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Research Article

# DISTRIBUTION, ISOLATION AND CHARACTERIZATION OF LYTIC BACTERIOPHAGES AGAINST MULTI-DRUG RESISTANT AND EXTENDED-SPECTRUM OF β-LACTAMASE PRODUCING PATHOGENS FROM HOSPITAL EFFLUENTS

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**ABSTRACT**

**Objective:** To isolate. Screen and characterize an effective phage for MDR and ESBL producing pathogenic bacterial strains.

**Methods:** Bacteriophages were isolated from hospital effluent samples by double layer agar method. Isolated phages were propagated by liquid enrichment technique and its host range was analyzed by double layer agar method. Morphology of the isolated phages was identified by Transmission Electron Microscope (TEM). Genomic and proteomic analysis was confirmed by electrophoresis technique.

**Results & Discussion:** 46 bacteriophages were isolated against 20 different MDR and ESBL strains of those 7 phages (Mm81, Ec84, Ps85, En833, Sal836, Ec8ATCC and Ec8PMG) were selected for further studies. According to the host range analysis result the 7 phage has been shown narrow host range. The phage genomic DNA and structural proteins were analyzed. In addition to based on the TEM analysis two phages viz., Mm81 and Ec84 were belongs to *Siphoviridae* and *Podoviridae* family respectively. Present study evaluates the extensive occurrence of phages in the hospital effluent. In addition, this is first report of isolation and characterization of *Morganella morganii* lytic phage in Tamil Nadu, India.

**Conclusion:** The study highlights the distribution of bacteriophages in the hospital effluent and it gives the therapeutic potential of isolated phages for the treatment of MDR and ESBL Producing Pathogens.

**Keywords:** Phage therapy, Transmission electron microscope, Extended-spectrum of β-lactamase, Multi-drug resistant.

## INTRODUCTION

The emergence of antibiotic-resistant bacterial pathogens is an increasing health hazard [1,2]. Production of extended-spectrum of β-lactamase (ESBL) and other resistance mechanism lead to the development of multi-drug resistance (MDR) by bacterial pathogens, due to the extensive usage of broad-spectrum antibiotics in hospitalized patients [3]. Gram-positive infections particularly methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus* has increased in critical care units of hospitals in India. Gram-negative infections are most predominant in hospitalized patients. These infections are difficult to treat, especially in the Intensive Care Units [4]. For the past six decades, the efficiency of antibiotics against pathogens has gradually decreased, because these bacteria generate an evolutionary pressure inherent to the mechanism of action of these antibiotics. This is mostly due to the indiscriminate usage of antibiotics [5]. Though the treatment of MDR bacterial infections are extremely important; during 1998-2003, only nine new antibiotics were approved by the Food and Drug Administration out of which two antibiotics have a novel mode of action on antibiotic resistant infections [6]. There are currently no new classes of effective antibacterial compounds, which can prevent or treat antibiotic resistant bacterial infections [7]. Therefore, there is an urgent need to develop novel therapies to control these pathogens.

Phage therapy could serve as a prophylactic and therapeutic alternative treatment against MDR strains that cause pathogenic infections [1,2,8,9]. Phage therapy is already evolved and developed concept, but there is a need to regenerate this concept to fight against the antibiotic resistant bacterial infections [10].

Bacteriophages constitute a group of viruses that can specifically infect and lyse bacteria. Phages could prove to be superior to antibiotics, since they are persisting, inactive and non-pathogenic outside their bacterial hosts [11,12]. Compared with other conventional therapeutic approaches, phage therapy could be more effective in treating emerging resistant pathogenic bacterial strains [13]. It is easily discovered and can kill the biofilm forming bacteria also have low inherent toxicities [14]. Phages during the lytic course are capable of increasing in number specifically where hosts are located [15]. It's generally will not affect beneficial bacteria, side-effects are uncommon in phages and do not affect eukaryotic cells [16]. The objectives of this study were to screen the bacteriophages isolated from the hospital effluents against MDR and ESBL producing bacterial strains.

## METHODS

### Bacterial strains and growth conditions

In this study, 20 MDR and ESBL producing strains (Table 1) were obtained from Rhizosphere Biology Laboratory, Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. These 20 strains were grown in Nutrient Agar (Hi-media, India) at 37°C and stored at 4°C. Growth of these bacterial cultures was monitored by measuring optical density at 600 nm.

### Sample collection

Sewage samples were collected from stagnant effluents at a depth of 1 feet, in sterile containers from 10 different hospital areas in Tiruchirappalli, Tamil Nadu, India, between December 2010 and March 2011. Precautions were taken while collecting the samples by wearing sterile gloves and masks. Approximately 100 ml of sewage

samples were collected and transported immediately to the laboratory and stored at 4°C, until processing.

**Bacteriophage isolation**

Bacteriophages were isolated from the collected sewage samples by enrichment technique [17]. Under sterile conditions, 4.5 ml of sewage sample was emulsified with 0.5 ml of ×10 tryptone broth and 0.5 ml of log-phase broth culture (optical density measured at 600 nm) of MDR and ESBL producing bacteria. Suspensions were incubated overnight at 37°C, with shaking (120 rev/minutes) and were centrifuged at 10,000 g for 10 minutes at 4°C. The supernatant was filtered through a 0.22 µm syringe filter (Nupore, India) to obtain a bacterial free filtrate (BFF) and analyzed for the presence of phages through spot test method [18]. Double layer plaque technique was performed for bacteriophage isolation [19,20]. 3 ml of 0.6% of trypticase soy agar (TSA: tryptone - 1.7 g, soytone - 0.3 g, dextrose - 0.3 g, NaCl - 0.5 g, $K_2HPO_4$ - 0.25 g, Agar - 0.6 g, D.$H_2O$ - 100 ml) was inoculated with 100 µl of 3-5 hrs old MDR and ESBL strains and 100 µl of the BFF. The suspension was plated on fresh 1.5% TSA plate and incubated in an upright position at 37°C overnight. Formation of plaques or zones of clearance indicated the presence of phages [21].

**Phage propagation in liquid media**

The bacterial host was grown at 10 ml trypticase soy broth (TSB) at 37°C with agitation (100 rev/minutes) until log phase growth was obtained (3-4 hrs). An inoculum from a concentrated phage stock from TSA plate was added to the TSB culture. The tubes containing the phage/host suspension were re-incubated at 37°C with vigorous shaking for 5 hrs. The suspension was centrifuged at 9000 g for 10 minutes and filtered through a 0.22 µm filter. The above procedure was repeated 5 times to obtain concentrated phages from centrifugation and filtration as described in Sambrook *et al*., 1989 [22].

**Concentration and purification of phages**

The lysate in TSB was centrifuged at 8000 g for 20 minutes to remove agar and it was further filtered through 0.22 µm filter to remove host bacteria. From the concentrated phage lysate, bacteriophages were purified by using the simplified polyethylene glycol (PEG) precipitation method [22]. In 20 ml of phage lysate, pancreatic digest DNaseI and RNase were added at the final concentration of 1 µg/ml and lysate was incubated at 30 minutes at room temperature. To the lysate, 1.16 g of NaCl was added to a final concentration of 1 M. The culture was swirled until the salt dissolved and incubated in ice for 1 hr. The supernatant was centrifuged at 8300 g for 10 minutes at 4°C to remove debris. Then 1 g of solid PEG 8000 was added, to a final concentration of 10% w/v and dissolved by stirring at room temperature. The PEG/phage solution was stored at 4°C for at least 1 hr to allow the phage particles to precipitate. These precipitated bacteriophages were recovered by centrifugation at 8300 g for 10 minutes at 4°C. The supernatant was discarded and the pellet was dried at room temperature for 5 minutes. The pellet was resuspended in SM buffer (50 mM Tris - HCl (pH 7.5), 100 mM NaCl, 10 mM $MgSO_4$, and 2% gelatin) and incubated at room temperature for 1 hr. The PEG and cell debris were removed from the suspension by adding equal volume of chloroform and vortexed for 30 seconds. The organic and aqueous phases were separated by centrifugation at 4300 g for 15 minutes at 4°C and the aqueous phase containing the bacteriophages was recovered.

**Table 1: Strains used in this study**

| Type of strains | Name of the strains |
|---|---|
| MDR and ESBL strains | *M. morganii* - VRK1, *P. aeruginosa* - VRK5, *Pantoea* sp. - VRK9, *E. cloacae* - VRK33, *P. sturatii* - VRK27, *Salmonella* sp. - VRK36, *S. paratyphi* - VRK12. *E. coli* - VRK4, *P. mirabilis* - VRK15, *K. pneumoniae* - VRK34 |
| MRSA strains | *S. aureus* - VRK17, *S. aureus* - VRK2, *S. aureus* - VRK 143 |
| MSSA strain | *S. aureus* - VRK3 |
| Control strains | *S. aureus* - MTCC-96, *Klebsiella pneumonia* - MTCC-432, *E. coli* - MTCC-724, *E. coli* - ATCC-25922, *P. aeruginosa* ATCC-27853, *E. coli* - J53P$^{MG298}$ |

MDR: Multi drug resistant, ESBL: Extended-spectrum of β-lactamase, MRSA: Methicillin resistant *Staphylococcus aureus*, MSSA: Methicillin sensitive *Staphylococcus aureus*, MTCC: Microbial type culture collection, ATCC: American type culture collection, *M. morganii: Morganella morganii, P. aeruginosa: Pseudomonas aeruginosa, E. cloacae: Enterobacter cloacae, P. sturatii: Providencia sturatii, S. paratyphi: Salmonella paratyphi, E. coli: Escherichia coli, P. mirabilis: Proteus mirabilis, K. pneumoniae: Klebsiella pneumoniae, S. aureus: Staphylococcus aureus*

**Host range**

The isolated phages were investigated for the host range specificity by screening against MDR and ESBL strains. Purified phages were inoculated with different bacteria as a host, to check whether the phages have the ability to lyse different bacteria. Seven MDR and ESBL bacterial lawns were propagated on TSA plates and 10 µl of purified phages were spotted onto the bacterial lawns [23]. After 18-22 hrs of incubation, the effect of the phage suspensions on the lawns was investigated. A positive response was defined as plaques or zones of lysis [24].

**Transmission electron microscope (TEM) analysis**

The morphological analyses of potential phages were done by TEM without enrichment or preservation. One drop of phage suspension was deposited directly onto a colladium coated 200 - mesh copper grid, stabilized with a thin layer of carbon. It was allowed to adsorb for 60 seconds, and then excess liquid was removed using filter paper. The sample was stained with 1% phosphotungstic acid for 60 seconds and dried in air before being examined by TEM [25].

**Isolation of phage DNA**

Phage DNA was extracted from the purified phages by using sodium dodecyl sulfate (SDS) and proteinase K and the DNA was precipitated by adding 3 M sodium acetate and absolute ethanol according to the method of Maniatis *et al*., 1982 [26]. The pellet was dried and resuspended in an appropriate volume of sterile high-purity water and stored at −20°C. The DNA samples were simultaneously checked on 0.8% agarose gel along with DNA ladder (λ DNA digested with EcoRI/HindIII).

**SDS-polyacrylamide gel electrophoresis (PAGE)**

Purified phage structural proteins were extracted by the acetone precipitation method and analyzed through SDS-PAGE as described by Laemmli *et al*., 1970 [27]. Briefly, samples were prepared with sample buffer and loaded on 12% PAGE precast gels and electrophoresed with tris-glycine buffer. After electrophoresis, the gel was stained with bio-safe coomassie.

**RESULTS**

**Isolation of bacteriophages**

In total, 46 bacteriophages were isolated from Tiruchirappalli, Tamil Nadu, India by processing 10 hospital effluent samples against 20 different MDR and ESBL strains (Table 2). The bacteriophages appear as transparent plaques when spotted on agar plates having a lawn of host bacteria and the plaque appearance confirms their infectivity. Among the 10 samples, S8 and S2 had the highest number of bacteriophages (7 and 6, resp.). And the sample numbers 1, 3, 4, 5, 6, 7, 9 and 10 contained 5, 5, 4, 3, 3, 4, 4 and 5 lytic phages, respectively (Fig. 1). In addition, sample 7 had the phage against *S. aureus* (VRK2), while other samples did not have the phages against *S. aureus* - VRK17 (MRSA), *S. aureus* - VRK143 and *S. aureus* - VRK3 (methicillin sensitive *S. aureus* - MSSA) strains.

Sample-2 exhibited potent lytic activity against *Morganella morganii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogens*, *Salmonella* sp. and *Klebsiella pneumoniae.* while sample-8 exhibited potent lytic activity against *E. coli*, *M. morganii*, *E. coli* - ATCC,

**Table 2: Lytic activity of phages against MDR and ESBL strains**

| MDR and ESBL strains | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| *M morganii* (VRK1) | + | + | + | + | − | − | − | + | − | − |
| *P. aeruginosa* (VRK5) | + | + | + | − | − | − | − | + | − | + |
| *Pantoea* sp. (VRK9) | − | − | − | − | − | − | − | − | − | − |
| *E. cloacae* (VRK33) | − | + | + | + | − | − | − | − | − | − |
| *P. stuartii* (VRK27) | − | − | − | − | − | + | + | + | + | − |
| *Salmonella* sp. (VRK36) | + | + | + | − | + | + | − | + | + | − |
| *S. paratyphi* (VRK12) | − | − | + | + | + | − | − | − | − | + |
| *E. coli* (VRK 4) | + | + | − | + | + | − | + | + | − | + |
| *P. mirabilis* (VRK 15) | − | − | − | − | − | − | − | − | − | − |
| *K. pneumonia* (VRK34) | + | + | -− | − | − | − | + | − | − | − |
| MRSA (VRK17) | − | − | − | − | − | − | − | − | − | − |
| MRSA (VRK2) | − | − | − | − | − | − | + | − | − | − |
| MRSA (VRK143) | ND | ND | ND | ND | − | − | − | − | − | − |
| MSSA (VRK3) | ND | ND | ND | ND | − | − | - | − | − | − |
| MTCC-96 | ND | ND | ND | ND | − | − | − | − | − | − |
| MTCC-432 | ND | ND | ND | ND | − | − | − | − | − | − |
| MTCC-724 | ND | ND | ND | ND | − | − | − | − | − | − |
| ATCC-25922 | ND | ND | ND | ND | − | − | − | + | + | + |
| ATCC-27853 | ND | ND | ND | ND | − | − | − | − | − | − |
| PMG$^{298}$ J53 | ND | ND | ND | ND | − | + | − | + | + | + |

+: Presence of phage, −: Absence of phage, ND: Not done, MDR: Multi drug resistant, ESBL: Extended-spectrum of β-lactamase, MRSA: Methicillin resistant *Staphylococcus aureus*, MTCC: Microbial type culture collection, ATCC: American type culture collection, MSSA: Methicillin sensitive *S. aureus, M. morganii: Morganella morganii, P. aeruginosa: Pseudomonas aeruginosa, E. cloacae: Enterobacter cloacae, P. sturatii: Providencia sturatii, S. paratyphi: Salmonella paratyphi, E. coli: Escherichia coli, P. mirabilis: Proteus mirabilis, K. pneumoniae: Klebsiella pneumoniae, S. aureus: Staphylococcus aureus*

*P. aeruginosa*, *E. aerogens*, *Salmonella* sp. and *E. coli* J53 P$^{MG298}$. These seven phages were selected for further analysis and the phages were named based on their host specificity, sample number and strain number Mm81, Ec84, Ps85, En833, Sal836, Ec8PMG and Ec8ATCC. Out of the 10 samples, 7 samples had phage against *E. coli,* 6 samples had phage against *Salmonella* and 5 samples had phages against both *Salmonella paratyphi* and *P. aeruginosa.*

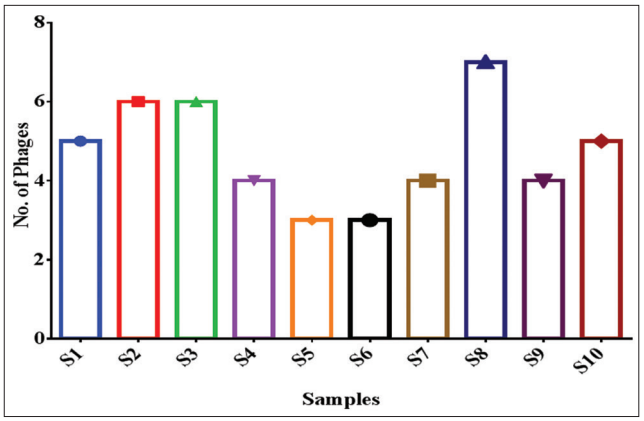


**Fig. 1: Isolated phages from the sewage sample**

### Host range analysis

Host ranges of the seven phages were analyzed for the evaluation of genus specificity. These host range analyses were carried out by spotting the phage lysates separately onto lawns formed by seven different clinical isolates respectively i.e., *M. morganii*, *E. coli*, *P. aeruginosa*, *Enterobacter cloacae* , *Salmonella* sp., *E. coli* ATCC and *E. coli* J53 P$^{MG298}$ - ATCC. Phages Mm81, Ec84, Ps85, En833 and Sal836 formed plaques only on lawns of their specific host bacteria indicating that they are highly genus specific with a narrow host range. In addition, these phages did not show lytic activity against other bacterial hosts. In case of phages, Ec8PMG and Ec8ATCC, they were seen to exhibit lytic activity in both the bacterial strains. This may be because the hosts belong to the same genus. These results indicate that these phages have host specificity and do not possess broad host range activities (Table 3).

### TEM analysis

Morphological characterization of bacteriophages was done by transmission electron microscopy. In this study, only two bacteriophages namely Mm81 and Ec84 were analyzed at high magnification in electron microscope and based on the head and tail characteristics, the phages Mm81 and Ec84 were assigned to the family of *Siphoviridae* and Podoviridae respectively. The Ec84 phage particle reveals a short tail of length (Fig. 2); In addition, the Mm81 phage reveals icosahedral head and long tail of length (Fig. 3). The measurements of these two phages were summarized in Table 4.

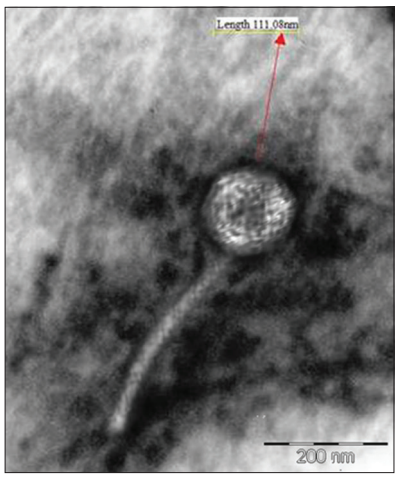


**Fig. 2: Electron microscope image of *Morganella morganii* phage Mm81**

### Phage DNA studies

Isolation of phage DNA allows the approximate determination of the genomic size of the phages with Mm81, Ec84, En833and Ec8PMG showing DNA in the range of 23 kb (Fig. 4). The two phages Sal836 and Ps85 did not yield DNA.

**Table 4: Features of phages**

| Phage | Order | Family | Size of the head | Tail | Genome | Indicator |
|---|---|---|---|---|---|---|
| Mm81 | Caudovirales | *Siphoviridae* | 111.08 nm | Long, non-contractile | ds DNA | *M. morganii* |
| Ec84 | Caudovirales | *Podoviridae* | 66.89 nm | Short, non-contractile | ds DNA | *E. coli* |

*M. morganii: Morganella morganii, E. coli: Escherichia coli*

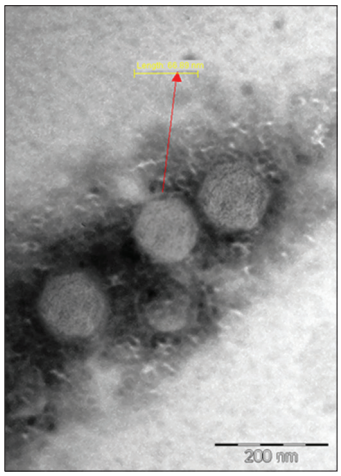


**Fig. 3: Electron microscope image of *Escherichia coli* phage Ec84**

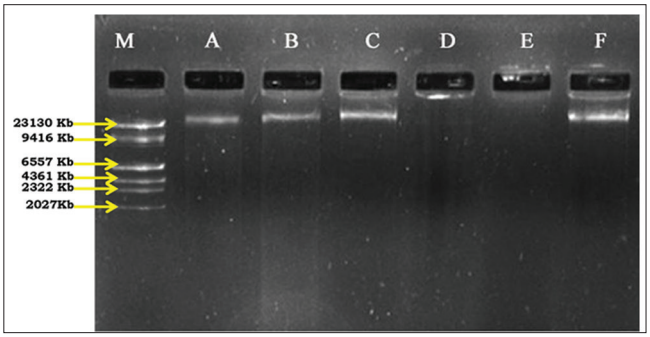


**Fig. 4: Agarose gel electrophoresis of DNA, M: Marker λ DNA digested with EcoRI/HindIII (in Kb), A: Mm81, B: Ec84, C: Ps85, D: En833, E: Sal836, F: Ec8PMG**

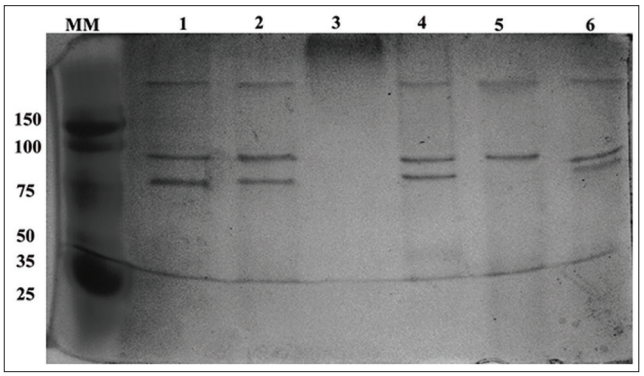


**Fig. 5: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of structural proteins of six isolated phages, MM: Medium range protein marker, 1: Mm81, 2: Ec84, 3: Ps85, 4: En833, 5: Sal836, 6: Ec8PMG**

**Table 3: Host range analysis of isolated phages**

| Phages | Bacterial host | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mm1 | Ec4 | Ps5 | En33 | Sal36 | EcPMG | EcATCC |
| Mm81 | # | – | – | – | – | – | – |
| Ec84 | – | # | – | – | – | – | – |
| Ps85 | – | – | # | – | – | – | – |
| En833 | – | – | – | # | – | – | – |
| Sal836 | – | – | – | – | # | – | – |
| Ec8PMG | – | – | – | – | – | # | + |
| Ec8ATCC | – | – | – | – | – | + | # |

+: Plaque formation, –: No plaque formation, #: Own host bacteria, Mm: *Morganella morganii*, Ec: *Escherichia coli*, Ps: *Pseudomonas aeruginosa*, En: *Enterobacter cloacae*, Sal: *Salmonella* sp., Ec8ATCC - *E. coli* ATCC, Ec8PMG - *E. coli* J53 $P^{MG298}$ - ATCC

**Structural protein analysis**

In addition, to characterize the phage proteins, they were analyzed through SDS-PAGE. 14 distinct bands with molecular weight ranging from 25 to 150 kDa, were observed in the gradient gel (Fig. 5) using the Coomassie Brilliant Blue staining. Phages Mm81, Ec84 and En833 showed proteins in the range of 75-90 kDa, while phages Sal836 and Ec8PMG showed proteins in the higher molecular weight range.

**DISCUSSION**

Only few reports have been published so far in India, particularly on characterization of the phages from Tamil Nadu. In tropical regions, bacteria have sufficient opportunity to survive and multiply in the environment. Consequently, this environment favors the evolution of bacteriophages against emerging drug resistant bacteria [28]. Numerous, bacteriophages were present ($10^8$-$10^{10}$ total phage particle) in sewage as compared to sea waters and soil [29]. The lytic phages were favorable for the phage therapy without transferring the antibiotic resistant gene among bacteria [30]. In the present study, 46 lytic bacteriophages were isolated from the hospital effluent samples and our results were similar to a study wherein 87 samples were reported from catheter washings, drainage and sewage from a hospital [31]. Mahadevan *et al.* (2009) have isolated five lytic phages against *Salmonella typhi*, *P. aeruginosa*, *E. coli*, *Klebsella* sp. and *Shigella* sp. from sewage water [32].

Oliveira *et al.* (2009) selected three (phiF78E, phiF258E and phiF61E) phages for further characterization from five isolates [33]. Our study correlated with previous reports and we selected only seven lytic phages for further characterization. These phages were isolated against three different *E. coli*, *Salmonella* sp., *P. aeruginosa, E. cloacae* and *M. morganii* strains. There were a number of reports about *E. coli*, *Salmonella sp*, *P. aeruginosa*, and *E. cloacae* phages worldwide, but there are only few reports about *M. morganii* phage.

In our study, we used NaCl and PEG 8000 for the precipitation of isolated phage particles using the simplified PEG precipitation method developed by Yamamoto *et al.*,1970 [34] wherein PEG provides a good alternative method for concentration and purification of phages. The highest recovery (100%) and a significant increase in phage concentration were obtained with NaCl and PEG 8000. Since this combination allowed the production of a dense phage pellet at the bottom of the centrifuge tube, it was easier to separate from the supernatant than the fuzzy white precipitate obtained otherwise [12].

Host range is an important characteristic factor, which makes the bacteriophage a potential therapeutic agent against bacterial infections [35]. We isolated seven lytic phages Mm81, Ec84, Ps85, En83, Sal836, Ec8PMG and Ec8ATCC which showed a narrow host range. The toxicity and dosage level studies are under progress for the successful

development of the phages as a good therapeutic agent. These results support the observations made earlier that multiple virulent phages were able to lyse the original host bacterium independently and that the bacteriophages are specific to different bacteria. The results of Yang *et al*., (2010) had found that the *Acinetobacter baumannii* specific phage AB1 had a narrow host range [36]. In general, phages exhibit very narrow host ranges, mostly infecting only one particular genus and species or in some cases, even specific strains [37].

The morphological chracteristics of the phages observed through TEM, revealed that Mm81 and Ec84 belonged to Siphoviridae and Podoviridae family respectively. Similar studies have isolated and morphologically characterized three Podoviridae families from 13 *E. coli* specific phages from sewage by TEM [38]. The present study also supported that the Podoviridae family of bacteriophages have *E. coli* as their host. In addition, the morphological characteristics of T7 phage categorize it under the Podoviridae family closely related to phage Ec84 [39]. Based on the TEM results, the phage Mm81 had an icosahedral head and long non-contractile tail and hence suggested that it belonged to the Siphoviridae family. However Zhu *et al*., have previously categorized the *M. morganii* phage MmP1 with icosahedral head and short non-contractile tail under the Podoviridae family [40].

The isolated DNA from the phages Mm81, Ec84, Ps85, En833 and Ec8PMG was 23 kb in size. Our results also supported the findings of a previous study, who have found five phages (Kpn5, Kpn12, Kpn13, Kpn17 and Kpn22), that are specific to *K. pneumoniae* and it had a genomic size of 23-24 kb [41]. Another study by Oliveira *et al*., showed that the avian pathogenic *E. coli* phage (phiF78E) genomic DNA digested with the restriction enzyme BseGI showed size in the range of 20-23 kb [35].

The protein pattern of these six phages showed similar structural proteins, which was used for the characterization and differentiation of these phages. In the present study, the phage proteins were in the range of 25-150 kDa. The above observation supports the results of Eyer *et al*., 2007) wherein the *Staphylococcal* phages 812 have proteins in the range of 10-150 kDa [42]. 14 distinct bands were observed in the gel in the range of 25-150 kDa; which corroborate with the findings of Sillankorva *et al*., 2008) who clearly distinguished the *Pseudomonas fluorescens* specific phage ϕIBB-PF7A having 16 distinct bands at the range of 10-140 kDa [20]. The results of our study correlated to the findings of Lin *et al*., 2010, where they reported the novel *A. baumannii* phage ϕAB2 having 7 distinct protein bands with molecular mass ranging from 20 to 110 kDa [31]. Phages Mm81, Ec84 and En833 showed 2 minor protein bands at 75 and 90 kDa resp. Similar results have been reported by Kumari et al. 2010 where *K. pneumoniae* specific phages (Kpn12) possessed 2 minor protein bands in the range of 43 and 55 kDa.

Isolated phages have lytic activity against the antibiotic resistant bacterial strains and it explains the extensive occurrence of phages in hospital effluents. In addition, the present study is the first report of isolation and characterization of *M. morganii* lytic phage in Tamil Nadu, India. This highlights the distribution of bacteriophages in hospital effluents and their therapeutic potential against MDR and ESBL producing pathogens by *in-vitro* studies. However, further studies have to be done on phages as a therapeutic agent using specific phages against these pathogens.

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