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Research Article

In Vitro Meropenem/Antibiotic and Meropenem/Bacteriophage Combinations against Carbapenem-Resistant Gram-Negative Uropathogens

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Abstract

Carbapenem antibiotics are among the last-resort treatments for infections caused by multidrug-resistant bacteria. The emergence and spread of carbapenem resistance (CR) have led researchers to seek alternative treatment approaches to antibiotic monotherapy. The goal of this study was to investigate the effect of combining meropenem, a carbapenem antibiotic, with other antibiotics and bacteriophages *in vitro* against CR Gram-negative uropathogens to detect potential synergism. The checkerboard assay was used to explore potential synergistic effects against 19 CR Gram-negative uropathogens when combining meropenem with amikacin, colistin, and tigecycline. Bacteriophages were isolated from hospital sewage, and morphologically classified, and the lytic phages were combined with meropenem for detecting potential synergistic effects against the tested CR Gram-negative uropathogens. Combining meropenem with amikacin, colistin, and tigecycline showed additive effects against 89.4%, 63.2%, and 68.4% of the tested CR uropathogens, respectively. Synergism was observed against a CR *K. terrigena* isolate when meropenem and amikacin were combined at doses of 4 and 8 µg/mL, respectively. The isolated phages belong to the *Myoviridae* family of the order *Caudovirales*. In plaque assay, translucent halos were formed around the clear plaques of phages infecting a CR *A. baumannii* isolate, suggesting the depolymerase-producing ability of these phages. Combining the isolated phages in this study with meropenem did not exhibit any synergistic effect *in vitro* against the tested isolates. In conclusion, Meropenem/amikacin combination showed *in vitro* synergistic activity against a clinical CR *K. terrigena* isolate. Synergism was absent upon combining meropenem with the isolated phages in this study against CR Gram-negative uropathogens.

Keywords: *meropenem; carbapenem; combination; carbapenem resistance; bacteriophage; synergism*.

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1. Introduction

Carbapenem antibiotics are currently regarded as the most effective treatment option for infections caused by multidrug-resistant (MDR) pathogens and extended-spectrum β-lactamase (ESBL)-producers **[1]**. There has been an increase in the administration of carbapenems over the past years to tackle the increased resistance rates to penicillins, cephalosporins, fluoroquinolones, and aminoglycosides **[2]**. Unfortunately, this has led to the development of carbapenem resistance (CR) in Gram-negative

bacteria (GNB), which in turn has caused a widespread public health crisis owing to the rapid dissemination of CR and the scarcity of alternative last-resort antibiotics **[3]**.

Antibiotic combination therapy is frequently considered for treating CR infections even in the lack of supporting clinical data **[4]**. Combination regimens generally include a carbapenem, an aminoglycoside, polymyxin, or tigecycline. Combination therapy has improved survival in patients with a high mortality score **[5, 6]**. Current Infectious Diseases Society of America (IDSA) guidance on the treatment of carbapenem-resistant *Acinetobacter baumanii* (CRAB) suggests combination antibiotic therapy due to lack of data with monotherapy, high bacterial burdens with delayed therapy, the fact that most patients with CRAB are critically ill, and the ability for inducible resistance **[7]**.

Phage therapy has become a promising approach to treating resistant bacterial infections, especially virulent phages **[8]**. The prevalence of MDR *A. baumannii* has increased in the past few years, leading to the introduction of tigecycline and colistin to the treatment regimens of infections caused by this pathogen. Unfortunately, *A. baumannii* isolates exhibiting colistin and tigecycline resistance have emerged, thus phage therapy was considered **[8]**. Several studies have been reported demonstrating the effectiveness of bacteriophage/antibiotic combination therapy when antibiotics either alone or in combination failed to eradicate the etiological bacteria **[9–11]**.

We aimed to explore the *in vitro* effect of combining meropenem, a carbapenem antibiotic, with other antibiotics often used in the treatment of resistant infections, and with lytic bacteriophages isolated from hospital sewage against CR-GNB uropathogens as an approach to overcome or reduce CR in such isolates.

2. Materials and Methods

2.1. Bacterial Isolates

The bacterial isolates used in this study were collected from the microbiology laboratories of Kasr Al-Ainy and El-Demerdash Tertiary Care Hospitals, Cairo, Egypt, from urinary tract infection (UTI) patients. The study was approved by the Ethics Committee of the Faculty of Pharmacy Ain Shams University (EN-REC-ASU-2019-98) and following the Declaration of Helsinki **[12]**. Additional information including the collection methods, antimicrobial susceptibility testing, phenotypic analysis, genotyping, PCR amplification, and transformation was previously published **[13]**.

2.2. Minimum Inhibitory Concentrations of the Tested Antibiotics

The minimum inhibitory concentrations (MICs) of meropenem, amikacin, colistin, and tigecycline were measured against 19 CR-GNB uropathogens by broth microdilution method following CLSI guidelines **[14, 15]**. The tested CR uropathogens included 15 *Klebsiella* spp., 2 *P. aeruginosa*, 1 *E. coli*, and 1 *A. baumannii* isolates. According to the findings of antimicrobial susceptibility testing, all the tested isolates were meropenem resistant and carried at least one carbapenemase gene (as revealed from PCR results). The results of antimicrobial susceptibility testing and PCR were previously published **[13]**.

2.3. Meropenem/Antibiotic Combinations

In an attempt to reduce or overcome CR in 19 meropenem-resistant uropathogens, meropenem was combined with other antibiotics often used in the treatment of resistant infections (amikacin, colistin, and tigecycline) to study the impact of such combinations on the activity of meropenem against the tested CR isolates *in vitro* using the checkerboard assay **[16]**. In brief, after the MICs of each antimicrobial agent

(meropenem, amikacin, colistin, tigecycline) against the tested isolates were determined, the stock solutions of the tested antimicrobial agent were prepared just before testing. Two-fold serial dilutions of the tested antimicrobials were prepared in 96-well microtiter plates with round-bottom wells where one of the antimicrobials of the combination was serially diluted horizontally, while the other was diluted vertically. Concentrations ranging from 4 MIC to l/16 MIC of the tested antimicrobials were prepared to observe the extent of synergism or antagonism. For the tested isolates, bacterial suspensions with turbidity equivalent to 0.5 McFarland were prepared in sterile saline (10^8 cfu/mL), and 1 mL from each suspension was transferred to 9 mL DS-MHB (10^7 cfu/mL). The antimicrobial stock was prepared at 32 MIC concentration of the tested antimicrobial. In a 96-well microtiter plate, 50 µL aliquots of sterile distilled water were added to the wells of columns 1 through 8. For antimicrobial (A), two-fold serial dilutions were prepared by adding 50 µL aliquots of stock (A) to wells of column 1, then transferring 50 µL from each well to the next in the sequence (columns 1 through 7), followed by discarding 50 µL aliquots from wells of column 7 (antimicrobial (A) was not added to wells of column 8). For antimicrobial (B), another 96-well microtiter plate was used, where 50 µL aliquots of sterile distilled water were added to the wells of columns 1 through 8. Two-fold serial dilutions of antimicrobial (B) were prepared by adding 50 µL aliquots of stock (B) to wells of row A, then transferring 50 µL from each well to the next in the sequence (rows A through G), followed by discarding 50 µL aliquots from wells of row G (antimicrobial (B) was not added to wells of row H). To prepare the combination plate, 50 µL from wells of plate (B) were transferred to wells of the plate (A) using a multichannel micropipette. The volume of the antimicrobial combination in each well was 100 µL. Column 8 contained antimicrobial (B) alone, and row H contained antimicrobial (A) alone (for calculating the MIC of each alone). Finally, 100 µL aliquots of the bacterial suspension were added to all the wells, and the final volume in each well was 200 µL (final inoculum size = $5x10^5$ cfu/mL). Well no. H8 was used as a positive control (100 µL bacterial suspension + 100 µL sterile distilled water). The plates were incubated at 37°C overnight **[16]**. The fractional inhibitory concentration index (FIC index) was then calculated according to the equation:

$$FIC = FIC\ (A) + FIC\ (B)$$

$$= \frac{\text{MIC of A in combination}}{\text{MIC of A alone}} + \frac{\text{MIC of B in combination}}{\text{MIC of B alone}}$$

The combination was regarded as synergistic when the FIC index was ≤ 0.5, additive when the FIC index was > 0.5 - ≤ 1, indifferent when the FIC index was > 1 - ≤ 4, and antagonistic when the FIC index was > 4 **[17]**.

2.4. Isolation and Identification of Bacteriophages Infecting CR-GNB Isolates

Five sewage samples labeled A, B, C, D, and E were collected from five separate wards at El-Demerdash Tertiary Care Hospital and kept at 4 °C until processing. Five meropenem-resistant isolates carrying CR genes (2 *K. pneumoniae* isolates, 24.SK and 37.AK; 1 *A. baumannii* isolate, 38.WA; 1 *P. aeruginosa* isolate, 54.WP; and 1 *E. coli* isolate, 48.WE) **[13]** were used as indicator bacterial host strains for the isolation of bacteriophages from each of the collected sewage samples. The sewage samples were separately filtered using filter paper to remove suspended particles, and 10 mL of the filtrate was then transferred to a sterile centrifuge tube and centrifuged at 4000 rpm for 15 min. After centrifugation, the supernatants were aseptically filtered via 0.22 µm low protein-binding polyvinylidene difluoride (PVDF) membrane filters to eliminate bacteria and cellular debris

yielding 5 potential viral suspensions to be tested **[18]**.

2.5. Phage Enumeration by Plaque Assay and Phage Purification

The presence of phages in the prepared viral suspensions was verified by plaque enumeration assay. The double-layer agar method was performed as previously described by Jofre et al. **[19]**. The plates were examined for plaques (clearing zones) on the bacterial lawn after a 24-hour incubation period to determine the presence of phages. The number of lytic plaques was counted to calculate the phage titer. Phage purification was carried out as previously described **[19]**, and the obtained phage lysates were enumerated by plaque assay.

2.6. Transmission Electron Microscopy and Nomenclature of Phages

High-titer phage lysates were prepared and enumerated as previously described by Jofre et al. **[19]** and were sent to NanoTech for Photo Electronics, Egypt, where they were negatively stained with 2% (w/v) phosphotungstic acid and observed using transmission electron microscopy (TEM). Head and tail lengths from TEM images were measured via ImageJ software **[20]**. Based on the morphological features of the virions, the phages were identified and classified following the guidelines of the International Committee on Taxonomy of Viruses (ICTV) **[21]**.

2.7. Bacteriophage Host Range by Spot Test

The phage lysates were tested against 65 CR-GNB uropathogens to determine the specificity of these phages. The tested isolates included *K. pneumoniae* (22; 33.8%), *K. terrigena* (6; 9.2%), *P. aeruginosa* (19; 29.2%), *A. baumannii* (8; 12.3%), *E. coli* (6; 9.2%), *P. mirabilis* (2; 3.1%), and *E. cloacae* (2; 3.1%) **[13]**. The spot test was conducted as previously described by Clokie et al., and the plates were examined for visible plaques within the spotting zones **[18]**. In brief, overnight bacterial cultures of the tested bacterial isolates were prepared in 9 ml TSB. Underlay TS agar was prepared (TSB + 1.5% agar) and poured into sterile Petri dishes and left to solidify. Overlay TS agar was prepared and distributed as 3 ml aliquots in test tubes, sterilized, and stored till the time of use. One ml of bacterial suspension (OD_{600}= 0.3) was added to 3 mL melted overlay TS agar, vortexed, then poured on top of the underlay agar. The overlay was allowed to solidify at room temperature. Then, 10 µL of each of the phage lysates were spotted on the plate of the tested isolate. The plates were allowed to dry at room temperature without disruption for 15 min before overnight incubation at 37 °C. After incubation, the plates were examined for visible plaques within the spotting zones **[18]**.

2.8. Phage Lytic Efficacy

The phage lytic efficacy was determined as described by Nikolic et al. **[22]** with few modifications. Briefly, 10-fold dilutions of phage lysates were prepared in 96-well microtiter plates in Mueller-Hinton broth. A suspension of the tested isolate with turbidity matching that of a 0.5 McFarland standard was prepared in saline ($1x10^8$ cfu/mL) and then diluted 1:20 in ($5x10^6$ cfu/mL). 10 µL of bacterial suspension (5×10^5 cfu/mL) were added to the wells. The final volume in each well was 100 µL. The plates were incubated overnight at 37 °C. The multiplicity of infection (MOI) that prevented bacterial growth was determined and assigned ‘minimal inhibitory MOI’ (MIM).

2.9. Meropenem/Bacteriophage Combinations

Meropenem/bacteriophage interactions were examined via the checkerboard assay in 96-well microtiter plates. Every plate was prepared with 2-fold phage MOI in columns 1 through 10 and 2-fold meropenem dilutions in rows A through G. Meropenem alone was in column 11, while phage

alone was in row H. Controls in column 12 included: bacterial growth, medium sterility, antibiotic stock sterility, and phage suspension sterility controls. The results were interpreted based on the calculated FIC index values **[22]**.

3. Results

3.1. Minimum Inhibitory Concentrations of the Tested Antibiotics

The MICs of meropenem, amikacin, colistin, and tigecycline were measured against 19 CR-GNB uropathogens by the broth microdilution method. Meropenem resistance was observed in 19 CR-GNB isolates (100%), amikacin resistance was observed in 15 out of 19 (78.9%) isolates, and colistin resistance was observed in 1 (5.3%) of the tested isolates. The 2 (10.5%) *P. aeruginosa* isolates examined in this assay were resistant to tigecycline because of the drug's low antibacterial activity against *P. aeruginosa*, **[23]** but none of the other tested isolates were resistant to tigecycline. None of the tested isolates were co-resistant to the four antibiotics in the assay; however, 2 (10.5%) isolates exhibited co-resistance to three antibiotics; 14 (73.7%) isolates exhibited co-resistance to two antibiotics, and 3 (15.8%) isolates exhibited meropenem resistance only. The MICs of antimicrobial agents against the tested CR isolates are listed in **Table 1**.

Table 1. The minimum inhibitory concentrations of antimicrobial agents against the tested carbapenem-resistant Gram-negative uropathogens

Isolate code	Species	MIC of antimicrobial agents (µg/mL)/Susceptibility			
		MEM	AK	COL	TGC
6.SK	*K. terrigena*	64/R	2048/R	1/I	2/S
11.SK	*K. terrigena*	32/R	32/I	2/I	2/S
12.SP	*P. aeruginosa*	64/R	32/I	1/I	128/R
18.SK	*K. pneumoniae*	8/R	2048/R	0.25/I	4/I
24.SK	*K. pneumoniae*	64/R	1/S	2/I	0.5/S
27.AK	*K. pneumoniae*	64/R	2048/R	1/I	4/I
28.AK	*K. terrigena*	8/R	2048/R	2/I	2/S
35.AK	*K. terrigena*	64/R	2048/R	0.5/I	2/S
37.AK	*K. pneumoniae*	8/R	4096/R	0.5/I	0.5/S
38. WA	*A. baumannii*	32/R	4096/R	2/I	2/S
41.WK	*K. terrigena*	32/R	2048/R	0.25/I	4/I
42.WK	*K. pneumoniae*	256/R	2048/R	0.5/I	0.5/S
44.WK	*K. pneumoniae*	256/R	1024/R	0.5/I	4/I
48. WE	*E. coli*	64/R	16/S	1/I	4/I
53.WK	*K. pneumoniae*	64/R	2048/R	0.25/I	4/I
54.WP	*P. aeruginosa*	256/R	4096/R	2/I	128/R
59.WK	*K. pneumoniae*	128/R	2048/R	32/R	2/S
60.WK	*K. pneumoniae*	128/R	1024/R	0.5/I	1/S
66.WK	*K. pneumoniae*	128/R	4096/R	0.25/I	1/S

MIC, minimum inhibitory concentration; MEM, meropenem; AK, amikacin; COL, colistin; TGC, tigecycline; R, resistant; I, intermediate sensitivity; S, susceptible.

3.2. Meropenem/Antibiotic Combinations

The effects of various combinations of meropenem with other antibiotics including amikacin, colistin, and tigecycline were examined *in vitro* on 19 selected CR uropathogens. Apart from one *K. terrigena* isolate (11.SK), where synergism was apparent when meropenem and amikacin were combined at doses of 4 and 8 µg/mL, respectively, combining meropenem with amikacin, colistin, and tigecycline showed additive effects against 89.4%, 63.2%, and 68.4% of the tested CR uropathogens, respectively. The calculated FIC index values of the meropenem/antibiotic combinations are shown in **Table 2**.

Table 2. The calculated FIC index values of the meropenem/antibiotic combinations in this study against 19 carbapenem-resistant Gram-negative uropathogens

Isolate code	Species	MEM + AK		MEM + COL		MEM + TGC	
		FIC index	Interpretation*	FIC index	Interpretation*	FIC index	Interpretation*
6.SK	*K. terrigena*	0.75	Additive	0.75	Additive	2.016	Indifferent
11.SK	*K. terrigena*	0.375	Synergism	0.75	Additive	1.25	Indifferent
12.SP	*P. aeruginosa*	0.625	Additive	2.063	Indifferent	1.063	Indifferent
18.SK	*K. pneumoniae*	0.516	Additive	0.508	Additive	1	Additive
24.SK	*K. pneumoniae*	1.5	Indifferent	1.031	Indifferent	1.031	Indifferent
27.AK	*K. pneumoniae*	0.531	Additive	0.75	Additive	1.031	Indifferent
28.AK	*K. terrigena*	0.625	Additive	1.016	Indifferent	0.516	Additive
35.AK	*K. terrigena*	0.75	Additive	1.016	Indifferent	1	Additive
37.AK	*K. pneumoniae*	0.75	Additive	1.063	Indifferent	0.531	Additive
38.WA	*A. baumannii*	0.625	Additive	0.563	Additive	1	Additive
41.WK	*K. terrigena*	1	Additive	1	Additive	1	Additive
42.WK	*K. pneumoniae*	0.75	Additive	0.75	Additive	1	Additive
44.WK	*K. pneumoniae*	0.625	Additive	1.016	Indifferent	1	Additive
48.WE	*E. coli*	1	Additive	1	Additive	0.75	Additive
53.WK	*K. pneumoniae*	0.75	Additive	1	Additive	0.531	Additive
54.WP	*P. aeruginosa*	1	Additive	1	Additive	1	Additive
59.WK	*K. pneumoniae*	0.625	Additive	0.75	Additive	0.75	Additive
60.WK	*K. pneumoniae*	1	Additive	0.75	Additive	1.031	Indifferent
66.WK	*K. pneumoniae*	0.75	Additive	1.016	Indifferent	0.75	Additive

*FIC index was calculated using the lowest concentration of the respective antimicrobial agents at which the lowest value of FIC was achieved. Synergism ≤0.5, additive >0.5 - ≤1, indifference >1 - ≤4, antagonism >4. FIC index, fractional inhibitory concentration index; MEM, meropenem; AK, amikacin; COL, colistin; TGC, tigecycline.

3.3. Isolation of Bacteriophages and Phage Enumeration by Plaque Assay

Four out of five examined sewage samples (sewage samples A, B, C, and D) yielded lytic phages that were successfully isolated and were infective to two indicators of CR bacterial host strains, namely *K. pneumoniae* (37.AK) and *A. baumannii* (38.WA). As a result, the phage lysates that infected isolate 37.AK was coded PL37-A, PL37-B, PL37-C, and PL37-D, while the phage lysates that infected isolate 38.WA were coded PL38-A, PL38-B, PL38-C, and PL38-D. It was noticed that the phage lysates infective to one of the isolates were not infective to the other. Since no phages were discovered in sewage sample E with any of the examined indicator bacterial host strains, the sample was excluded from the investigation.

3.4. Phage Purification

Single plaques of phage lysates PL38-A, PL38-B, PL38-C, and PL38-D were successfully excised and isolated from overlay agar of plaque assay plates while preserving their infectivity to their host strain isolate *A. baumannii* (38.WA) as indicated by clear zones in spot test. However, only phage lysate PL37-C was able to maintain its infectivity to its host strain *K. pneumoniae* (37.AK), but regrettably, infectivity was spontaneously lost after one week of storage at 4°C. Therefore, additional experiments involving bacteriophages were conducted exclusively on phage lysates infective to *A. baumannii* (38.WA).

3.5. Transmission Electron Microscopy

The images of the phages obtained using TEM are shown in **Fig. 1**. The phages' non-enveloped head-tail configurations prompted our conclusion that they belong to the *Myoviridae* family of the order *Caudovirales*. The four phages were given the names Acinetobacter phage vB_AbaM-P38-A, Acinetobacter phage vB_AbaM-P38-B, Acinetobacter phage vB_AbaM-P38-C, and Acinetobacter phage vB_AbaM-P38-D. In the parts that follow, the names P38-A, P38-B, P38-C, and P38-D are used as the phages' common names.

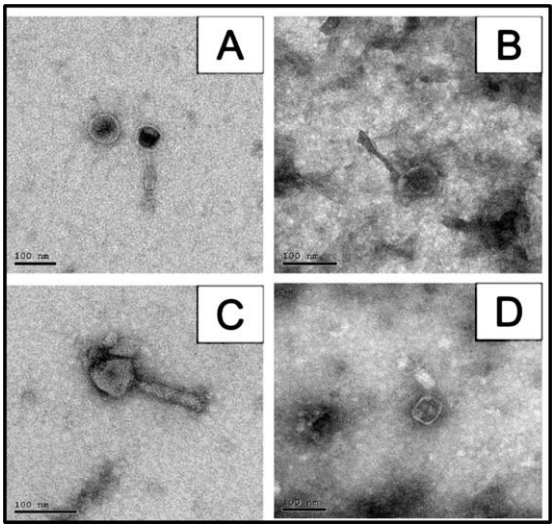

Fig. 1. Transmission electron microscopy of bacteriophages (A) Acinetobacter phage vB_AbaM-P38-A virion, (B) Acinetobacter phage vB_AbaM-P38-B virion, (C) Acinetobacter phage vB_AbaM-P38-C virion, and (D) Acinetobacter phage vB_AbaM-P38-D virion. Scale bar represents 100 nm.

3.6. Bacteriophage Host Range by Spot Test

The phages P38-A, P38-B, P38-C, and P38-D were tested against 65 CR-GNB uropathogens to determine the specificity of the phages. It was found that the phages could infect only 1 isolate; their original host strain *A. baumannii* (38.WA), indicated by clear visible plaques within the spotting zones **(Fig. 2)**. It was thus concluded that the phages had a narrow host range.

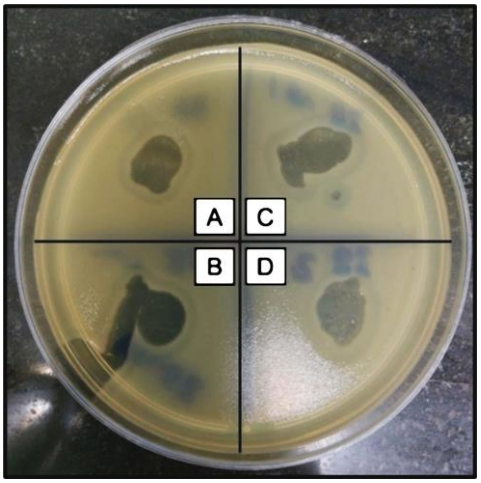

Fig. 2. Spot test of (A) phage P38-A, (B) phage P38-B, (C) phage P38-C, (D) phage P38-D infecting their original host strain *A. baumannii* (38.WA).

3.7. Phage Lytic Efficacy

The phage lytic efficacy of bacteriophages was determined by the method described by Nikolic et al. **[22]**. The MOI that prevented bacterial growth was recorded and assigned 'minimal inhibitory MOI' (MIM). The obtained MIM values of phages against *A. baumannii* (38.WA) isolate used as a basis for the synergy experiments (meropenem/bacteriophage combinations) were 37.6, 4.78 x 10^5, 2.62, and 89 for phages P38-A, P38-B, P38-C, and P38-D, respectively.

3.8. Meropenem/Bacteriophage Combinations

The *in vitro* combinations of meropenem with the phages in this study showed indifferent effects against the tested CR *A. baumannii* (38.WA) isolate. The FIC index values are listed in **Table 3.**

Table 3. FIC index values were calculated for four different phage combinations with meropenem against the carbapenem-resistant uropathogen *A. baumannii* (38.WA)

Combination	FIC index	Interpretation*
MEM / P38-A	1.25	Indifferent
MEM / P38-B	1.25	Indifferent
MEM / P38-C	2.25	Indifferent
MEM / P38-D	1.25	Indifferent

*FIC index was calculated using the lowest concentration of the respective antimicrobial agent and bacteriophages at which the lowest value of FIC was achieved. Synergism ≤0.5, additive >0.5 - ≤1, indifference >1 - ≤4, antagonism >4. FIC index, fractional inhibitory concentration index; MEM, meropenem.

4. Discussion

The increased reliance on carbapenems for treating resistant infections has led to the emergence of CR-GNB such as *Enterobacterales, Acinetobacter* spp., and *Pseudomonas* spp. We aimed to investigate the impact of combining a carbapenem antibiotic, meropenem, with other antibiotics and with bacteriophages against CR-GNB uropathogens *in vitro*, in an attempt to reduce or overcome such resistance in these isolates.

Meropenem was combined with amikacin, colistin, and tigecycline to study the effect of such combinations on the activity of meropenem against the tested CR uropathogens *in vitro*. Synergism was observed against a CR *K. terrigena* isolate (11.SK) when meropenem and amikacin were combined at concentrations of 4 and 8 µg/mL, respectively, with an obtained FIC index value of 0.375. Similar to our results, a study conducted by Ota et al. to evaluate the efficacy of meropenem/amikacin combination therapy in a mouse model of pneumonia infected by CR *K. pneumoniae* revealed the *in vivo* efficacy of meropenem/amikacin combination therapy which caused a significant decrease in the bacterial count of the lungs in the group receiving combination therapy compared to meropenem and amikacin control groups **[24]**.

Currently, phage therapy is emerging as a promising treatment option for MDR bacterial infections **[25]**. Thus, in this study we isolated lytic bacteriophages from hospital sewage samples, hoping to find phages with the potential for treating CR infections **[26]**. Some of the isolated phages in the present study lost their infectivity to their host strain. However, four bacteriophages infective with CR-*A. baumannii* were successfully propagated and morphologically characterized.

The ability of some *A. baumannii*-infecting phages to produce translucent halos around the plaques on bacterial lawns is one of the key features indicating the depolymerizing activity of the phage, which cleaves the bacterial surface polysaccharides via phage-associated enzymes known as depolymerase **[26]**. When phages P38-A, P38-B, P38-C, and P38-D infected *A. baumannii* (38.WA), these halos were observed around the clear plaques, suggesting that these phages produce depolymerase.

A host range restricted to a single species is useful when phage therapy is being considered since it keeps the phage from killing other species, allowing the host's microbiome to remain unaffected and preventing diluting the effective concentration of the phage toward the target bacteria **[27]**. The phages P38-A, P38-B, P38-C, and P38-D could only infect one of the tested CR-GNB isolates (n= 65) in the host range determination experiment of the current work, which was their original host strain *A. baumannii* (38.WA). These findings revealed that the phages have a limited host range and would be beneficial in phage therapy without jeopardizing the host's normal flora or diluting the effective concentration of the phage toward the target bacteria. However, *in vivo* testing is required before the application of these phages in phage therapy.

Several studies have been reported demonstrating the effectiveness of bacteriophage/antimicrobial combination therapy when antimicrobials either alone or in combination failed to eradicate the etiological bacteria **[9–11, 28, 29]**. A study conducted by Jansen et al. aimed to investigate the antibacterial outcome of combining the phage vB_AbaM-KARL-1 (with lytic activity against MDR *A. baumannii*) with meropenem, ciprofloxacin, and colistin antibiotics. The study found that a complete clearance of liquid cultures was achieved when the phage KARL-1 (MOI=10^{-1}) was combined with meropenem (>128 µg/mL), and the antibacterial activity was significantly augmented with meropenem **[30]**. Another study by Luo et al. tested the bactericidal effect of the phage YC#06 combined with antibiotics commonly used to treat MDR *A. baumannii* infections. Phage-antibiotic synergism was observed with chloramphenicol, minocycline, imipenem, and cefotaxime **[31]**. Accordingly, we studied the impact of combining bacteriophages and meropenem *in vitro* to determine whether there might be any synergism between them against the MDR CR-*A. baumannii* isolate in our study. However, all the meropenem/bacteriophage combinations in the current study showed indifferent effects against the tested CR *A. baumannii* uropathogen.

Limitations

There are some limitations in our study. For the identification of phages at present, genetic characterizations have more weight than morphological ones. Our future perspectives include the characterization of phage genomes for full identification and a better understanding of the isolated phages in this study.

Conclusions

Synergism was observed when meropenem was combined with amikacin *in vitro* at concentrations of 4 and 8 µg/mL, respectively, against a CR *K. terrigena* isolate recovered from the urine specimen of an Egyptian patient with a complicated urinary tract infection. Additive effects were observed when meropenem was combined *in vitro* with amikacin, colistin, and tigecycline against 89.4%, 63.2%, and 68.4% of the tested carbapenem-resistant uropathogens, respectively. In the plaque assay of phages, translucent halos were formed around the clear plaques of phages P38-A, P38-B, P38-C, and P38-D when infecting a CR *A. baumannii* isolate,

suggesting the depolymerase-producing ability of these phages. Phages P38-A, P38-B, P38-C, and P38-D have a narrow host range and are thus potentially beneficial in phage therapy without impairing the host's normal flora or diluting the effective concentration of the phage toward the target bacteria. Combining phages P38-A, P38-B, P38-C, and P38-D with meropenem *in vitro* did not exhibit any synergistic effect against the tested CR *A. baumannii* isolate in our study.

Recommendations

Alternatives to antimicrobial therapy should be considered to reduce the rate by which resistance is increasing.

List of Abbreviations

CLSI, Clinical and Laboratory Standards Institute; CR, carbapenem resistance; CRAB, carbapenem-resistant *Acinetobacter baumanii*; DS-MHB, double strength Mueller-Hinton broth; ESBL, extended-spectrum β-lactamase; FIC, fractional inhibitory concentration; GNB, Gram-negative bacteria; ICTV, the International Committee on Taxonomy of Viruses; IDSA, Infectious Diseases Society of America; MDR, multidrug-resistant; MHB, Mueller-Hinton broth; MIC, minimum inhibitory concentration; MIM, the minimum inhibitory multiplicity of infection; MOI, the multiplicity of infection; PCR, polymerase chain reaction; PVDF, polyvinylidene difluoride; TEM, transmission electron microscopy; UTI, urinary tract infection.

Declarations

Ethics approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Faculty of Pharmacy Ain Shams University Ethics Committee (ENREC-ASU-2019-98).

Consent to participate

Informed consent was obtained from the patients after explaining the purpose of the study.

Consent to publish

Not applicable

Data availability statement

All the data supporting the findings are included in the manuscript.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' contributions statement

All authors contributed to the conceptualization of the study. Material preparation, data collection, and analysis were performed by Ann A. Elshamy, Sarra E. Saleh, Khaled M. Aboshanab, and Mohammad M. Aboulwafa. The original draft of the manuscript was written by Ann A. Elshamy and Sarra E. Saleh. The revision and editing of the manuscript were done by Khaled M. Aboshanab, Mohammad M. Aboulwafa, and Nadia A. Hassouna. All authors have read and agreed to the published version of the manuscript.

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