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Anti-biofilm Activity of Phage ΦKAB and Colistin Against Carbapenem Resistance *Acinetobacter baumannii*

Kareema Ali Bahr [a], Ban O. Abdulsattar [b], Susan A. Ibrahim [b], and Ashraf A. Abd EL-Tawab [c]

[a] *Department of Biology, College of Science, Tikrit University, Tikrit, Iraq*
[b] *Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq*
[c] *Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Benha University, Egypt*

CORRESPONDANCE
Susan A. Ibrahim
dr.susan.ibrahim@uomusansiriyah.edu.iq

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ABSTRACT: ***Background:*** *Carbapenem-resistant A. baumannii (CRAB) bacterium is difficult to treat with available antimicrobial agents leading to use a few antibiotics such as colistin as an option for treatment. However, the use of the colistin has serious side effects and leads to developing bacterial resistance.* ***Objective:*** *This study aimed to determine effectiveness of phage/colistin combination to inhibit biofilm formation of CRAB.* ***Methods:*** *Sixty clinical A. baummanii isolates were identified with the VITEKII system and 16S rRNA gene. The antibiotic susceptibility and detection of Oxacillinases genes were tested for all isolates.* ***Results:*** *The antibiotic sensitivity of A. baumannii isolates showed a high resistance percentage to Ceftriaxone (CRO) with 92% (55 isolates), and Cefotiam (CTF) with 87% (52 isolates), while the lowest percent related to Colistin (CO) with 17% (10 isolates). The results of antibiotic resistance and Oxacillinases genes reported that only 14 isolates from the current study were CRAB. Phage and host A. baumannii of phage were isolated and characterized previously. Biofilm production assay of CRAB isolates were showed that among 14 isolates including the phage host: 57% (8) was weak and 43% (6) was moderate biofilm producer. Therefore, the synergistic effect of a combination ΦKAB phage in MOI (10) and Colistin with MIC=8 µg/ml against CRAB isolates was evaluated and showed complete transfer of isolates by 100 % to weak biofilm producer compared with CRAB isolates.* ***Conclusions:*** *Among 60 isolates of A. baumannii, most isolates were MDR 44 (73.3%) and only 14 (23.3%) isolates were CRAB that were 57% weak and 43% moderate biofilm producers. The mixture of Colistin/ΦKAB phage could inhibit and reduce biofilm forming in CRAB isolates. Overall, the present study may provide evidence on the capability of the isolated phage to serve as a novel strategy to treat infections caused by MDR A. baumannii.*

KEYWORDS: *Acinetobacter baumannii; Carbapenem resistance; Bacteriophage; Anti-biofilm; Oxacillinases genes*

INTRODUCTION

Acinetobacter baumannii's (*A. baumannii*) infection is associated with the modern healthcare system, which is spreading around the world as an opportunistic pathogen that is responsible for hospital-acquired infections (nosocomial infections), particularly in intensive care units [1]. *A. baumannii* is a part of the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) pathogens, which are responsible for the majority of antibiotic-resistant nosocomial infections around the world [2]. Recent local studies indicated that antibiotic-resistant bacteria including *A. baumannii* were spreading rapidly and uncontrollably [3], [4]. Carbapenems are the most effective broad-spectrum antibacterial from the β-lactam family. They were previously used to treat *A. baumannii* infections due to their high antibacterial activity and low toxicity [5]. Carbapenems have a strong affinity for the

highest molecular weight penicillin binding proteins (PBPs) of both gram-positive and gram-negative bacteria. *A. baumannii* has evolved resistance to this antibiotic. The hospital's intensive care unit (ICU) has seen an increase in carbapenem-resistant *A. baumannii* intra and inter-hospital transmission [6], [7]. CRAB is usually associated with the advent of Carbapenem-hydrolyzing β-lactamases of class D known as OXAs (Oxacillinases) and less frequently with Metallo β-lactamases [8]. The available antimicrobial agents are currently almost difficult to treat carbapenem-resistant *A. baumannii* (CRAB) isolates [7]. Colistin is the most frequent component of antimicrobial combinations even against colistin-resistant *A. baumannii*, which revealed that the most effective combinations against colistin-resistant *A. baumannii* are colistin-rifampin and colistin-teicoplanin [9]. Nevertheless, higher doses of colistin might cause side effects such as renal and neurological [10], [11]. The ability of *A. baumannii* to form biofilm on different surfaces increases infection persistence and bacterial survival [12]. The formed biofilms decrease susceptibility to antibiotics significantly and that would enhance *A. baumannii* pathogenicity and develop resistance to a broad spectrum of antimicrobial agents [13]. There is an increasing interest in finding multiple alternatives to reduce and control infections mediated by antibiotic-resistant *A. baumannii*. Bacteriophages are typically highly specific and may be active against antibiotic-resistant Gram-positive and Gram-negative bacteria. When multiple antibiotic drugs failed to treat *A. baumannii* infections, phage therapy was applied with exceptionally. Phage therapy is proposed as an effective treatment for wound, ulcer, and skin infections caused by gram-negative nosocomial pathogens including *A. baumannii* [14]. Recently, studies indicate that phages could also be effective on cells forming a biofilm, which marks phages an alternative antibiotic treatment [15]. This study detected CRAB isolates using Kirby-Bauer methods and Oxacillinases genes. In addition, a combination of novel phage ΦKAB with colistin was assessed against CRAB. Further investigation to evaluate anti-biofilm activity *in-vivo* is needed.

MATERIALS AND METHODS

Bacterial Isolates Morphological Identification

A total of 85 clinical isolates were collected between 2022 and 2023 from different sources. Each sample was cultured on MacConkey agar, Blood agar plates and Chrom agar for primary identification. The initial identification of each isolate depended on its morphological characteristics based on its growth that appeared on selected medium and biochemical tests [16]. As well as identification of bacterial isolates was performed by VITEKII compact System and *16S rRNA* gene.

Molecular Identification

Extraction of Genomic DNA

All *A. baumannii* isolates' genomic DNA was were extracted using the boiling and freeze-thawing method [17].

Primers Stock Preparation

The lyophilized primers (Alpha DNA, Canada) were dissolved in ddH_2O for final concentration of 100 Picomol/μl as a stock solution recommended by the provider and stored at -20 °C. Working solution 5 Picomol/μl was utilized.

Amplification of *16S rRNA* Gene

The coding sequence of the conserved region in the *16S rRNA* gene of *A. baumannii* isolates was amplified with specific primers as shown in Table 1. The PCR reaction mixture contained 12.5 μl Go Taq®Green Master Mix (Promega, USA). Then, 1μl forward, 1 μl reverse primers and 5 μl DNA template were added and the volume completed to 25μl with ddH_2O. The amplification program was 5 min at 95 °C for initial denaturation, 30 Sec at 95 °C (30 cycles), 30 Sec at annealing temperature 56 °C and 30 sec at 72 °C followed by 10 min at 72 °C for final extension. Negative control was added with the entire component except genomic DNA, which ddH_2O was used instead.

Table 1. The sequences of primers used for Multiplex PCR to detect *A. baumannii 16S rRNA* and *Oxacillinases genes*

Genes	Sequences	Size (bp)	Reference
16S rRNA	**F**-5'-TTTAAGCGAGGAGGAGG-3 **R**-5'-ATTCTACCATCCTCTCCC-3'	240	[22]
$bla_{OXA\text{-}51\text{-}Like}$	**F**-5'-TAATGCTTTGATCGGCCTTG-3' **R**- 5'-TGGATTGCACTTCATCTTGG-3'	353	[23], [24]
$bla_{OXA\text{-}23\text{-}Like}$	**F**-5'-GATCGGATTGGAGAACCAGA-3' **R**- 5'-ATTTCTGACCGCATTTCCAT-3'	501	
$bla_{OXA\text{-}24\text{-}Like}$	**F**-5'-GGTTAGTTGGCCCCCTTAAA-3' **R**-5'-AGTTGAGCGAAAAGGGGATT-3'	246	
$bla_{OXA\text{-}58\text{-}Like}$	**F**-5'-AAGTATTGGGGCTTGTGCTG-3' **R**-5'-CCCCTCTGCGCTCTACATAC-3'	599	

F= Forward, **R=** Reverse primer

Polymerase Chain Reaction (PCR) Techniques

Multiplex PCR Assay

Multiplex PCR was done to amplify different fragments of genes under this study in a single tube to detect genes encoding oxacillinases (Carbapenem resistance genes; Table 1) in clinical and environmental isolates of *A. baumannii*. Multiplex PCR was used to amplify the coding sequence of the four families of Carbapenem resistance genes (Oxacillinases) including: $bla_{OXA51\text{-}Like}$ genes (which also used for identification of isolates to species level), $bla_{OXA23\text{-}Like}$ genes, $bla_{OXA24\text{-}Like}$ genes and $bla_{OXA58\text{-}Like}$ genes with specific primers as listed in Table 1.

Agarose Gel Electrophoresis

The PCR products were subjected to 1% gel electrophoresis to analyze the PCR products and Red safe dye was used. The first well is loaded with 5 μl of 1Kb DNA marker (Clever scientific company, UK) to determine the band size. After electrophoresis, the gels were imaged using a gel-imaging system UV-transilluminator [18].

Antibiotic Susceptibility Test

The Kirby-Bauer method was followed for performing antibiotic susceptibility tests for antibiotics selected for this study according to CLSI (2022) Guidelines [19].

The Minimum Inhibitory Concentration (MIC) of Colistin

The antimicrobial activity of colistin against the Carbapenem-resistant *A. baumannii* (CRAB) was assessed. Therefore, MIC of colistin against CRAB was evaluated using serial dilution of colistin concentrations (1-64 μg/ml) in Mueller Hinton Broth (MHB) with equal volume of CRAB growth (100μl) that equaled to 0.5 McFarland in microtiter plate. Then, the 96-well plate was incubated at 37 °C for 16-18 hrs. After incubation, the lowest concentration that inhibited the bacterial growth was recorded as colistin MIC [15].

ΦKAB Phage

The Phage used for anti-biofilm activity was previously characterized. The optimal multiplicity of infection (MOI) of ΦKAB phage was determined according to [20] and MOI 10 was optimal for the phage.

In-vitro Activity of ΦKAB Phage Against CRAB Strains

Acinetobacter baumannii isolates were resistance to Carbapenems (Imipenem, and Meropenem); were tested for ΦKAB phage anti-biofilm activity using microtiter plate method [21]. Briefly, overnight culture of CRAB isolates were inoculated in 5 ml of tryptone soya broth (TSB) with 1% glucose

grown for 24 hr at 37 °C. A 200 μl volume of CRAB resistance *A. baumannii* growth equivalent to 0.5 McFarland was transferred to each well of microtiter plate in triplicate and incubated at 37 °C for 48 hr, which was considered as plate 1 to evaluate the biofilm formation for CARB only. TSB (Uninoculated) was used as a negative control.

To evaluate ΦKAB phage anti-biofilm activity *in-vitro* (plate 2), a 100 μl volume of CRAB resistance *A. baumannii* growth equivalent to 0.5 McFarland was transferred to each well of microtiter plate in triplicate and mixed (1:1) with the ΦKAB phage at optimal MOI (10) and incubated at 37 °C for 48 hrs. TSB (Uninoculated) was used as a negative control.

To assess ΦKAB phage and colistin mixture anti-biofilm activity *in-vitro* (Plate 3), the ΦKAB phage at optimal MOI (10) and colistin at 8 μg/ml as MIC concentration (50 μl for each one) were mixed (1:1) with CRAB growth (100 μl) in each well of a sterile 96-well microtitration plate in triplicate. TSB (Uninoculated) was used as a negative control.

The plate was incubated under aerobic conditions for 48 hrs at 37 °C. After incubation for of the three plates, the contents were gently removed by tapping. The plate was washed three times with 200 μl of PBS pH 7.2. Heat fixation at 60 °C for 1 hr was applied to bacterial adhesive film and then stained with 150 μl for each well of 0.1% crystal violet (Sigma-Aldrich, Gillingham, UK) at room temperature for 15 min. The excess stain was washed with tap water. Ethanol (100 μl) was added to each well, and the biofilm biomass was measured at an optical density (OD) of 630 nm using a spectrophotometer.

The ability of bacteria to form biofilm before and after phage and phage/ colistin mixture were divided into four groups as shown in Table 2 [21]:

Table 2. Groups for biofilm formation with OD value

Biofilm formation	**OD value**
Non-adherent	OD≤ODc
Weakly adherent	ODc<OD≤2×ODc
Moderately adherent	2xODc<OD ≤ 4 × ODc
Strongly adherent	4xODc < OD

RESULTS AND DISCUSSION

Identification and Isolation of *Acinetobacter baumannii*

In the current study, samples from different sources were collected from the laboratories of Salah Al-deen General, Ghazi Al-hariri, and Medical City hospitals from 2022 to 2023. Then isolates were cultured on Blood agar, MacConkey agar and Chrom agar^{TM} for 24 hrs at 37 °C to identify according to the morphology of the colony. As well as the VITEK2 system (BioMerieux, France) was used for phenotypic identification. All isolates that were obtained from Chrom agar were subjected to Gram stain examination by Microscopic, which showed as Gr-ve coccobacilli and arranged in diplococci.

Molecular Identification of *A. baumannii* using *16S rRNA*

For molecular identification, PCR was performed to amplify the *16S rRNA* to determine the *A. baumannii* species. The PCR products were 242bp as shown in Figure 1. The PCR is a more effective way to diagnose *A. baumannii* in clinical laboratories rather than Chrom medium [25]. According to the finding, 60 (70.6%) out of 85 isolates were diagnosed as *A. baumannii*.

Antibiotic Susceptibility of *A. baumannii*

In the current study, eighteen antibiotics of different groups were used to detect the antibiotic sensitivity of 60 isolates of *A. baumannii* as illustrated in Figure 2. The highest resistance percentage were to Ceftriaxone (CRO) with 92% (55 isolates), and Cefotiam (CTF) with 87% (52 isolates), while the lowest percent related to Colistin (CO) with 17% (10 isolates). Other results showed antibiotic resistance percentages as 75% (45 isolates), 73% (44 isolates), 72% (43 isolates), and 70% (42 isolates) for Gentamicin (GN), Cefixime (CFM), Piperacillin (PRL), Cefotaxime (CTX) respectively. As well as, results appeared intermediate antibiotic sensibility for each one of the following:- 68% (41 isolates) for Trimethoprim-sulphamethoxazole (SXT), 67% (40 isolates) for each of Ceftazidime (CAZ), and

Ciprofloxacin (CIP), 65% (39 isolates) for Piperacillin/Tazobactum (PTZ), 63% (38 isolates) for Levofloxacin (LEV), 62% (37 isolates) for each one of Amikacin (AK), and Imipenem (IMI), 60% (36 isolates) for Aztreonam (AZM) and 53% (32 isolates) for Tetracycline (TET). At the same time, Meropenem (MEM) and Bacitracin (BAC) recorded lower antibiotic sensibility at 23% (14 isolates) and 22% (13 isolates) respectively (Figure 2).

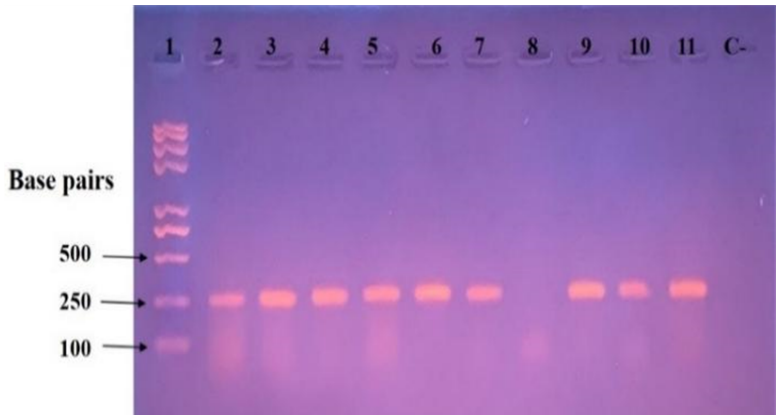

Figure 1. Genetic detection of *A. baumannii* of *16S rRNA* on agarose gel electrophoresis. The 1% of agarose gel was used with 7 V/cm /30 min and 0.125 μg/ml of Syber safe stain as a final concentration. Line 1:100 bp DNA ladder, line 2-7 and 9-11: positive result for *16S rRNA* with 242 bp bands, line 8: negative result and line C-= negative control. The DNA bands was visualized under UV light

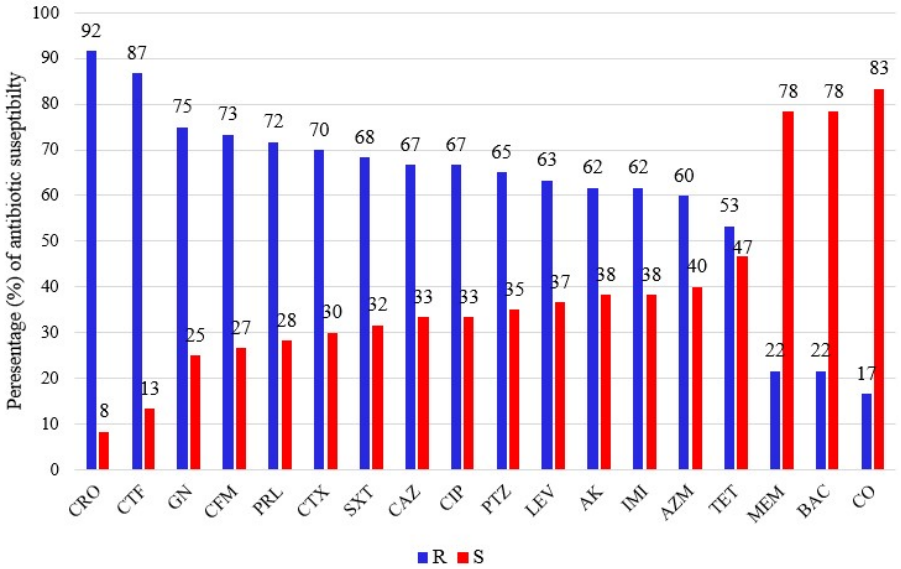

Figure 2. Percent of antibiotic susceptibility of *A. baumannii* isolates. CRO= ceftriaxone, CTF=Cefotiam, GN= Gentamicin, PRL= Piperacillin, CFM=Cefixime, CTX= Cefotaxime, SXT= Trimethoprim/ Sulfamethoxazole, CAZ= Ceftazidime, CIP= Ciprofloxacin, PTZ= Piperacillin/Tazobactum, LEV= Levofloxacin, AK= Amikacin, IMI= Imipenem, AZM= Aztreonam, TET= Tetracycline, MEM= Meropenem, BAC= Bacitracin, and Co= Colisten.R= Resistance and S= Sensitive

The percentage of its resistance to Piperacillin (PRL) was 72% and 65% for Piperacillin/Tazobactum (PTZ), which is in disagreement with [26] as the resistance rate to PRL was 94%. The development of ß –lactamase enzyme leads to cause ß-lactam antibiotics resistance. The ß –lactamase enzyme can attack the ring in ß-lactam antibiotic [27]. The current study noted that most isolates had recorded a high resistance rate to cephalosporin when reaching 92%, 87%, 73%, 70%, and 67% for Ceftriaxone (CRO), Cefotiam (CTF), Cefixime (CFM), Cefotaxime (CTX) and Ceftazidime (CAZ) respectively. In Egypt, a study reported 91.3% and 87% for Cefotaxime (CTX), and Ceftazidime (CAZ) antibiotics respectively [28]. Otherwise, an Iran study reported a 100% resistance rate against

Cefotaxime (CTX), but Ceftazidime (CAZ) reached a 92.5% resistance rate [29]. Resistance to most Cephalosporin antibiotics tested indicates these bacteria possess multiple resistance mechanisms. In addition to their production of modifying enzymes; they might be able to alter outer membrane proteins and harboring efflux pump system that acts on pumping the antibiotic out extracellular [30]. Intermediate resistance of Sulfa drug (SXT) was observed in 68% (41 isolates) in the current study, which agreed with [31] who reported 62.5%. However, previous studies recorded a high resistance rate of 96% and 87.03% to SXT respectively [32], [33]. Overall, these results disagreed with the current study. The results of aminoglycoside resistance in this study disagreed with the researcher, who concluded that *A. baumannii* had resistance percentages of 54.5% to Gentamicin and 52.5% to Amikacin [34]. The primary resistance route to aminoglycoside is enzymatic modification through the development of aminoglycoside-modifying enzymes (AMEs) [35]. Amikacin resistance was caused by high-affinity binding to the A-site on the 30S ribosomal RNA, inhibiting protein synthesis [36].

For Ciprofloxacin, the resistance rate to this antibiotic was 65% (39 isolates) but it disagreed with the researcher found, the resistance rate to Ciprofloxacin was 92% [37]. In addition, isolates showed 63% for Levofloxacin (LEV), which is a little higher than reported with 50% resistance [31]. The cause of resistance to the Fluoroquinolone group in *A. baumannii* is the occurrence of spontaneous mutations in the target DNA gyrase and Topoisomerase IV enzymes. Another mechanism for the occurrence of resistance to this antibiotic is a decrease in drug accumulation as a result of increased expression in efflux pumps [38].

The results of resistance to Carbapenem, including Imipenem (IPM) with 62% and 23% for Meropenem, disagree with a previous study that reported *A. baumannii* had a resistance of 36.1% and 40.90% for Imipenem [39], [40]. *Acinetobacter baumannii* resistance to Carbapenem in clinical isolates has been identified as a serious danger. This indicates that if Carbapenem is overused, early treatment discontinuation will likely result in a rapid increase in resistance and treatment failure [41]. Due to bacteria's ability to acquire antibiotic resistance genes and survive for days in the hospital environment and on the hands of healthcare workers (HCWs), endemic *A. baumannii* strains may be transferred and persist in hospitals [42]. The isolates showed the lowest percentage of resistance to Colistin, with 17% converging with what was previously found as the isolates were highly sensitive to Colistin [43]. Colistin is still one of the most effective single antimicrobial agents against multidrug-resistant *A. baumannii*, and it's frequently employed as a last resort [28], [44]. However, Colistin has a lot of disadvantages, including neurotoxicity and nephrotoxicity, and low lung tissue penetration, which restricts its efficacy [45]. Overall, antibiotic resistance results suggested that *A. baumannii* isolates in the current study were Multi-drug resistant (MDR), which has been increasing recently [46].

Colistin MIC concentration

The MIC of colistin against CRAB was determined by Broth micro-dilution method. The MIC value of colistin against CRAB was 8μg/ml.

Prevalence of *Oxacillinases genes* (Carbapenem Resistance Genes)

To detect the prevalence of *Oxacillinases genes* "Carbapenem hydrolyzing class D β–lactamases genes" (CHDLs) in all *A. baumannii* isolates. The PCR products were of $bla_{OXA\text{-}23\text{-}Like}$ with 501bp, $bla_{OXA\text{-}24\text{-}Like}$ with 246bp, $bla_{OXA\text{-}51\text{-}Like}$ with 353bp and $bla_{OXA\text{-}58\text{-}Like}$ with 599bp comparing with DNA ladder (Figure 3).

The results in Figure 3 illustrated the prevalence of $bla_{OXA\text{-}51\text{-}like}$ gene with 40 (66.67 %) in *A. baumannii* studied isolates. The $bla_{OXA\text{-}51}$ class D-carbapenemase has been reported from globally Carbapenem resistance *A. baumannii* strains [47]. The $bla_{OXA\text{-}51\text{-}like}$ enzymes were first identified in *A. nosocomialis* in Taiwan. The $bla_{OXA\text{-}51\text{-}like}$ an intrinsic carbapenemase genes in *A. baumannii*, suggesting that the spread of these resistance genes among baumannii-calcoaceticus complex [48]. Previous research concluded that The $bla_{OXA\text{-}51}$ genes are unique and related to *A. baumannii*, which provides an easier and more useful method for identification [49]. In addition, results indicated that the $bla_{OXA\text{-}23}$ genes were 23 (57.5%) in MDR *A. baumannii* (Figure 3) out of 37 (92.5%) imipenem resistant *A. baumannii*, which 20 (50 %) of 40 *A. baumannii* isolates had both $bla_{OXA\text{-}51}$ genes and $bla_{OXA\text{-}23}$ genes. The $bla_{OXA\text{-}23}$ enzymes were first identified as carbapenem-resistant OXA-type β-lactamases in Scotland, which are encoded by plasmid [50]. This enzyme can confer the mechanisms of antibiotic resistance to Imipenem, Meropenem, Amoxicillin and Ticarcelin *in vivo* [51].

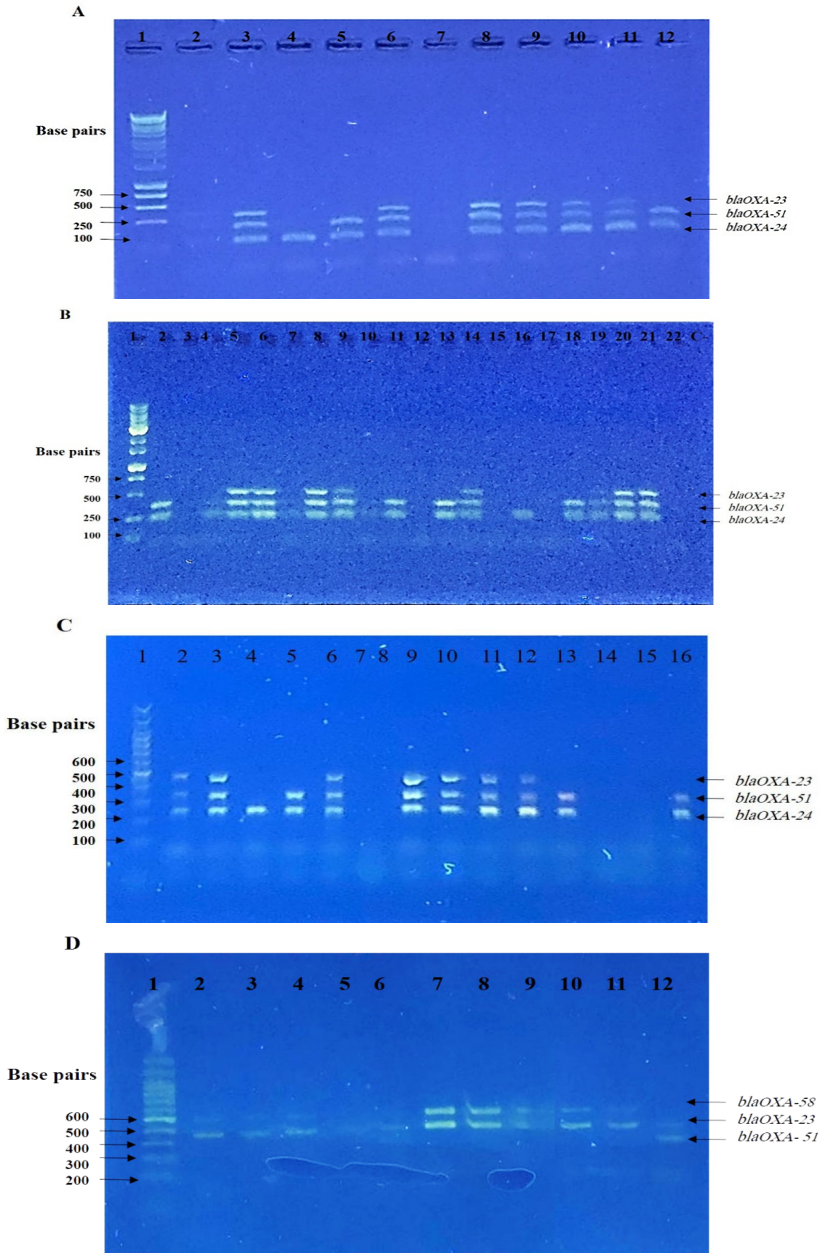

Figure 3. The prevalence of *Oxacillinases genes* in *A. baumannii*. Multiplex PCR of *A. baumannii* isolates from different samples to detect of $bla_{OXA\text{-}23\text{-}Like}$ with 501bp, $bla_{OXA\text{-}24\text{-}Like}$, $bla_{OXA\text{-}51\text{-}Like}$ and $bla_{OXA\text{-}58\text{-}Like}$ on 1% agarose gel at 10 V/cm for 30 min and 0.125 μg/ml of Syber safe stain as a final concentration and visualized under UV light. The A, B, and C presented line 1, 100 bp DNA ladder; lanes 2-12, 2-22, and 2-16 *A. baumannii* $bla_{OXA\text{-}23}$ with 501bp, $bla_{OXA\text{-}51}$ with 353bp and $bla_{OXA\text{-}24}$ with 246bp; lane C-, Negative control. As well as the D showed line 1, 100 bp DNA ladder, lines 2-12, $bla_{OXA\text{-}58}$ with 599 bp

Moreover, another study reported that the main method to transfer $bla_{OXA\text{-}23\text{-}like}$ among *A. baumannii* was via Tn2008 transposon that $bla_{OXA\text{-}23\text{-}like}$ genes flanked by upstream insertion sequence ISAba1 [52]. As well as, it has been found that $bla_{OXA\text{-}24}$ genes were 35 (87.5%) in MDR *A. baumannii* isolates and 17 (42.5 %) out of 40 *A. baumannii* isolates had both $bla_{OXA\text{-}51}$ and $bla_{OXA\text{-}24}$ genes. Furthermore, 20 (50 %) out of 40 *A. baumannii* isolates had all three genes, which were $bla_{OXA\text{-}51}$, $bla_{OXA\text{-}23}$ and $bla_{OXA\text{-}24}$ genes (Figure 3). In addition, it has been noticed that $bla_{OXA\text{-}58\text{-}like}$ was 9 (22.5%) in *A. baumannii* isolates and $bla_{OXA\text{-}51\text{-}like}$ genes (Figure 3). These results might be related to the un-transferable plasmid, which can reduce Carbapenem sensibility. Initially, the $bla_{OXA\text{-}58}$ enzymes were identified in CRAB strain in France that can hydrolysis Imipenem, penicillin and Oxacillin

but not expended-spectrum Cephalosporins [53]. In addition, *bla$_{OXA\text{-}58\text{-}like}$* pseudo-enzymes were reported in *Acinetobacter*, which carried out by plasmids; however, the chromosomal location was also reported [54]. Isolates with *bla$_{OXA\text{-}58\text{-}like}$* have been reported form different places such as Europe, South America, North America, Anatolia, Asia and Australia [53], [55].

Overall, the results of antibiotic and *Oxacillinases genes* reported only 14 isolates from the current study were CRAB (Imepenem and Meropenem).

Anti-Biofilm Activity of Phage ΦKAB Against CRAB Isolates

To detect biofilm formation in CRAB isolates, all CRAB 14 isolates were subjected to microtiter plate assay for detection of biofilm production. Results indicated that among 14 isolates: 57% (8) were weak and 43% (6) were moderate biofilm producer illustrated as positive results (Figure 4A). For anti-biofilm activity of ΦKAB phage against CRAB isolates, CRAB culture was incubated with phage ΦKAB at MOI=10. Results concluded that after the addition of phage ΦKAB to CRAB culture the biofilm formation was reduced to weak biofilm producer by 86% (12 isolates) and non-biofilm producer by 14% (2 isolates) only (Figure 4B), which suggested that phage ΦKAB can reduce and prevent biofilm formation in CRAB isolates at MOI 10. In addition, ΦKAB phage could be used as an alternative therapeutic treatment pathway for CRAB. To detect the synergistic effect of phage and colistin by microtiter plate assay, MIC of colistin against CRAB was 8 μg/ml. As well as the MOI for ΦKAB phage was 10. The mixture was done by combining ΦKAB phage at MOI of 10 and Colistin (MIC=8 μg/ml) by microtiter plate assay to determine the synergistic effect. The Results in Figure 4C showed that a complete transfer of isolates by 100 % to weak biofilm producer compared with Figure 4A, which suggested that the synergistic activity of combination ΦKAB phage and Colistin might be useful to treat CRAB than using antibiotic or phage alone [56].

The mechanism of the phage-antibiotic combination is still unclear. The action of colistin on the outer membrane of gram-negative bacteria might enhance both phage adsorption and phage DNA injection [57]. A previous study has indicated that the synergism of phage and antibiotic affected bacterial morphological changes, leading to rapid phage maturation and acceleration of cell lysis [58].

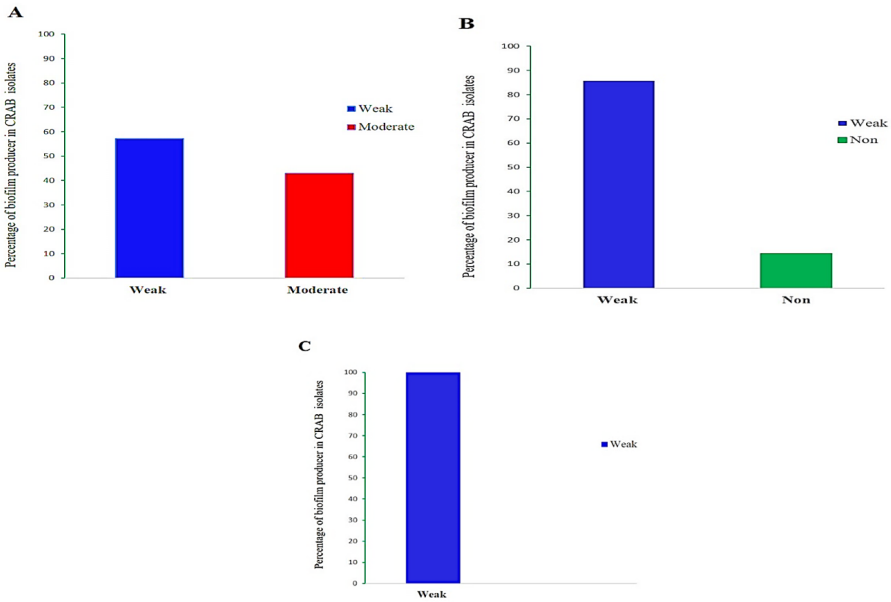

Figure 4. Activity of phage ΦKAB against biofilm formation of CRAB isolates by microtiter plate assay. (A) Biofilm formation in CRAB isolates only. (B) Anti-biofilm activity of ΦKAB phage at MOI (10) against CRAB isolates. (C) The synergistic effect of combination of colistin/ΦKAB phage against CRAB isolates. Experiments were done independently in triplicate

CONCLUSION

In summary, among 60 isolates of *A. baumannii*, most isolates were MDR and only 14 isolates were CRAB. The 14 CRAB isolates were 57% (8) weak and 43% (6) moderate biofilm producers. The

Colistin/ΦKAB phage mixture could inhibit and reduce biofilm forming in CRAB isolates. Overall, the present study may provide evidence on the capability of the isolated phage to serve as a novel strategy to treat infections caused by MDR *A. baumannii*.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Kareema Ali Bahr and Susan A. Ibrahim performed the experiments. Susan A. Ibrahim interpreted the results. Ban O. Abdulsattar, Susan A. Ibrahim and Ashraf A. Abd EL-Tawab wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

FUNDING

None.

DATA AVAILABILITY STATEMENT

None.

ETHICAL APPROVAL

This study did not involve direct contact with humans, and all clinical isolates were obtained from hospital laboratories. Therefore, no ethical approval was needed.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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