperature for infection ranged from <4 to 95 ^{o}c (Delisle and Levin, 1972), Optimum PH ranged from PH 1 to PH 11 (Schleper *et al*., 1992; Jarrell *et al*., 1997) and it can infect halophilic microorganisms which are stable in up to 5M salt (Nuttall and Dyall-Smith, 1993; Bath and Dyall-Smith, 1998).

Bacteriophages can attach to their bacterium during the replication cycle which may be lytic or lysogenic. The first step of the replication process is binding to the corresponding host and injecting bacteriophage its genomic material into a bacterial cell. This process is finished by lysis of bacterial cells and a new phage progeny is produced. However, in the lysogenic replication cycle phage genome integrates with the bacterial chromosome and is replicated without killing or lysis for bacterial cells (Ptashne *et al.*, 2006; Clokie *et al.,* 2011). The polysaccharides cell wall is an important virulence factor for Bacteriophages wall which enable them from protection against the invading; Bacteriophages can invade bacterial cell by the attachment to specific receptors on its surface including lipopolysaccharides, proteins, flagella and Teichoicacid.(Drulis *et al.,* 2015).

Antibiotics and lytic phages almost have the same Function that they aim to get rid of bacteria (Adamia *et al.,* 1990). However; some studies reported that phages can treat human infections better than Antibiotics (Meladze *et al.,* 1982; Kochetkova *et al.,* 1989; Sakandelidze *et al.,* 1991). Due to the usage of phage as a biocontrol against antibiotic resistance through virus genes that can encode enzymes responsible for bacterial biofilm degradation. (Wommack *et al.,* 2000). Bacteriophages are very specific to their host, therefore; they can't attack humans but it excellently used in overcoming bacterial human diseases (Boyd *et al.,* 2012).

Bacteriophages are used for medical cases in a large number of countries such as Poland, Russia, Georgia and where it is available to use (Sulakvelidze *et al.,* 2001; Abedon *et al.,* 2011). In recent century phages have been achieved as an antimicrobial tool against MDR Bactria including their use to *Acinetobacter baumannii* (Twort *et al.,* 1915; Lin *et al.,* 2017). In this study, the isolation and identification of bacteriophages were a new indicator for bacteriophage therapy against Multidrug-resistant *Acinetobacter baumannii.*

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions:

This study was performed on antibiotic-resistant isolates that were isolated previously from patients in Qalubiya governorate, Egypt. All strains were stored at -80°C in Brain-Heart-Infusion broth complemented with 20% (v/v) glycerol. Freshly overnight grown cultures were prepared by inoculating a single colony into 10 ml of Basal salt medium with yeast extract and incubating for 16 h at 37°C with shaking at 200 rpm.

Morphological and Biochemical Identification of *Acinetobacter baumannii:*

Morphological and Biochemical identification (Table S1, Supplementary data) of isolated *A. baumannii*-S-MH bacteria were carried out according to Bergey's Manual of Systematic Bacteriology. These isolates were confirmed by VITEK® 2 COMPACT automated instrument for ID/AST testing (Pinus *et al.,* 2006).

Antibiotic Sensitivity Test:

Antibiotics sensitivity testing was performed on Mueller-Hinton agar by the disc diffusion method (Biemer *et al.,* 1973). For the following antibiotics (Oxoid, Hampshire, UK); cefoxitin (FOX, 30 µg); Ceftriaxone (CRO, 30 µg), Amoxicillin (AX, 25 µg), Imipenem (IPM, 30 µg), Ciprofloxacin (CIP, 5 µg), Tobramycin (TOB, 10 µg), Norfloxacin (NOR, 30 µg), Streptomycin (S, 10 µg), Cephalexin (CL, 30 µg), Ceftazidime (CAZ, 30 µg), Aztreonam (ATM, 30 µg), Cefaclor (CEC, 30 µg), Ofloxacin(OFX, 5 µg), Cefotaxime (CTX, 30 µg) and Trimethoprim\Sulphamethazole (SXT, 25 µg). The results were interpreted conferring to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (Wayne *et al.,* 2011).

Isolation of Bacteriophages:

The clinical isolates *Acinetobacter baumannii's phage* (VB_AciM-AM-M) was used as a host for the isolation and propagation of bacteriophages. *A. baumannii* samples from 11 healthy individuals' patients and drainage samples from Benha hospital were collected from Benha city, Qalubiya governorate, Egypt for bacteriophage screening. The collected samples were centrifuged at 10,000 rpm for 10 min, supernatant fluids were collected, and membrane filtered using 0.22 µm membrane filters (Millipore, Ireland).

Enrichment of phages and isolation were performed as described previously (Van Twest and Kropinski 2009). Briefly, 5 ml of a 0.22 µm-filtered sample was mixed with 20 ml double-strength Tryptic Soybean Broth (TSB) medium and 100 µl of a mid-log culture of *Acinetobacter baumannii*'s phage (VB_AciM-AM-M) and incubated for 48 h at 37°C with shaking at 200 rpm. Later, bacteria were harvested by centrifugation at 10,000 rpm for 10 min, supernatant fluids were recovered, and membrane-filtered using 0.22-µm Millipore filters (Millipore, Ireland). Phage Was screened by spotting five microliters of the enriched samples onto double-layered plates containing a lawn of the indicator bacteria strain and incubated for 48 h at 37°C (Huang *et al.,* 2018). Plates were inspected for the presence of clear lysis zones; the clear zone was cut and propagated in a fresh culture. Thislysate was serially diluted, spotted onto double-layered plates, and incubated as described above.

Transmission Electron Microscopy of Bacteriophages:

Ten microliters of highly purified phage suspension were fixed onto 300- by 300-mesh copper grids (Electron Microscopy Sciences) supported by carbon-coated Formvar film *(*Ackermann *et al*., 2012). After 5 minutes, fixed phages were negatively stained with 2% (w/v) aqueous phosphate tungsten acid, pH 7.2 for 1 min, and air-dried at room temperature for 1 h. A transmission electron microscope (A JEOL JEM-2100) was used for attaining the phage particle images at the Electron Microscope Facility, Al-Azhar University, Egypt.

One-step Growth Curve:

Phage's growth phases and burst size were determined as described previously (Huang *et al.,* 2018). A known number of an exponential-phase culture (ca. 1×107 CFU/ml) of each bacterial host was infected with each specific phage individually at an MOI of 1, phages were allowed to adsorb for 5 min at room temperature. The mixture was then centrifuged at 5,000 rpm for 5 min and the supernatant was decanted to remove free-unbound phages. The bacteria-phage pellet was then washed twice and resuspended in 10 ml of TSB and maintained at 37°C with continuous shaking. At appropriate times phage titers were enumerated using plaque assay (Kropinski *et al.,* 2018).

Determination of the Bacteriophages Host Range:

The host range for the isolated phage (VB_AciM-AM-M) was determined against a

collection of twenty bacterial isolates (Table 3) as previously described with some modifications *(*Ackermann *et al*., 2009; Karthik *et al*., 2014). Ten microliters of each phage suspension (about 10^{11} PFU/ml) were spotted, in duplicate, onto the TSA bacterial lawn plates and incubated at 37 °C for 16–18.

Thermal and pH Stability:

Thermal and pH-stability of the isolated phages were tested as described before. For the assessment of thermal stability, 900 µl of preheated 0.22 µ m filter-sterilized SM buffer (5.8 g NaCl, 2.0 g $MgSO_4$ •$7H_2O$, 50 ml 1 M Tris-HCl pH 7.4, in 1-liter dH_2O) were added to 100 µl of each of phage lysates (8 log10 PFU/ml). Tubes were incubated at 10°C, 40°C, 50°C, 60°C,70°C and 80°C for 1 h. Aliquots were collected After 60 min of incubation to determine phage titers. For pH-stability assessment, phage lysates (11 log10 PFU/ml) were added to tubes containing sterile SM buffer with pH values ranging from 2-12 adjusted with NaOH and HCl. The tubes were incubated at 37°C for 60 min. Subsequently, the phage solutions were serially diluted and the recovered phage titers were determined using bacterial hosts employing the double-layer agar method. Each temperature and pH treatment was performed in triplicates and the average of triplicate counts was calculated. Phage thermal/pH stability (%) = (Recovered phage titers following the treatment / Initial Phage titer before treatment) ×100%.

Biocontrol of *A. baumannii*-S-MH Bacteria Using Bacteriophages:

The efficacy of phages to inhibit the growth of their hosts was assessed in broth medium using different MOIs. Phages were separately mixed with bacterial suspension that was diluted to103 CFU/ml from an overnight culture of *A. baumannii*- S-MH to obtain MOIs of 1, 3, and 5 incubated at 37°C for 24 h. Times of collecting samples were at 1, 3, and 24 h. Surviving bacterial cells were counted using serial dilutions in sterile saline. Then, dilutions were plated onto nutrient plates and incubated at 37°C for 24 h. Bacterial growth was measured by monitoring optical densities at 600 nm.

RESULTS

Bacteria and antibiotic sensitivity testing:

In the current study, *A. baumannii*- S-MH was isolated from Benha University Hospital. The isolates were identified biochemically using conventional methods (Table S1, Supplementary Data) and were confirmed by Vitek 2 system.

An antibiotic sensitivity test was performed for these isolated bacteria against a selection of fifteen antibiotics (Fig.1). Qualitative data from the antibiograms (Table 1) revealed that *A. baumannii*-S-MH was resistant to at most antibiotics with a resistance percentage of 87% against the tested antibiotics, *A. baumannii*-S-MH resisted for Amoxicillin (AX, 25 µg), Ciprofloxacin (CIP, 5 µg), Norfloxacin (NOR, 30 µg), Ofloxacin (OFX, 5 µg), Tobramycin (TOB, 10 µg), Cefaclor (CEC, 30 µg), Aztreonam (ATM, 30 µg), Cephalexin (CL, 30 µg), Trimethoprim\ Sulphamethazole (SXT, 25 µg), Cefotaxime (CTX, 30 µg), Ceftazidime (CAZ, 30 µg), Ceftriaxone (CRO, 30 µg), Cefoxitin (FOX , 30 µg). this isolate was intermediated to Imipenem (IPM, 30 µg), Streptomycin (S, 10 µg).

Table 1. Antibiotic sensitivity pattern of the isolated *A.baumannii*- S-MH bacteria against a selection of fifteen antibiotics.

Bacteria	AX	CIP	NOR	OFX	IPM	S	TOB	CEC	ATM	CL	SXT	CTX	CAZ	CRO	FOX
A. baumannii-S-MH	R	R	R	R	I	I	R	R	R	R	R	R	R	R	R

Amoxicillin (AX, 25 µg), Ciprofloxacin (CIP, 5 µg), Norfloxacin (NOR, 30 µg), Ofloxacin(OFX, 5 µg), Imipenem (IPM, 30 µg), Streptomycin (S, 10 µg), Tobramycin (TOB, 10 µg), Cefaclor (CEC, 30 µg), Aztreonam (ATM, 30 µg), Cephalexin (CL, 30 µg), Trimethoprim\ Sulphamethazole (SXT, 25 µg), Cefotaxime (CTX, 30 µg), Ceftazidime (CAZ, 30 µg), Ceftriaxone (CRO, 30 µg), Cefoxitin (FOX , 30 µg). * Denotes for Resistant (R), Sensitive (S), Intermediate (I).

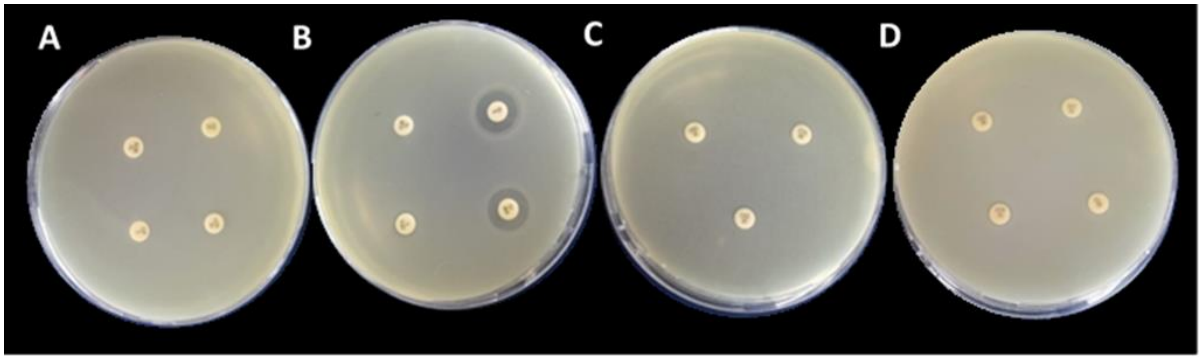

Fig.1. Antibiotic susceptibility test showing zones of clearance surrounding Antibiotic Discs: Multidrug resistance *A. baumannii*-S-MH (A-D) against a selection of 15 antibiotics.

Morphology of Bacteriophages:

Spot assay of (VB_AciM-AM-M) on *A. baumannii*-S-MH was shown in (Fig. 2A). Phage with plaque morphology (Fig. 2B) targeted bacteria, *A. baumannii*-S-MH was successfully obtained from Benha University Hospital. Successive double soft-layer agar assays led to pure phages isolation, titrated and processed at 4°C. (Fig. 2B) shows clear plaques produced by (VB_AciM-AM-M) Phage on *A. baumannii*-S-MH as host strain. Phage (VB_AciM-AM-M) produces circular clear plaques with a diameter of 2 mm. The concentrations of phages were $5*10^{11}$ PFU/ml.

Transmission Electron microscopy (Fig.2C) allowed us to infer that Phage (VB_AciM-AM-M) belongs taxonomically to order Caudovirales. Dimensions of the isolated phage were measured and summarized in Table 2. The particle of Phage (VB_AciM-AM-M) had a contractile tail with 95.8 ±2 nm in length and head with a diameter of about 60.8 ±1 nm a typical member of the Myoviridae family.

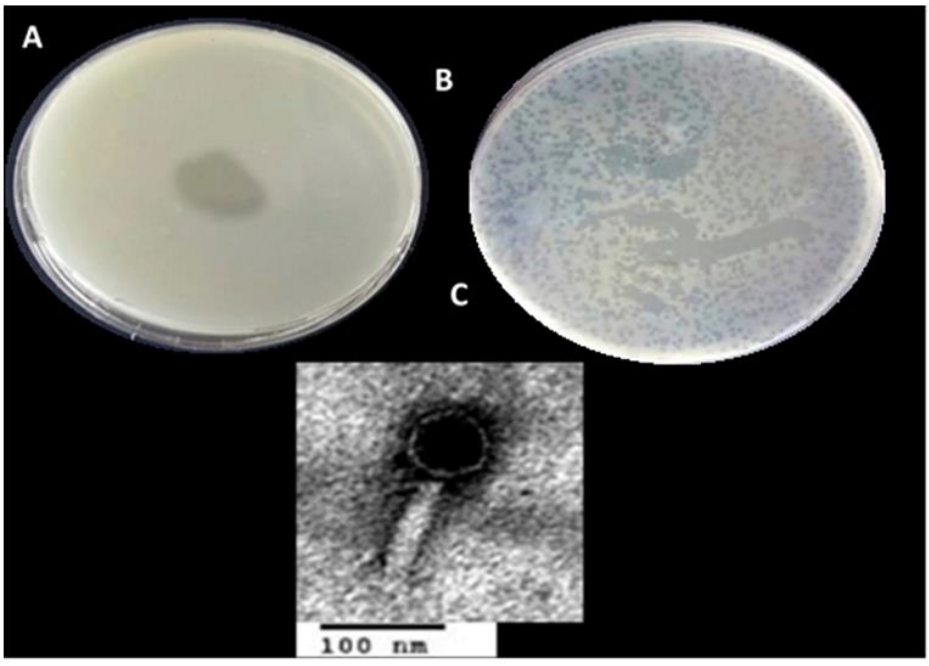

Fig. 2. Spot assay, Plaques phenotypes and TEM morphology of the isolated phage. (A-B-C) Images of bacterial spot and plaques respectively formed by the isolated phage in top-agar lawns of *A. baumannii*-S-MH plaque appearance was detected and imaged after culturing 24 h on their hosts. (C) TEM micrographs of phage (VB_AciM-AM-M) were negatively stained with 0.2% uranyl acetate as described in Materials and Methods. Scale bar= 100 nm).

Table 2. Dimensions of the isolated phages

Phage	Bacterial Host	Plaques Diameter(mm)	Head Diameter (nm)	Tail length (nm)	Proposed Family
VB_AciM-AM-M	*A. baumannii*-S-MH	2 mm	60.8 nm	95.8 nm	Myoviridae

Growth-kinetics and Host Range:

One-step growth kinetics of the isolated phages (Fig. 3) exhibited typical growth kinetics of Most bacteriophages. Phage (VB_AciM-AM-M) gave burst sizes of 290 PFU/cell with a latent period of 20 minutes. The host range of the isolated phages was estimated, and results were summarized in (Table 3) phage established a narrow spectrum of lytic activity.

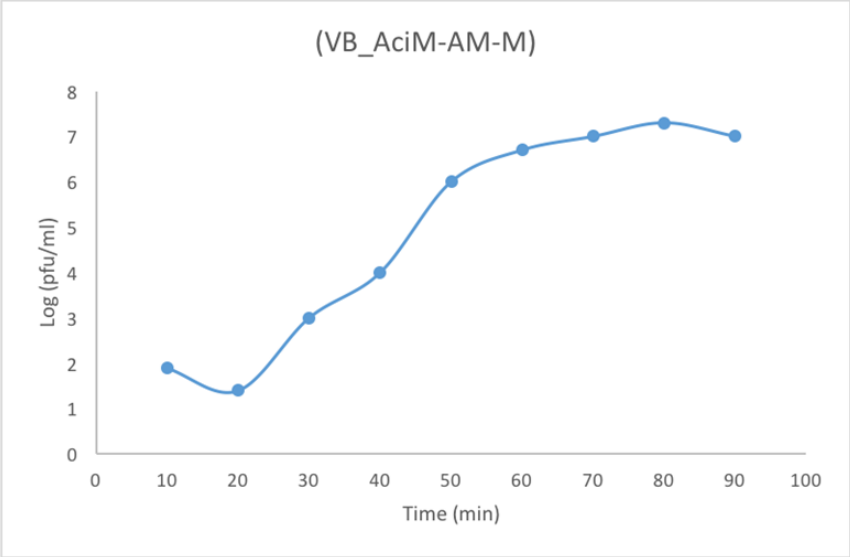

Fig. 3. One-step growth curves of phage VB_AciM-AM-M on their corresponding hosts.

Table 3. Host ranges of the isolated phages**.**

Species	Strain ID number	lysis by bacteriophage M
Acinetobacter baumannii	***Acineto-z***	+
	Acineto-G	+
	Acineto-M	+
	Acineto-N	-
	Acineto-E	-
	Acineto-MEL	-
	Acineto-MA	-
	Acineto-HA	-
	Acineto-AS	-
	Acineto-AA	-
	Acineto-AE	-
Proteus	***Proteus 1***	-
	Proteus 2	-
Shigella spp	***Sh1***	-
E-coli	***E-1***	-
Staphylococcus aureus	***S.au 1***	-
Salmonella entrica	***Sa 1***	-
Pseudomonas	***Seudo 1***	-
aeruginosa	***Seudo 2***	-
Klebsiella pneumonia	**Kleb 1**	-

"- "No clearing; "+" Completely clear.

Thermal and pH Stability:

Thermal and pH stability patterns of Phage (VB_AciM-AM-M) were tested based on residual phage titers after incubation under different pH values and temperatures (**Fig. 4**). The *A. baumannii*'s phage (VB_AciM-AM-M) was thermostable at temperatures ranging from 10 to 70 °C, but no viable phages were detected after heating at ≥80°C for 60 min. Phage (VB_AciM-AM-M) resisted a pH range between pH 2 and 11 for 1h.

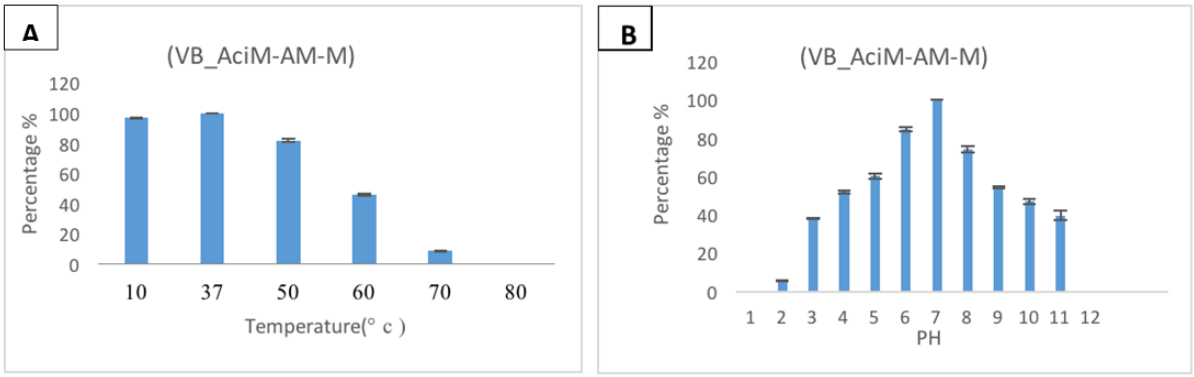

Fig. 4. Thermal and pH tolerance test of phage (VB_AciM-AM-M) (A) Thermal tolerance, and (B) pH stability of (VB_AciM-AM-M) phage. Temperature experiments were performed for 1 h at pH 7. pH tolerance was performed for 1 h at 37°C. Data showed the percentages of the remaining phages after each treatment as normalized from the control. Data shown are the mean of three replicates and error bars show the deviation in the values.

Biocontrol of *A. baumannii*-S-MH Bacteria Using the Bacteriophages:

In the current study, different MOIs were used to control targeted pathogens in broth medium (Fig. 5). High values of MOI were used to be enough to infect bacterial cells and to reduce the chance of bacterial cells regrowth. In broth medium, complete inhibition of bacterial growth was achieved using phages with higher and lower MOIs of 1, 3 and 5 after 1, 3 and 24 hr at 37°C compared to control counts. Based on the stability and lytic activity of the isolated phage against *A. baumannii*-S-MH, they were applied to control such hosts in broth medium using different MOIs. Findings showed that MOIs of 1, 3 and 5 appeared efficient to eradicate the bacterial growth and prevent their regrowth in broth medium. Thus, the involvement of these phages in therapy could be promising as alternatives to antibiotics.

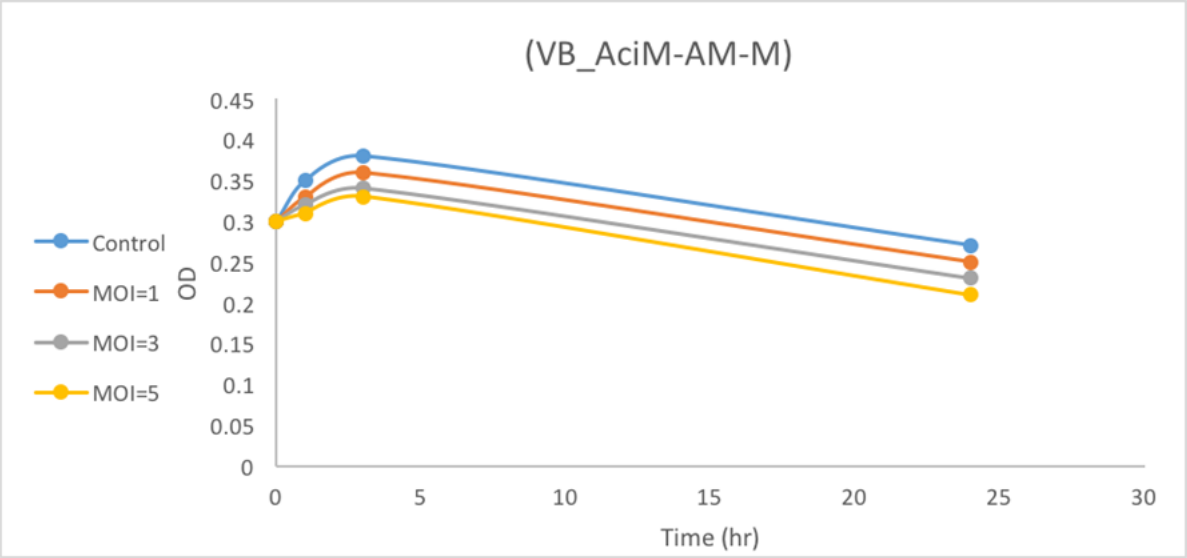

Fig. 5. *In vitro* biocontrol assay of the isolated phages on their corresponding hosts at different MOIs. Lytic activity of (VB_AciM-AM-M) phage on *A .baumannii*-S-MH. bacteria that challenged with the corresponding phage at different MOIs of 1, 3, and 5. Bacterial growth was determined by measuring the optical density at 600 nm.

DISCUSSION

Now; most infections in hospitals are caused by *Acinetobacter baumannii*. In the current study, the pathogenic bacteria, *A. baumannii*-S-MH, were isolated from Benha University Hospital. The isolated bacteria were characterized microscopically and identified biochemically using conventional methods and were confirmed by Vitek 2 system according to previous studies.

Recently, most bacteria had the potential to develop resistance against different classes of antibiotics. Antibiotic resistance is one of the top concerns that threaten global health (Dadgostar *et al*., 2019). Egypt is one of the countries where antibiotic remedies have fewer extreme limitations (Esmael *et al.,* 2020; Esmaet *et al.,* 2017) that enhance the chance for bacteria to resist antibiotics. In the current study, antibiotic sensitivity testing of *A. baumannii*-S-MH against a selection of fifteen antibiotics showed that the isolate resisted at most of the tested antibiotics. Resistance mechanisms against antibiotics by *A. baumannii*-S-MH were reported.

Antibiotic resistance can be developed through mutations in chromosomal genes or by mobile genetic elements (horizontally acquired resistance). In that view, a resistance that is acquired through mutation, mechanism of horizontally acquired resistance, or overexpression of the drug efflux were discussed previously. Hence, the current study used bacteriophages as an alternative strategy to control the spread of these organisms.

Bacteriophages have been sought as one of the novel therapeutic approaches to control antibiotic-resistant pathogenic bacteria (Shlezinger *et al.,* 2017; Vickers *et al.,* 2017). In the current study, phage (VB_AciM-AM-M) targeted infections caused by bacteria, *A. baumannii*-S-MH were isolated from Benha University Hospital. Phage (VB_AciM-AM-M) belongs to the family Myoviridae.

The stability of phages under stressful environmental conditions promotes the application of phages as a bio-control agent in *A. baumannii*-S-MH bacterial therapy. At temperatures between 10 and 70°C and pH range between pH 2 and 11 for 1h. *A. baumannii*-S-MH 's phage was stable. Previous studies showed stability of Phage (VB_AciM-AM-M) against *A. baumannii*-S-MH under a wide range of temperature degrees (4–70°C) and resisted in acidic or

alkaline pH (pH 2–11). These findings make the isolated phage highly potent to be used in clinical settings.

Growth kinetics of the isolated phages exhibited typical growth kinetics of most bacteriophages. Phage (VB_AciM-AM-M) gave burst sizes of 290 PFU/cell with a latent period of 20 Minutes. These values, similar to those obtained in other studies with *A. baumannii*-S-MH 's phages where the average burst size of such phages was approximately 200 PFU/ infected cell and latent period 9 min (Jin *et al.,* 2012).

Based on the stability and lytic activity of the isolated phages against *A. baumannii*-S-MH, they were applied to control such hosts in broth medium using different MOI. Findings showed that MOI 1, 3 and 5 appeared efficient to eradicate the bacterial growth and prevent their regrowth in broth medium. Thus, the involvement of these phages in therapy could be promising as alternatives to antibiotics.

CONCLUSION

Health concerns related to diseases have been exacerbated by antibiotic-resistant *A. baumannii*. Hence, bacteriophages could be proposed as an alternative strategy to mitigate the causative bacterial pathogens. In this study, virulent phage was isolated for antibiotic-resistant *A. baumannii*-S-MH's phage (VB_AciM-AM-M) belonged to the family *Myoviridae* morphologically. The phage has narrow host ranges, low latent periods, strong pH and thermal resistance. More importantly, our findings demonstrate the efficacy of phage (VB_AciM-AM-M) for the inhibition of multidrug-resistant *A. baumannii*-S-MH growth *in vitro*. This research forms the basis for the therapeutic application of phage (VB_AciM-AM-M) to manage *A. baumannii*-S-MH infections.

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Supplementary Data:

Table. s1, Biochemical characterization of the isolated *A. baumannii* S-MH

Morphological characteristics and Biochemical tests	
Test	Result
Morphology & arrange	Coccobacilli
Motility	Non motile
Gram stain	-
Hemolysis production	- (ɣ hemolysis)
Oxidase production	-
Catalase	+
Lactose fermentation	-
Indole	-
Urease	-
Nitrate reduction	-

*Negative result (-ve) , positive result (+ve).

Table. S2, Bacterial strength of this study.

species	Strain ID number	Sources
Acinetobacter baumannii	*Acineto-z*	BUH
	Acineto-G	BUH
	Acineto-M	BUH
	Acineto-N	BUH
	Acineto-E	BUH
	Acineto-MEL	BUH
	Acineto-MA	BUH
	Acineto-HA	BUH
	Acineto-AS	BUH
	Acineto-AA	BUH
	Acineto-AE	BUH
Proteus	*Proteus 1*	BUH
	Proteus 2	BUH
Shigella spp	*Sh1*	FSCBU
E-coli	*E-1*	FSCBU
Staphylococcus aureus	*S.au 1*	FSCBU
Salmonella entrica	*Sa 1*	FSCBU
Pseudomonas aeruginosa	*Pseudo 1*	BUH
	Pseudo 2	BUH
Klebsiella pneumonia	*Kleb 1*	BUH

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*2 FSCBU (Microbiology lab, Botany and Microbiology Dept. Faculty of Science, Benha Univ.).