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Novel Phage Cocktail for the Treatment of Bacteria Causing Chronic Suppurative Otitis Media

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ABSTRACT

Chronic suppurative otitis media (CSOM) is a chronic inflammation of the middle ear cavity and mastoid cavity. It occurs in any community in a percentage of 0.5-33% for all age group. The objective of this study was to isolate and prepare phage cocktail active against bacteria causing chronic suppurative otitis media from sewage water. Bacteria were isolated from non-drug responsive cases of CSOM. A total of 73 ear swabs were collected from patients of CSOM and showed bacterial growth in 63 (86.3%) and fungal growth in 10 (13.7%). The most prevalent bacterium was *Pseudomonas aeruginosa* which was found in 33 swabs (45.2%), followed by *Staphylococcus aureus* in 15 (20.5%). Species of *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, and *Moraxella catarrhalis* were isolated in 5 (6.8%), 5 (6.8%), 4 (5.5%) and 1 (1.3%), respectively. The most potent phages were 7, 3, 1, 2, 1 and 1 for species of *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *E. coli*, *P. mirabilis* and *M. catarrhalis*, respectively. Phages were isolated after enrichment of each CSOM bacterial pathogen with sewage water by spot lysis method and characterized morphologically by top agarose plaque assay. Most virulent isolated phages were selected for formulation of phage cocktail against isolated bacterial pathogens of CSOM. A number of 17 phages cocktail were formulated and showed 60-100% inhibition on all tested isolates of *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *E. coli*, *P. mirabilis* and *M. catarrhalis* in a growth inhibition assay.

Keywords: Chronic suppurative otitis media, Phage therapy, Drug resistance, Phage cocktail.

Introduction

Chronic suppurative otitis media (CSOM) is a chronic inflammation of the middle ear cavity and mastoid cavity, which manifested as otorrhoea (recurrent pus discharge) after tympanic perforation.[1] This infection starts in childhood as a spontaneous perforation of tympanic membrane as a result of acute infection of the middle ear, which is called acute otitis media (AOM), or as an outcome of milder types of otitis media (such as secretory OM).[2] The case of CSOM is diagnosed when mucoid discharge from perforated tympanic membrane continues for 3 weeks to 3 months in spite of medical treatment.[3] The World Health Organization demonstrated a duration of 2 weeks for active case of CSOM.[1] This infection may extend to the temporal bone and leads to intracranial complication, in addition to extracranial complication, which includes Leakage of cerebrospinal fluid, temporal abscess, encephalocele, Petrositis, Sigmoid sinus thrombosis, Postauricular abscess, Paresis of facial nerve, Mastoiditis, Labyrinthine fistula, Labyrinthitis, Meningitis.[4] This infection is common in all age groups, but more prevalent in low socioeconomic population and affects 0.5-33% of any community.[5] CSOM infection mostly result from bacterial and fungal infection, and the most frequent species are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebseilla pnueumoniae*, *Escherichia coli* and *Proteus mirabilis*, *Apergillus spp.* and *Candida* albicans.[6] It is difficult to control cases of CSOM due to two problems; first is the emergence of drug-resistance in CSOM bacterial pathogen;[7] second is the formation of biofilm in most cases of this infection making it difficult to control the pathogens by antibiotics.[8] Phage therapy represents an ideal therapy for cases of different forms of otitis media including CSOM with its great ability in resolving the crisis of antibiotic-resistance in bacterial pathogens plus its outstanding ability to act as potent anti-biofilm.[9,10]

Phage therapy is defined as the treatment of bacterial infection by using lytic phages.[11] Increasing number of case reports for phage therapy for human bacterial infections were documented worldwide in recent years as treatment of *Pseudomonas aeruginosa* bacteremia,[12] *P. aeruginosa* urinary tract infection,[13] *Escherichia coli* diarrhoeal diseases,[14] Staphylococcal infections as infective endocarditis and septic shock,[15] and *P. aeruginosa* burn wound infection.[16] The current study aimed to isolate pathogens of CSOM from different age groups, to isolate phage(s) active against each bacterial pathogens and to prepare phage cocktail that is active against main bacterial pathogens of CSOM to be a broad-spectrum cocktail against bacterial pathogens of CSOM.

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Materials and Methods

Sample collection

A total of 73 ear swabs for mucoid discharge were collected from patients in Otorhinolaryngology outpatient clinic in Baghdad Hospital in Medical city directory from December 2015 to March 2016. Age of patients ranged from 1 up to 73 years old. Ear canal was washed with sterile normal saline prior to taking a swab. Three Swabs were taken from each patient and directly transferred to the laboratory for direct examination and detection on bacterial and fungal pathogens. The samples were taken under the approval of the commission of research ethics of Iraq ministry of health and environment.

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Direct examination
One swab was used for preparation of direct smear of the sample for detection of pus cells, bacteria and/or fungi.

Culture and Identification
For bacterial culture, one swab was streaked on Blood agar, MacConkey agar and Chocolate agar (HiMedia, India). The Blood agar and MacConkey agar were incubated aerobically whereas the Chocolate agar was incubated under microaerophilic condition at 37°C for 24-48 hrs. The positive cultures were purified and diagnosed by VITEK® 2 Automated instrument for ID/AST testing (bioMérieux, France). For fungal culture, one swab was streaked on Sabouraud dextrose agar plates and incubated at 28°C for 7 days. Positive fungal cultures were diagnosed in Mycology unit in Central public health laboratory in Baghdad.

Antibiotic susceptibility test
Antibiotic susceptibility patterns of bacterial isolates towards 14 antibiotics were detected by Kirby-Bauer method according to the Clinical and Laboratory Standards Institute criteria. The antibiotics discs (HIMEDIA, India) were Azithromycin (15 mcg) , Ceftriaxone (30 mcg), Cefotaxime (30 mcg), Ceftazidime (30 mcg), Cefixime (5 mcg), Gentamicin (10 mcg), Augmentin (20/10mcg), Methicillin (10 mcg), Chloramphenicol (30 mcg), Amikacin (30 mcg), Ciprofloxacin (10 mcg), Cotrimoxazole (25 mcg- (1.25/23.75mcg)), Streptomycin (10 mcg) and Polymyxin B (300 Units/disc).[17]

Water sample
Sewage water samples from 15 water processing plants around Baghdad were collected and filtered through a 0.22 μm membrane filter to remove any particles larger than phage.[18]

Phage isolation
A volume of 10X of Lauria Bertani broth (5 mL), membrane filtered sewage water sample (40 mL) and overnight broth culture of host bacterial isolates (5 mL) were mixed and incubated under shaking at 37°C for 18 hr. Culture was centrifuged at 10000 rpm for 5 min. Supernatant was further membrane filtered to remove the possible remaining bacterial cell. This filtrate is supposed to be a potential phage preparation.[19]

Phage spot lysis assay
A host bacterium was enriched by culturing on Lauria Bertani broth (LB) at 37°C for 24 hr. One mL of LB of host bacterium was spread on the surface of nutrient agar and left for 20-30 min for drying. A volume of 10 μL of phage preparation was spotted over bacterial lawn and allowed to dry before incubation at 37°C for 24 hr. Development of clear, semi-clear or hazy zone at the spot was considered positive for the presence of lytic phage towards host bacterial cell.[20]

Phage propagation
Zone of lysis (phage plaque) on nutrient agar was picked up by a sterile loop and transferred into 1 mL of SM buffer (Sodium Chloride-100 mM, Magnesium Sulfate- 8 mM , gelatin- 0.01%, Tris-HCL- 50 mM, pH 7.5- Sigma-Aldrich- USA) in 1.5 mL eppendorf tube. The eppendorf was shaked gently for 5 min by hand. The supernatant was transferred into other eppendorf tube. Chloroform was added to the cell lysate in a ratio of 1:10 v/v and shaked gently for 5-7 min to kill any remaining bacterial cells. Bacterial lysate was centrifuged at 10000 rpm for 5 min to remove host cell debris. Supernatant was transferred into new eppendorf tube and called the transient phage stock suspension.[20]

Phage titration
A single colony of host bacterium was cultured on Lauria Bertani broth (5 mL) and incubated with shaking till the OD600 was about 0.5. Top agarose (1% Bacto-tryptone, 0.5% yeast extract, 0.5% NaCl, 0.1% $MgCl_2.6H_2O$, 0.7% agarose) was heated to 45°C in water bath. A volume of 10 μL of phage preparation and mid-log phase host bacterial cell were added to the heated top agarose, mixed well, poured and distributed evenly onto Lauria Bertani agar plate. Plates were allowed to cool and incubated at 37°C for 24 hr. Plaques formed were counted and the amount of phages were expressed as plaque forming units (PFU).[19]

Determination of plaque properties
Properties of plaques were determined by top agarose plaque assay. Properties of plaque shape (Circular or oval), Size of plaque (diameter) in millimeter, margincut of plaque (regular or irregular) and clarity or turbidity of the plaque were recorded for plaques of each phage towards its host bacterium.[20]

Preparation of secondary phages
A Phage isolated through enrichment of phage source (sewage water) with primary host bacterium are considered to be primary phage for that host bacterium, whereas same phage was considered secondary phage against each bacterial isolate sensitive to its lytic action other than its primary host bacterium. Each of the phage isolates were tested by spot lysis method towards all bacterial isolates isolated in this study and morphological properties of plaques of each phage towards all bacterial isolates were determined.[20]

Formulation of phage cocktail
Each bacteriophage has been prepared in a concentration of 10^6 PFU/mL in SM buffer, and mixed with other phages in equal volumes to prepare phage cocktail of the desired phages.

Growth inhibition assay
Each indicator bacterial isolate was cultured on LB broth and incubated at 37°C in a shaker incubator at 150 rpm/min for 24 hr. Bacterial culture was diluted to a concentration of 1% into LB broth and incubated in a shaker incubator at 150 rpm/min until OD650 reached 0.4. A 50 μL (approximately 2×10^8 CFU/mL) of bacterial culture was added to 50 μL of 2X LB broth in 96- well flat bottom microtitre plate (Nunc, USA). Then, A volume of 100 μL of bacteriophage preparation was added to the microtitre plate followed by the addition of 50 μL of filter sterilized 0.1% TTC (2, 3, 5-triphenyl tetrazolium chloride) (Hi-media). The microtitre plate was incubated in the dark at 37°C for 24 hr. Result of absorbance at OD540 has been measured by a Micro-plate reader (Fisher Scientific, USA). The percentage inhibition of bacteriophage preparation(s) towards bacterial isolates was calculated using the following formula:[21]

$$\% \text{ inhibition} = 100(\frac{The\ absorbance\ of\ controls The\ absorbance\ of\ treated\ wells}{The\ absorbance\ of\ controls}$$

Results and Discussion

Results for culture of 73 ear swab of CSOM patients showed bacterial growth in 63 (86.3%) and fungal growth in 10 (13.7%). Figure 1 illustrates the number and percentage of each bacterial and fungal pathogens of the current study. The most prevalent bacterial pathogens was *Pseudomonas aeruginosa* which was found in 33 (45.2%), followed by *Staphylococcus aureus* in 15 (20.5%). Species of *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, and *Moraxella catarrhalis* were isolated in 5 (6.8%), 5 (6.8%), 4 (5.5%) and 1 (1.3%), respectively. Fungal growth showed 3 (4%) isolates of *Candida albicans* and 7 (9.5%) isolates of *Aspergillus spp.*
Most isolates of *S. aureus*, *P. mirabilis*, *E. coli*, *K. pneumoniae* and *M. catarrhalis* showed multi-drug resistance (MDR) and most isolates of *P. aeruginosa* showed extensive drug resistance (XDR). One isolate of *P. aeruginosa* showed pan-drug resistance (PDR). Figure 2 illustrates the percentage resistance of bacterial isolates against the 14 antibiotics.
A total of 17 of the most potent phages against all bacterial isolates were selected for formulation of phage cocktail. Each one of those isolates showed broader activity towards all isolates of the same species of its host bacterium comparing with other phages active against same species. Table 1 shows plaque properties of 15 phage isolates which showed high to moderate and low activity towards bacterial pathogens.

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Phages were screened for its lytic activity towards isolates other than its primary host bacterium. Table 2 shows the list of secondary phages active against 3 isolates of *P. aeruginosa*, 3 isolates of *S. aureus*, 2 isolates of *E. coli* and 2 isolates of *K. pneumoniae*.

Host range was varied from one phage isolate to another against all bacterial isolates of the same bacterial isolates. The phage isolates showed lytic activity against isolates of each of the bacterial species in a percentage of 30-40%. Phages PPA2, PPA14 and PPA29 that were active against *P. aeruginosa*, and phages PSA6, PSA10 and PSA12 that were active against *S. aureus* showed 50-60% lytic activity against the bacterial isolates. Table 3 shows the host-range activity of 11 phage isolates active against isolates *P. aeruginosa*, *S. aureus*, *E. coli*, *K. pneumoniae*, *Proteus mirabilis* and *M. catarrhalis*.

A phage cocktail composed of 17 phages active against CSOM bacterial pathogens were formulated. Those phages were PPA2, PPA4, PPA7, PPA14, PPA26, PPA29 and PPA32 that were active against *P. aeruginosa*; Phages PSA6, PSA10 and PSA12 that were active against *S. aureus*; Phages PEC3 and PEC4 that were active against *E. coli*; Phages PKP2 and PKP5 that were active against *K. pueumoniae*; Phages PPM4 and PPM5 that were active against *P. mirabilis* and phage PMC1 that were active against *M. catarrhalis*.

Phage cocktail activity against CSOM bacterial pathogens by spot lysis method

Results of the 17 phages cocktail towards CSOM bacterial pathogens showed 100% lytic activity to all 63 bacterial isolates of *P. aeruginosa*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. mirabilis* and *M. catarrhalis*, whereas no single phage showed host-range activity more than 50-60% towards isolates of same bacterial species.

Growth inhibition assay for phage cocktail versus single phage lytic activity

A single phage PPA14 active against *P. aeruginosa* and a single phage PSA12 active against *S. aureus* were selected to evaluated for its ability to inhibit the growth of 33 isolates of *P. aeruginosa* and 15 isolates of *S. aureus*, respectively, versus the ability of the 17 phages cocktail in inhibiting growth of same isolates. A single phage PPA14 active against *P. aeruginosa* and a single phage PSA12 active against *S. aureus* were selected to evaluate their ability to inhibit the growth of 33 isolates of *P. aeruginosa* and 15 isolates of *S. aureus*, respectively, versus the ability of the 17 phages cocktail in inhibiting growth of same isolates. Results showed that the percentage inhibition of *P. aeruginosa* isolates by PPA14 ranged from 0 to 100%, whereas the 17 phages cocktail showed 100% inhibition against the same isolates (Figure 3).

Similar results were obtained for growth inhibition assay of PSA12 on 15 isolates of *S. aureus* (Figure 4).

Evaluation of phage cocktail activity towards routine isolates in public hospitals

The 17 phages cocktail was submitted to three public hospitals in Baghdad which were the educational laboratories in Baghdad Medical city, Al-Kindy public teaching hospital and Al-Sader public hospital to be evaluated against routine bacterial isolates of CSOM isolated in bacteriology laboratories of those hospitals. Results of evaluation showed that the percentage inhibition for *P. aeruginosa* routine isolates ranged from 60-100%, whereas those for *S. aureus* , *E. coli*, *K. pneumoniae* and *P. mirabilis* ranged from 70-100%, 60-73%, 70-85% and 66-90%, respectively (Figures 5 and 6).

The prevalence of *P. aeruginosa* in 45.2% of CSOM cases of this study is predominant in all etiological studies of CSOM.[22-25] This high percentage of *P. aeruginosa* in CSOM cases is attributed to the outstanding ability of *P. aeruginosa* to produce a biofilm in the middle ear cavity and mastoid cavity which is considered the first virulence factor for pathogens of CSOM as *P. aeruginosa*, *S. aureus*, *E. coli*, *K. pneumonia* and *P. mirabilisI*.[8-10,26]

All isolates of bacterial pathogens of CSOM were MDR, and most of *P. aeruginosa* isolates were XDR. One isolate of *P. aeruginosa* was shown to be PDR. This prevalence of MDR and XDR in CSOM

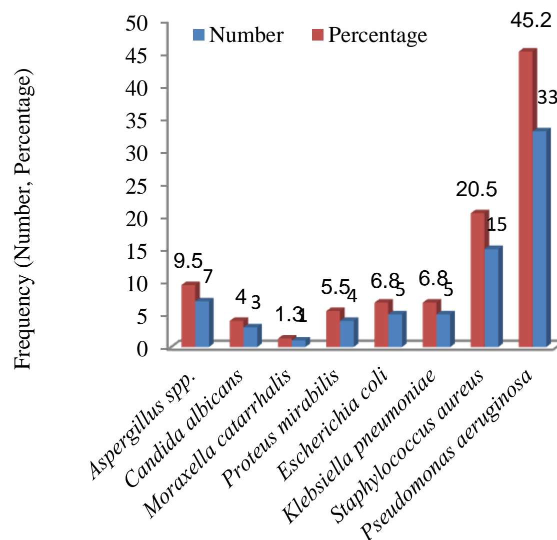

Figure 1: Frequency of bacterial and fungal pathogens of Chronic Suppurative Otitis Media represented by number and percentage of each pathogens to total specimens.

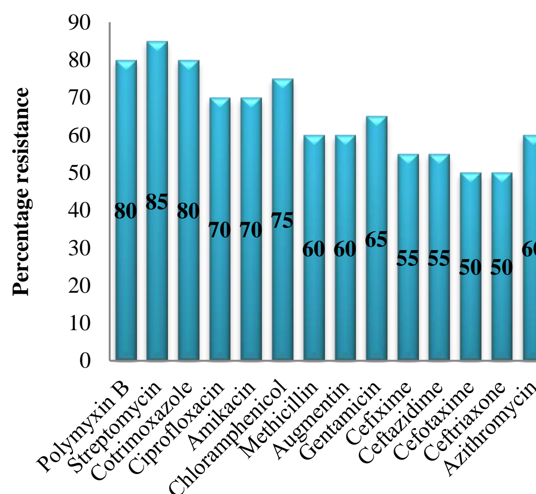

Figure 2: Percentage of antibiotic resistance of bacterial pathogens causing chronic suppurative otitis media against 14 antibiotics of different categories.

pathogens was a reflection of the pathology of CSOM which is dependent on the formation of biofilm of bacterial pathogens and the consequent events, pressure effect of antibiotic therapy and selection of drug-resistant mutants in population of biofilm during the course of infection.[26] As a result of biofilm formation during the pathology of CSOM, antibiotic therapy became inefficient in controlling such pathogens without anti-biofilm agent.[8-10] If we combine problem of biofilm formation with prevalence drug resistance among CSOM bacterial pathogens, it becomes necessary to employ phage therapy for the treatment of otitis media.[27]

All phages isolated in this study showed extreme narrow-host range. Lytic activity and were confined only to isolates of the same bacterial species. No phage showed neither intergeneric nor interspecies lytic activity. The narrow-host range of phages is arisen from their specificity to adsorb to certain receptor on the cell wall of the host bacterium, e.g. LPS in the gram-negative bacteria. When phage

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adsorbed to S-LPS (smooth- LPS), it shows an extreme-narrow host range due to high variation of O-antigen structure in different biotypes, serotypes and pathotypes of same bacterial species, whereas phage adsorption to R-LPS makes it broader-host range due to conservative structure of R-LPS in different genera and species of gram-negative bacteria.[28] It is worthless to employ phages with an extreme narrow-host range in phage therapy without formulation of phages in a cocktail to increase their host-range to encompass all, or at least as much as many of the serotypes, biotypes and pathotypes of the same bacterial species. Furthermore, with the formulation of phages lytic for different bacterial species and genera in one phage cocktail, we could attain broad host-range activity for the cocktail.

The broadened host-range of phage cocktail is due to adsorption of phages to different receptors at the same time on the surface of host bacterium as LPS, Protein-LPS complex, outer membrane proteins, enzymes localized on outer membrane, and selective transport protein(s),[28-31] Whereas each phage alone could be only adsorb to a single receptor on the surface of its host bacterium.

Evaluation of the percentage inhibition of phage cocktail against routine isolates of CSOM bacterial pathogens in 3 public hospitals showed that phage cocktail had broad-spectrum activity which ranged from 60-100% against such pathogens. High activity of this phage cocktail makes it good and efficient candidate for alternative therapy of CSOM cases.[32,33]

Table 1: Phage plaque characteristics of 17 phage isolates lytic on isolates of *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *E. coli*, *P. mirabilis* and *M. catarrhalis*

#	Bacteriophage isolate	Host bacterium	Plaque size (mm)	Plaque shape	Margin cut	Plaques clarity
1	PPA2*	***P. aeruginosa***	3.5	Circular	Regular	Clear
2	PPA3	***P. aeruginosa***	2	Circular	Regular	Clear
3	PPA9	***P. aeruginosa***	2	Oval	Irregular	Clear
4	PPA14*	***P. aeruginosa***	4	Circular	Regular	Clear
5	PPA15	***P. aeruginosa***	2.5	Oval	Irregular	Clear
6	PPA22	***P. aeruginosa***	3	Oval	Regular	Clear
7	PPA29*	***P. aeruginosa***	3.5	Circular	Regular	Clear
8	PSA6	***S. aureus***	3	Oval	Regular	Clear
9	PSA10	***S. aureus***	4	Circular	Regular	Clear
10	PSA12	***S. aureus***	3.5	Circular	Regular	Clear
11	PEC1	***E. coli***	3	Oval	Regular	Clear
12	PEC4*	***E. coli***	2.5	Oval	Irregular	Clear
13	PKP2*	***K. pneumonia***		Circular	Regular	Clear
14	PKP4	***K. pneumonia***				
15	PPM2*	***P. mirabilis***	4	Circular	Regular	Clear
16	PPM5*	***P. mirabilis***				
17	PMC1*	***M. catarrhalis***	3.5	Circular	Regular	Clear

*: Phages selected for phage cocktail preparation.

Table 2: The secondary phages active against 3 isolates of *P. aeruginosa*, 3 isolates of *S. aureus*, 2 isolates of *E. coli* and 2 isolates of *K. pneumonia*

#	Bacterial isolates	Secondary phage Isolates
1	***P. aeruginosa* 2**	PPA4, PPA5, PPA9, PPA11, PPA12, PPA 17, PPA18, PPA21, PPA23, PPA28, PPA30, PPA33
2	***P. aeruginosa* 13**	PPA1, PPA4, PPA5, PPA7, PPA9, PPA14, PPA16, PPA17, PPA20, PPA22, PPA26, PPA29, PPA32
3	***P. aeruginosa* 21**	PPA2, PPA3, PPA10, PPA14, PPA17, PPA21, PPA23, PPA25, PPA29, PPA32
4	***S. aureus* 3**	PSA2, PSA4, PSA8, PSA9, PSA12, PSA13, PSA15
5	***S. aureus* 8**	PSA1, PSA2, PSA6, PSA10, PSA11, PSA14
6	***S. aureus* 14**	PSA1, PSA6, PSA9, PSA11, PSA12, PSA15
7	***E. coli* 1**	PEC3, PEC4, PEC5
8	***E. coli* 5**	PEC1, PEC4
9	***K. pneumoniae* 3**	PKP1, PKP4
10	***K. pneumoniae* 5**	PKP1, PKP3, PKP4

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Table 3: The host range of 12 phage isolates against bacterial isolates

#	Phage isolate	Host isolates
1	PPA2	***P. aeruginosa*** 2 , ***P. aeruginosa*** 6, ***P. aeruginosa*** 7, ***P. aeruginosa*** 10, ***P. aeruginosa*** 15, ***P. aeruginosa*** 17, ***P. aeruginosa*** 18, ***P. aeruginosa*** 21, ***P. aeruginosa*** 24, ***P. aeruginosa*** 26, ***P. aeruginosa*** 29, ***P. aeruginosa*** 30, ***P. aeruginosa*** 32,
2	PPA14	***P. aeruginosa*** 1, ***P. aeruginosa*** 4, ***P. aeruginosa*** 6, ***P. aeruginosa*** 7, ***P. aeruginosa*** 9, ***P. aeruginosa*** 11, ***P. aeruginosa*** 13, ***P. aeruginosa*** 15, ***P. aeruginosa*** 16, ***P. aeruginosa*** 19, ***P. aeruginosa*** 21, ***P. aeruginosa*** 24, ***P. aeruginosa*** 26, ***P. aeruginosa*** 27, ***P. aeruginosa*** 29, ***P. aeruginosa*** 30, ***P. aeruginosa*** 31, ***P. aeruginosa*** 33
3	PPA29	***P. aeruginosa*** 3, ***P. aeruginosa*** 5, ***P. aeruginosa*** 7, ***P. aeruginosa*** 8, ***P. aeruginosa*** 10, ***P. aeruginosa*** 12, ***P. aeruginosa*** 13, ***P. aeruginosa*** 18, ***P. aeruginosa*** 19, ***P. aeruginosa*** 21, ***P. aeruginosa*** 23, ***P. aeruginosa*** 27, ***P. aeruginosa*** 30, ***P. aeruginosa*** 32, ***P. aeruginosa*** 33
4	PSA6	***S. aureus*** 3, ***S. aureus*** 5, ***S. aureus*** 6, ***S. aureus*** 8, ***S. aureus*** 9, ***S. aureus*** 11, ***S. aureus*** 12, ***S. aureus*** 15
5	PSA10	***S. aureus*** 1, ***S. aureus*** 6, ***S. aureus*** 7, ***S. aureus*** 8, ***S. aureus*** 10, ***S. aureus*** 12, ***S. aureus*** 13
6	PSA12	***S. aureus*** 2, ***S. aureus*** 3, ***S. aureus*** 5, ***S. aureus*** 8, ***S. aureus*** 9, ***S. aureus*** 12, ***S. aureus*** 13, ***S. aureus*** 15
7	PEC3	***E. coli*** 1, ***E. coli*** 3, ***E. coli*** 4
8	PEC4	***E. coli*** 4, ***E. coli*** 2, ***E. coli*** 4, ***E. coli*** 5
9	PKP2	***K. peumoniae*** 1, ***K. peumoniae*** 2, ***K. peumoniae*** 3, ***K. peumoniae*** 4
10	PKP5	***K. peumoniae*** 1, ***K. peumoniae*** 3, ***K. peumoniae*** 4, ***K. peumoniae*** 5
11	PPM4	***P. mirabilis*** 1, ***P. mirabilis*** 2, ***P. mirabilis*** 4
12	PPM5	***P. mirabilis*** 1, ***P. mirabilis*** 3, ***P. mirabilis*** 5

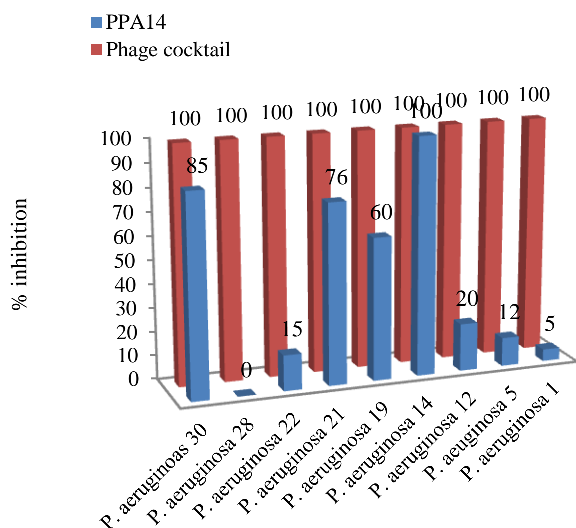

Figure 3: A % inhibition for growth inhibition assay of a single phage PPA14 versus the 17 phages cocktail on isolates of *P. aeruginosa*.

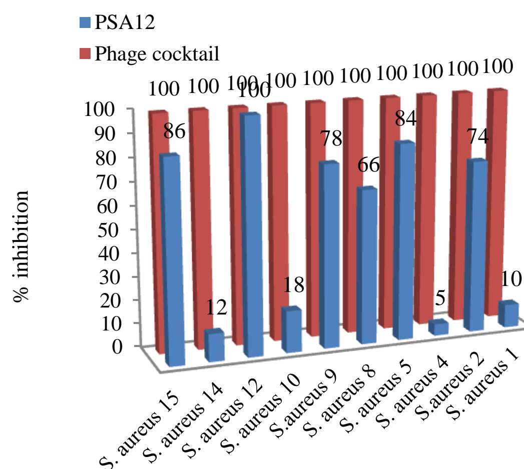

Figure 4: A % inhibition for growth inhibition assay of a single phage PSA12 versus the 17 phages cocktail on isolates of *S. aureus*.

Conclusion

Phage therapy is a potent alternative therapy for cases of CSOM owing to the ability of phage cocktail oriented against bacterial pathogens to control and eradicate the pathogens in a range of 60-100%. Further studies should be oriented towards isolation and characterization of more of the lytic phages towards another CSOM pathogens of Gram-positive and Gram-negative bacteria. Animal model studies for the treatment of experimental CSOM by phage cocktails and preparation of successful phage cocktails in pharmaceutical form for human application and subsequent clinical trials on human patients must be done to prove the clinical and pharmaceutical significance of such phage cocktails.

Conflict of interest

The authors declare no conflict of interest.

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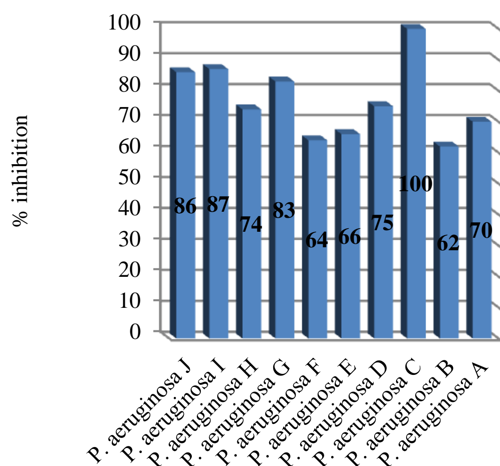

Figure 5: The % inhibition for growth inhibition assay of a phage cocktail against routine cultures of ***P. aeruginosa*** in 3 public hospitals in Baghdad

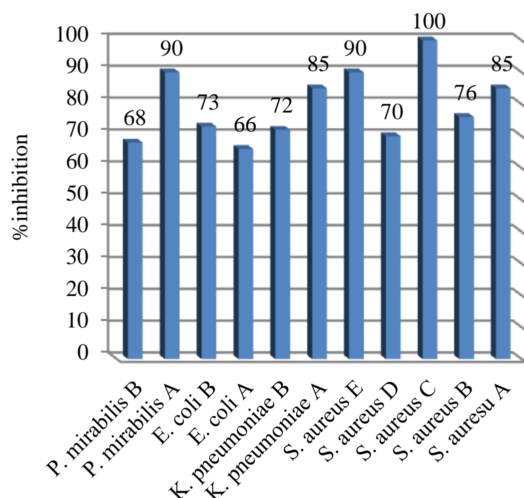

Figure 6: The % inhibition for growth inhibition assay of a phage cocktail against routine cultures of *S. aureus*, *K. pneumoniae*, *E. coli* and *P. mirabilis* in 3 public hospitals in Baghdad.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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