International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 722-730
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

# Role of Inanimate Objects in Nosocomial Infections and their Prevention by Specific Bacteriophage Treatment in Intensive Care Unit

**Rahul Katiyar[1], Arvind Bhalekar[1,*], Shekhar Verma[1], Alakh Narayan Singh[2], Gopal Nath[2], Ravi Kumar Verma[1], Rajeev Kumar Dubey[1]**

**[1]Department of Anaesthesiology, Sir Sundar Lal Hospital (Institute of Medical Sciences), Banaras Hindu University, Varanasi-221005, Uttar Pradesh; INDIA.**
**[2]Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh; INDIA.**

Received: 12 Mar 2019 / Accepted: 16 Apr 2019 / Published online: 1 Jul 2019
***Corresponding Author Email:** minearvind@gmail.com

## Abstract

Intensive care unit (ICU) acquired infections are challenging health care problems worldwide, especially multidrug-resistant (MDR) pathogens. In ICUs, inanimate surfaces and equipment e.g. stethoscopes, medical charts, ultrasound machine, bedrails etc may be contaminated by bacteria and MDR pathogens. Cross-transmission of microorganisms from inanimate surfaces may have a major role in ICU acquired infections and colonization. Contamination can happen from healthcare worker's hands or by patients themselves which are able to survive several months on dry surfaces. The practice of phage therapy, which uses bacterial viruses to treat bacterial infections, has been used for almost a century. Further, the decline in the effectiveness of antibiotics (i.e. drug resistance) has generated renewed interest in revisiting this practice. Conventionally, bacteriophage therapy reckons on the use of naturally occurring bacteriophage to infect and lyse bacteria at the site of infection. In the present study, bacteriophages were used as an adjuvant disinfectant for environmental cleaning and the efficacy of a phage aerosol on nosocomial transmission in ICUs was evaluated. The relevant clinical samples from patients admitted during first six months of study along with swab samples from environment and inanimate objects from ICU were collected for culture isolation. During next six months, *Klebsiella pneumoniae* specific phages were applied on the surface and common objects present in ICU. After this, spraying of phages was continued fortnightly for six months. The specimens from patients and environment were processed for culture isolation. The result showed significant reduction in isolation rate of *Klebsiella pneumoniae* from inanimate objects after *Klebsiella pneumoniae* specific phage spray. Furthermore, the effect of this reduction on inanimate objects leads to reduced isolation rate from patient's samples also. These observations showed definitive role of phage therapy of inanimate objects in ICU and in reduction of incidence of infections.

**Keywords**

Nosocomial infections; bacteriophages; intensive care unit; *Klebsiella pneumoniae;* multidrug-resistant

*****

## INTRODUCTION

As per reports of Centre for disease control (CDC), out of every hundred hospitalized patients at any given point of time, seven in developed and ten in developing countries acquire at least one health care-associated infection. The frequency and severity vary with the type of patient, nature of treatment and duration of stay in the hospital.[1-7] Intensive care unit (ICU) acquired infections take place by contact transmission (most and frequent mode), droplet transmission, airborne transmission, common vehicle transmission and vector borne transmission etc.[8-10] There are increasing evidences that contaminated inanimate surfaces, especially those frequently touched by hand, can significantly contribute to the spread of healthcare-associated pathogens. Nevertheless, it is difficult to eradicate the organisms from contaminated surfaces using conventional cleaning and disinfection methods.[11-17] However, for environmental cleaning, bacteriophages are a potential alternative to deal with surface contaminants.[18,20] In the present study, bacteriophages were used as an adjuvant disinfectant for environmental cleaning and the efficacy of a phage aerosol on nosocomial transmission in ICUs was evaluated.

## MATERIAL AND METHODS

This study was conducted in the ICU of Sir Sunder Lal Hospital, BHU, Varanasi, India. This is a tertiary care hospital providing super-specialty health care services. This prospective interventional study was conducted from 1st January 2017 to 31st December 2018 in a 16 bedded ICU having both medical and surgical patients. Ethical clearance for the study was obtained from Ethics Committee of the Institute and as per the University protocol prior to the study. Patients of either gender, staying in ICU for more than 48 hours were selected for the study. Well informed written consent was obtained from each of the patients or from their attendants as appropriate. The exclusion criteria for the study were:

1. Patients who stayed for less than three days in ICU.
2. Patients who were culture positive with some infection at the time of admission.
3. Patients whose attendants refused to give consent.
4. Patients who were admitted in ICU for monitoring only.

***Collection of data and culture from patients***

APACHE II (Acute Physiology and Chronic Health Evaluation II) score for all the patients were obtained at the time of admission.[21] Routine investigations along with culture samples such as blood, urine, sputum or endotracheal aspirate were obtained from all the patients at the time of admission. Also, sequential cultures were sent on day 3, day 7, day 14, and day 21 and so on as per our ICU protocols. Proper aseptic precautions were taken while collecting and transporting the samples. The samples were then sent for culture isolation. All the patients were followed up till their total duration of stay in ICU and records of all the culture reports were compiled in study record sheets. Outcome of all the patients were measured in terms of their total duration of stay in ICU and discharge to ward/death. Patients getting readmitted in ICU after more than 72 hours of their previous ICU stay were considered as new admissions.

***Collection of culture from inanimate objects***

Swab culture were obtained weekly from common inanimate objects in ICU such as Bain's circuit, Ambu bag, bed rails, ventilator's expiratory ports, command buttons of cardiac monitors, medical charts papers and caregiver's hands for consistent 52 weeks of study duration. All the samples were taken in duplicate by surface sampling method with the help of sterile swab and were inoculated in MacConkey's agar and blood agar plates. The isolated organisms from inanimate objects and that of patient's samples were then compared for any possible correlation.

**Study was conducted in two phases:**

1. Pre-interventional phase (P1) for 6 months
2. Interventional phase (P2) for 6 months

In P1 phase, incidence of nosocomial infections and prevalence of various bacteria obtained from culture reports of patients were recorded. Simultaneously, the prevalence of various bacteria contaminating the inanimate objects of ICU were recorded.

In P2 phase, bacteriophages isolated against *Klebsiella pneumoniae* were sprayed over inanimate objects of ICU. Again, prevalence of bacteria contaminating the objects of ICU and incidence and prevalence of bacteria infecting the patients were recorded. Results were compared between two groups (P1 and P2) and statistical analysis were done to find out effects of bacteriophage treatment of inanimate objects on nosocomial infections.

***Environmental decontamination***

After routine decontamination as per ICU protocols, bacteriophage aerosols generated through humidifier were sprayed all over the ICU. After dilution bacteriophage concentration was found to be $10^8$ PFU/ml in stock. Amount of bacteriophage chosen for aerosol spray was such that the surface

density of at least $10^4$ PFU per $cm^2$ of area could be achieved.

***Processing of samples for microbiological assessment***

Identification of bacteria was done by morphological and microscopic observations. In the morphological identification, the shape, size and cell wall structures were observed. Gram staining technique was used for microscopic observations. Biochemical tests included for identification were indole test, motility test, sugar test-glucose, lactose, mannose, sucrose, urease test, citrate utilizing test and $H_2S$ production test.

***Isolation of bacteriophages from different sources of water samples*** [22]

***Collection of water sample***

Sewage water from Sir Sundarlal Hospital, BHU, Varanasi, was collected in the sterilized rubber capped bottles with the help of thread string.

***Removal of contaminants from water***

Contamination was removed by centrifugation at 10,000 rpm for 10 min at 4°C. After centrifugation supernatant was taken out and treated with 1% chloroform solution for 15min (micro centrifuge tubes were shaken for 15min.). Centrifugation was repeated again at 10,000 rpm for 10min at 4°C and supernatants were taken for the processing.

***Sensitization of Klebsiella pneumoniae Bacteriophage***

*Klebsiella pneumoniae* culture was inoculated in the 5 ml LB broth and incubated at 37°C for overnight. Then, in 2ml micro centrifuge tubes 200µl of purified water, 1.2 ml of 2XLB broth and 20µl overnight culture of bacteria were added and incubated overnight at 37°C in incubator. After incubation, the medium was centrifuged, and supernatant was collected. Then 1 ml supernatant was taken from each micro centrifuge tube and treated with 1% chloroform and centrifuged three times at 10,000 rpm for 10 min. The resulting supernatant was collected for the phage generation.

***Plaques formation***

Phages appeared in the form of plaque. In the sterile test tubes 100µl above supernatants were added with the 890µl of TMG buffer and 10µl of *Klebsiella pneumoniae* culture suspensions. The contents in test tubes were mixed and incubated in 37°C at least for 4 hrs in the water bath shaker. After incubation, 4.5ml soft agar (cooled at 45-50°C) was added in the test tubes. The whole contents were mixed properly and poured on the Muller Hinton Agar media plates. Soft agar was allowed to solidify, and plates were incubated at 37°C for overnight in inverted position and were observed next day.

***Harvesting and purification of Bacteriophage***

After obtaining the confluent plaques on the plates, the surface of the lawn culture containing plaques on the plates were washed with the TMG buffer with the help of cotton swab streak. Washed out material was collected in the micro centrifuge tubes and treated with 1% chloroform for 15 min by shaking. The centrifuge tubes were centrifuged at 10,000 rpm for 10 min at 4°C. The supernatants collected were further centrifuged three-times. The final supernatant was collected and stored in the refrigerator at 4°C for further use.

***Bulk production of bacteriophages***

15µl of phages were taken from harvested phages stored at 4°C and diluted with 135µl TMG buffer and serial dilutions were performed from $10^{-1}$ to $10^{-15}$. 100µl of diluted phages from each dilution factor were added to different test tubes containing 890µl TMG buffer and 10µl bacterial suspension. The test tubes were incubated in a water bath shaker at 37°C for 4 hrs. After incubation, 4.5ml soft agar was added to each test tube and mixed properly. Then the whole contents of test tubes were poured in the MH agar containing media plates. The plates were swirled to spread the soft agar onto entire surface and the soft agar was allowed to dry. The plates were incubated for overnight at 37°C. Next day, the plates were observed for confluent plaques.

Confluent plaques obtained were used for the bulk production of bacteriophages. In the sterile test tubes 10µl of bacterial suspension and 100µl of phage suspension were taken and mixed properly. Then the test tubes were incubated 37°C for 4 hrs. After incubation 3ml soft agar was added and whole contents were poured in MH agar plates. The plates were incubated at 37°C for overnight. Next day plaques were observed on the plate. The upper layer, containing plaques were scrapped by using sterile cotton swabs and TMG buffer. The washed-out material was collected in the micro centrifuge tubes and treat with 1% chloroform for 15 min. Then the tubes were centrifuged at 10,000 rpm for 10 min at 4°C. The supernatants were collected and re-centrifuged for 2 time and resulting supernatants were stored at 4°C in the refrigerator.

***Sensitivity analysis of bacteriophages on host***

Isolated bacteriophages were checked for the activity over *Klebsiella pneumoniae*. MH agar plates were prepared, and bacterial suspension was swabbed over the media plates by using sterile cotton swabs. The swabbed plates were incubated at 37°C for 4 hrs to maintain the bacterial load in the form of log phase. After incubation 5µl of bacteriophages with the help of micropipette drop

on the swabbed MH agar plates according to the bacteriophages numbering.

***Concentration of Bacteriophages***

For concentration of bacteriophage sample, Polyethylene glycol (PEG)-8000 solution was used. The supernatant, collected in previous step, were treated with PEG-8000/2.5M NaCl solution. The final concentration of PEG solution was 25% of the phage supernatant (7.5ml PEG+30ml supernatant). Then it was incubated for overnight at 4°C. Next day the supernatants were centrifuged at 11,000 rpm for 20 mins and milky pellets were collected. The centrifugation processes were continued 2-3 times to remove all the PEG solutions. The milky pellets were dissolved in STE buffer solution and then stored for further usage.

**Statistical analysis**

Statistical analyses were performed using the statistical software SPSS version 23 (SPSS Inc., Chicago, IL, USA). Descriptive frequencies were expressed using mean (standard deviation) and median (range). Difference between means of continuous variables were compared using the unpaired student t-test and analysis of variance as applicable and that of categorical variables with the Chi-Square test. The critical value of 'p' indicating the probability of significant difference was taken as < 0.05 for comparison.

**RESULTS**

Total admissions were 763 during the above-mentioned study duration. After exclusion of the patients as per predetermined exclusion criteria, total 475 patients were included in study (Table 1). Study was conducted in two phases-

1. Pre-interventional phase (P1)
2. Interventional phase (P2)

**Table 1: Comparison of demographic and clinical parameters in P1 and P2 phase**

| | **Pre-interventional phase (P1)** (n=245) | **Interventional phase (P2** (n=230) | **P value** |
|---|---|---|---|
| Mean age (in years) (Mean age in years ± SD) | 48 ± 19.7 | 45.9 ± 17.9 | 0.218 |
| Male: Female Ratio | 130:115 (53.1%:46.9%) | 121:109 (52.6%:47.4%) | 0.927 |
| APACHE II SCORE (Mean score ± SD) | 23.42 ± 10.9 | 22.34 ± 10.43 | 0.273 |
| MEAN DAYS IN ICU | 11.43 | 8.49 | 0.360 |

**SD=standard deviation**

Total patients in pre-interventional phase (P1) were 245 and in interventional phase (P2) were 230. Mean age of group P1 was 48 and group P2 was 45.9 years (p value- 0.218) and male: female ratio 53.1:46.9 and 52.6:47.4 for group P1 and P2, respectively (p value- 0.927). To assess the severity of illness on the 1$^{st}$ day in the ICU, APACHE II score was used. APACHE II Score of all the patients at time of ICU admission was calculated. Mean APACHE II Score for P1 and P2 was noted 23 ± 10.9 and 22.34 ± 10.43, respectively (p value- 0.273). Mean duration of ICU stay was 11.43 and 8.49 for group P1 and P2, respectively (p value- 0.360). This statistical analysis shows that both the groups were matched for age, gender, APACHE II SCORE at time of admission and during the stay in ICU (Table 1).

In the second phase of study (P2), isolation of bacteriophages from different sources of water samples (Ganga river water and sewage, ponds) was done. These bacteriophages were then sensitized against *Klebsiella pneumoniae* bacteria which were isolated in first phase of study (P1). Further purification and bulk production of these bacteriophages was done. These concentrated *Klebsiella pneumoniae* specific bacteriophages were then selected for aerosol spray.

After routine decontamination of ICU, aerosol spray of *Klebsiella pneumoniae* bacteriophage was done fortnightly. Swab cultures from selected seven inanimate objects was taken as in phase P1. Prevalence of various bacteria present over inanimate objects were noted and compared with prevalence in phase P1 (Table 2).

**Table 2: Impact of bacteriophage treatment of inanimate objects on incidence and prevalence of different species of bacteria contaminating ICU**

| BACTERIA | P1 | P2 | P value |
|---|---|---|---|
| *Acinetobacter spp.* | 13 (7.14%) | 16 (8.79%) | 0.561 |
| *Klebsiella pneumoniae* | 17 (9.34%) | 6 (3.30%) | **0.018*** |
| *Staphylococcus aureus* | 33 (18.13%) | 29 (15.93%) | 0.577 |
| *Pseudomonas aeruginosa* | 10 (5.49%) | 11 (6.04%) | 0.822 |
| *Enterococcus spp.* | 6 (3.30%) | 7 (3.85%) | 0.778 |
| *Micrococcus spp.* | 7 (3.85%) | 6 (3.30%) | 0.778 |
| *Streptococcus spp.* | 3 (1.65%) | 5 (2.75%) | 0.475 |
| *Escherichia coli* | 4 (2.20%) | 3 (1.65%) | 0.703 |
| *Proteus spp.* | 3 (1.65%) | 5 (2.75%) | 0.475 |
| Others | 3 (1.65%) | 3 (1.65%) | 1.000 |

After aerosol spray of inanimate objects with bacteriophage against *Klebsiella pneumoniae*, there was significant reduction in prevalence of *Klebsiella pneumoniae* bacteria in phase P2 as shown in Table 2, Figure 1 (p value= 0.018). No significant difference was found in prevalence of other bacteria found over inanimate objects.

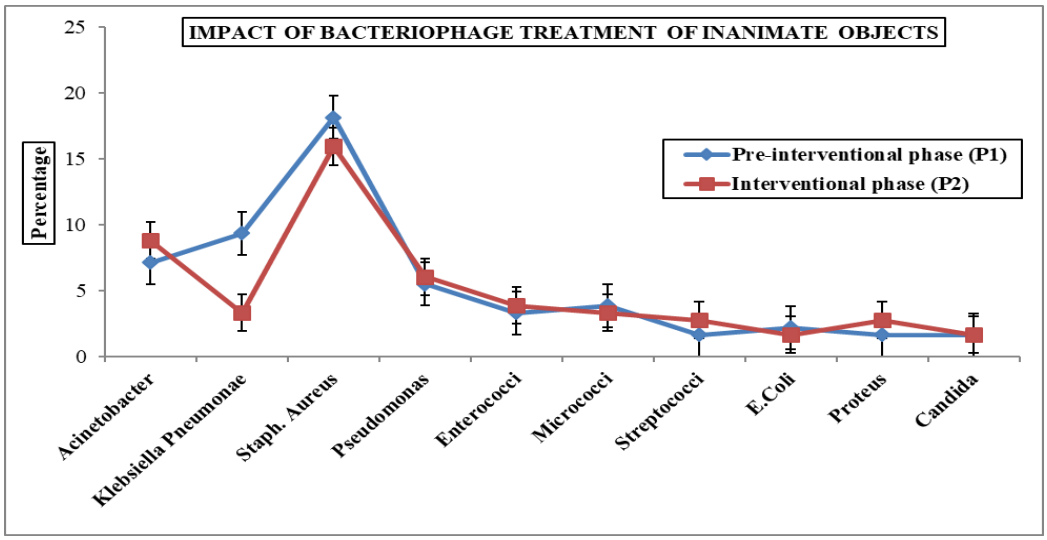


**Figure 1. Impact of bacteriophage treatment of inanimate objects on incidence and prevalence of different species of bacteria contaminating ICU**

**Table 3: Prevalence of different species of bacteria causing nosocomial infection in P1 and P2 phases**

| Bacteria | P1 | P2 | P value |
|---|---|---|---|
| *Acinetobacter spp.* | 50 (42.73%) | 54 (44.62%) | 0.485 |
| *Klebsiella pneumoniae* | 30 (25.64%) | 14 (11.57%) | 0.031 |
| *Staphylococcus aureus* | 9 (7.7%) | 12 (9.91%) | 0.552 |
| *Pseudomonas aeruginosa* | 12 (10.26%) | 20 (16.52%) | 0.142 |
| *Enterococcus spp.* | 2 (1.70) | 2 (1.65%) | 0.949 |
| *Micrococcus spp.* | 2 (1.70%) | 4 (3.30%) | 0.625 |
| *Streptococcus spp.* | 5 (4.27%) | 3 (2.48%) | 0.789 |
| *Escherichia coli* | 2 (1.70%) | 5 (4.13%) | 0.397 |
| *Citrobacter spp.* | 2 (1.70%) | 3 (2.47%) | 0.943 |
| Others | 3 (2.56%) | 4 (3.30%) | 0.933 |
| Total | 117 (100%) | 121 (100%) | -- |

Prevalence of various bacteria causing nosocomial infections in ICU in P1 and P2 phases was *Acinetobacter baumannii* (42.73% and 44.62%), *Klebsiella pneumoniae* (25.64% and 11.57%), *P. aeruginosa* (10.26% and 16.52% ), *S. aureus* (7.7% and 9.91), *Streptococci* (4.27% and 2.48%), *Enterococci* (1.7% and 1.65%), *Micrococci* (1.7% and 3.30%), *E.coli* (1.7% and 4.13%), *Citrobacter* (1.7% and 2.47%) and others (2.56% and 3.30%), respectively as shown in Table 3, Figure 2.

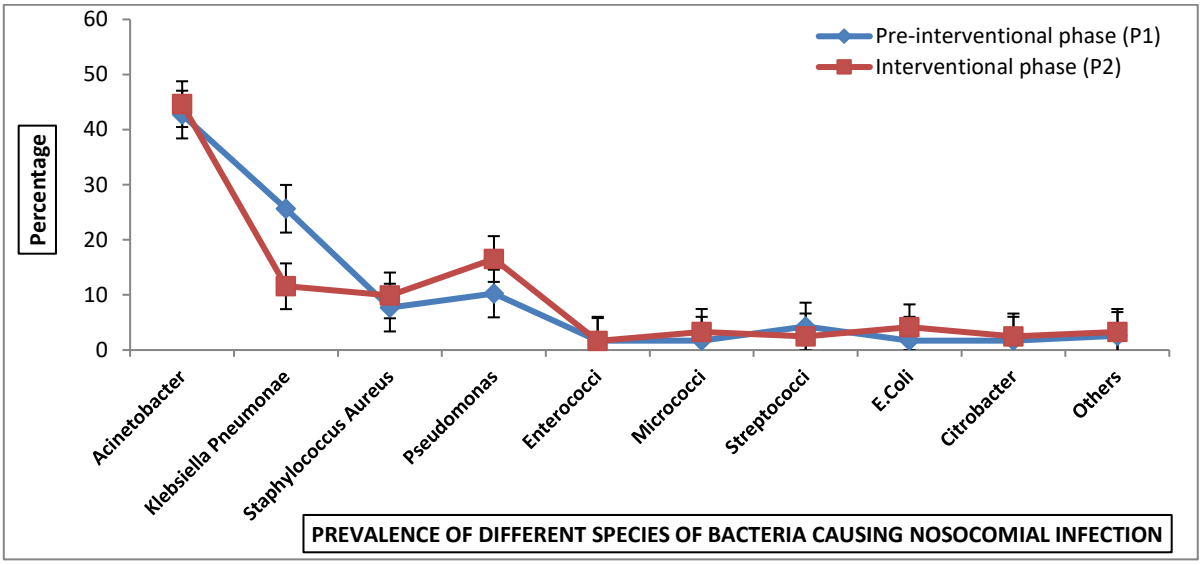


**Figure 2. Prevalence of different species of bacteria causing nosocomial infection in P1 and P2 phases.**

This study showed that there was significant difference in prevalence of *Klebsiella pneumoniae* infections as 25.64% and 11.57% in P1 and P2 phases, respectively (p value= 0.031) (Table 3). There was no change in prevalence of various other bacteria responsible for nosocomial infections as no particular intervention was done to stop the transmission of these bacteria. Thus, *Klebsiella pneumoniae* bacteriophage cocktail aerosol spray over inanimate objects of ICU significantly reduces the contamination of these objects with *Klebsiella pneumoniae* bacteria. Results shows that there was significant decrease in incidence and prevalence of nosocomial infections caused by *Klebsiella pneumoniae*.

## DISCUSSION

Increasing evidence supports the contribution of inanimate surface and equipment's contamination for transmission of pathogens to ICU patients.[1-8] Also, contaminated hands of healthcare workers after contact with inanimate surfaces surrounding a patient's bed can further transmit these pathogens to different inanimate surfaces and also to patients directly.[4] Health care associated infections are major burden to patients, society and health care management system. This burden significantly increases in ICU because of underline pathologies i.e. chronic lung disease, diabetic mellitus, hypertension and renal failure, along with local and systemic infections and immuno-compromised patients due to advanced age. Often patients admitted in ICU are already on immunosuppressive drugs due to various reasons. In addition to above factors several invasive procedures are also carried out such as central venous pressure (CVP) line insertion, endotracheal intubation, tracheostomy, mechanical ventilation, etc. Moreover, number of equipments and other factors related to environmental engineering (space ventilation, traffic flow, air conditioning) are responsible for high prevalence of nosocomial infections in ICU.[15]

It has been observed that the infections might be transmitted to the patient from the contaminated environment and inanimate objects while doing invasive procedures. This study, therefore, was planned to evaluate the effect of bacteriophage spray on the inanimate objects on the incidence of infections in ICU. *Klebsiella pneumoniae*, one of the most resistant bacteria, prevalent in ICU was selected for this purpose.[17]

In the present study, the relevant clinical samples from patients admitted during first six months of study (P1 Phase) along with swab samples from environment and inanimate objects from ICU was collected for culture isolation. During next six months, *K. pneumoniae* specific phages were applied on the surface and common objects present in ICU.

After this, spraying of phages was continued fortnightly for whole six months. The specimens from patients and environment were processed for culture isolation as mentioned above.

It was intriguing to note that there was significant reduction in isolation rate of *Klebsiella pneumoniae* from 9.3% (17/182) to 3.3% (6/182) (p value=0.018) from the inanimate objects and environment. Furthermore, the effect of this reduction on inanimate objects could be observed from isolation rate from patient's samples also. Wherein, significant reduction was observed in isolation of *K. Pneumoniae* from 25.6% (30/117) to 11.6% (14/121) (p value-0.031). This observation shows definitive role of phage therapy of inanimate objects in ICU and in reduction of incidence of infections. These results were in support with the study conducted by Yu-Huai Ho *et.al* where bacteriophages were successful in decreasing the rates of infection caused by carbapenam-resistant *Acinetobacter baumannii* after application of bacteriophage-containing aerosol against these bacteria. [23]

In this study, a total of 475 patients were included of which 245 belong to pre-interventional phase (P1) and 230 belong to interventional phase (P2). The commonest bacterial which could be isolated from human subjects was *Acinetobacter baumannii* (42.2%, 50/107) followed by *Klebsiella pneumoniae*25.6%, 30/107), *Pseudomonas aeruginosa* (10.3%, 12/107), *Staphylococcus aureus* (7.7%, 9/107), *Streptococci* (4.3%, 5/107) and other miscellaneous bacteria. These results were similar to Doyle *et al.*[2] and Patwardhan RB *et al.*[16] but the studies conducted by Xie *et al.* [24] has reported *Pseudomonas aeruginosa*, and *E. coli* as the most prevalent germs consistent with nosocomial infection. Further, these results were supported by a study from a tertiary care center ICU from North India where bacteriological culture came positive in 28% of enrolled patients. Gram-negative bacteria were the most common and included *Acinetobacter baumannii K. pneumoniae*, *E. coli*, and *Pseudomonas aeruginosa.*[25]

However, from inanimate objects in pre-interventional phase *Staphylococcus aureus* with the isolation rate 18.1% (33/182) was the commonest isolate followed by *Klebsiella pneumoniae,* 9.34% (17/182); *Acinetobacter baumannii*, 7.1% (13/182); *Pseudomonas aeruginosa* 5.4%, (10/182); *Enterococci,* 3.3% (6/182) and other miscellaneous bacteria. Although *Acinetobacter baumannii* was the commonest bacteria isolated from patients but we picked up *Klebsiella pneumoniae,* a Gram- negative bacteria, facultative fermenter and resistant to most of the antibiotics, for this interventional study. The significantly higher prevalence of *Staphylococcus aureus and Enterococci* may be explained in the basis of their cell wall structure known for resistant to desiccation. Interestingly, *Acinetobacter baumannii* despite being a Gram-negative bacterium are quite resistant to desiccation.[26] Furthermore of the other gram-negative bacteria *Klebsiella* spp. and *Enterobacter spp.* are also known for their viability for several days on inanimate objects. [27]

In the present study, only *Klebsiella pneumoniae* were targeted among variety of bacterial isolates obtained both from the patients and objects. The real impact of bacteriophage spray of inanimate objects on clinical infection in ICUs would have been seen when cocktail of the bacteriophages against all the bacterial strains isolated would have been used. This is why, we could not see the obvious reduction in incidence of ICU infections from pre-interventional to interventional period. Furthermore, frequency of spraying fortnightly might have affected the outcome in terms of *K. pneumoniae* in pre-interventional to interventional phases. It might be possible that if the frequency of spraying could have been increased, the incidence of *K. pneumoniae* infection in interventional period might have been decreased further. However, if the bacterial species causing infections in these patients were part of indigenous flora such type of spray would not be effective. Therefore, it is of utmost importance to determine the origin of implicated bacteria in ICU infections.

There are infrequent reports on this aspect from India as well as abroad. The present study is unique, and first time carried out in our university hospital. Significant reduction in the incidence of *K. pneumoniae* infection from pre-interventional and interventional phase is very encouraging.

The present study strongly suggests that further evaluation of the cocktail of bacteriophages against prevalent bacteria in the ICU should be carried out. The dose and frequency of bacteriophages in cocktail should also be decided. The further studies on the proposed line will definitely reduce the incidence of infections in places like ICU.

## CONCLUSION

Hospital acquired infections (HAIs) are more common in intensive care unit (ICU). Contaminated inanimate surfaces, especially those frequently touched by hand, can contribute to the spread of healthcare-associated pathogens. [28,29] For environmental cleaning, bacteriophages are a potential alternative strategy against bacterial

contamination. [30-34] Here, we used bacteriophages as an adjuvant disinfectant for environmental cleaning and evaluated the efficacy of a phage aerosol on nosocomial transmission in ICUs. [35-38] There was significant reduction in isolation rate of *Klebsiella pneumoniae* from inanimate objects after *Klebsiella pneumoniae* specific phage spray. [39-41] Furthermore, the effect of this reduction on inanimate objects leads to reduced isolation rate from patient's samples also. This observation shows definitive role of phage therapy of inanimate objects in ICU and in reduction of incidence of infections.

## CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

## REFERENCES

1. Allegranzi B, Bagheri Nejad S, Combescure C. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet. 2011; 377(9761):228–41.
2. Doyle JS, Buising KL, Thursky KA, *et al*. Epidemiology of infections acquired in intensive care units. Semin Respir Crit Care Med. 2011; 32(2):115-38.
3. Boyce JM. Environmental contamination makes an important contribution to hospital infection. J Hosp Infect. 2007; 65(Suppl 2):50–4.
4. Pittet D, Dharan S, Touveneau S, *et al*. Bacterial contamination of the hands of hospital staff during routine patient care. Arch Intern Med. 1999; 159 (8):821-6.
5. Caselli E. Hygiene: microbial strategies to reduce pathogens and drug resistance in clinical settings. Microb Biotechnol. 2017; 10(5):1079–83.
6. Dancer SJ. The role of environmental cleaning in the control of hospital-acquired infection. J Hosp Infect. 2009; 73(4):378–85.
7. Getchell-White SI1, Donowitz LG, Gröschel DH. The inanimate environment of an intensive care unit as a potential source of nosocomial bacteria: evidence for long survival of Acinetobacter calcoaceticus. Infect Control Hosp Epidemiol. 1989; 10(9):402-7.
8. Huang SS, Datta R, Platt R. Risk of acquiring antibiotic-resistant bacteria from prior room occupants. Arch Intern Med. 2006; 166(18):1945–51.
9. Manian FA, Griesnauer S, Senkel D, *et al*. Isolation of Acinetobacter baumannii complex and methicillin-resistent Staphylococcus aureus from hospital rooms following terminal cleaning and disinfection: can we do better? Infection control and Hospital Epidemiology. 2011; 32(7):667-72.
10. Manian FA, Griesnauer S, Senkel D. Impact of terminal cleaning and disinfection on isolation of Acinetobacter baumannii complex from inanimate surfaces of hospital rooms by quantitative and qualitative methods. Am J Infect Control. 2013; 41: 384–5.
11. Neely AN. A survey of gram-negative bacteria survival on hospital fabrics and plastics. J Burn Care Rehabil. 2000; 21(6):523-7.
12. Jawad A, Seifert H, Snelling AM, *et al*. Survival of Acinetobacter baumannii on dry surfaces: comparison of outbreak and sporadic isolates. J Clin Microbiol. 1998; 36(7):1938-41.
13. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect. Dis. 2006; 6:130.
14. Martinez JA, Ruthazer R, Hansjosten K, *et al*. Role of environmental contamination as a risk factor for acquisition of vancomycin-resistant enterococci in patients treated in a medical intensive care unit. Archives Int. Med. 2003; 163:1905-12.
15. Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. Infect Control Hosp Epidemiol. 2011; 32(7):687-99.
16. Patwardhan RB, Dhakephalkar PK, Niphadkar KB, *et al*. A study on nosocomial pathogens in ICU with special reference to multiresistant Acinetobacter baumannii harbouring multiple plasmids. Indian J Med Res. 2008; 128(2):178-87.
17. Suetens C, Hopkins S, Kolman J, *et al*. European Centre for Disease Prevention and Control. Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals – 2011–2012. Stockholm: ECDC; 2013.
18. Caselli E, Antonioli P, Mazzacane S. Safety of probiotics used for hospital environmental sanitation. J Hosp Infect. 2016; 94(2):193-4.
19. Caselli E, D'Accolti M, Vandini A, *et al*. Impact of a probiotic-based cleaning intervention on the microbiota ecosystem of the hospital surfaces: focus on the resistome remodulation. PLoS One. 2016; 11(2): e0148857.
20. D'Accolti M, Soffritti I, Piffanelli M, *et al*. Efficient removal of hospital pathogens from hard surfaces by a combined use of bacteriophages and probiotics: potential as sanitizing agents. Infection and Drug Resistance. 2018; 11:1015-26.
21. Knaus WA, Draper EA, Wagner DP. APACHE II: a severity of disease classification system. Crit Care Med. 1985; 13(10):818-29. PMID 3928249
22. Sambrook J, Russell D (2001) Molecular Cloning: A Laboratoty Manual, Edn.: CSHL, New York.
23. Ho YH, Tseng CC, Wang LS, *et al*. Application of Bacteriophage-containing Aerosol against Nosocomial Transmission of Carbapenem-Resistant *Acinetobacter baumannii* in an Intensive Care Unit. PLOS ONE 2016; 11(12)
24. Xie DS, Xiong W, Xiang LL, *et al.* Point prevalence surveys of healthcare-associated infection in 13 hospitals in Hubei Province, China, 2007-2008. J Hosp Infect. 2010; 76:150–5.
25. Ghanshani R, Gupta R, Gupta BS, *et al*. Epidemiological study of prevalence, determinants, and outcomes of infections in medical ICU at a

tertiary care hospital in India. Lung India. 2015; 32(5): 441–8.
26. Tankovic J, Legrand P, de Gatines G, *et al*. Characterisation of a hospital outbreak of imipenem-resistant Acinetobacter baumannii by phenotypic and genotypic typing methods. J. Clin. Microbiol. 1994; 32:2677–81
27. Weber DJ, Rutala WA, Miller MB, *et al*. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, Clostridium difficile, and Acinetobacter species. Am J Infect Control. 2010; 38(5 Suppl 1):S25–S33.
28. Weber DJ, Anderson D, Rutala WA. The role of the surface environment in healthcare-associated infections. Curr Opin Infect Dis. 2013; 26(4):338–44.
29. Dancer SJ. Importance of the environment in methicillin-resistant Staphylococcus aureus acquisition: the case for hospital cleaning. Lancet Infect. Dis. 2008; 8:101-13.
30. Abuladze T, Li M, Menetrez MY, *et al*. Bacteriophages reduce experimental contamination of hard surfaces, tomato, spinach, broccoli, and ground beef by Escherichia coli O157:H7. Appl Environ Microbiol. 2008; 74(20):6230-8.
31. Capparelli R, Parlato M, Borriello G, *et al*. Experimental phage therapy against Staphylococcus aureus in mice. Antimicrob. Agents Chemother. 2007; 51:2765-73.
32. Caselli E, Antonioli P, Mazzacane S. Safety of probiotics used for hospital environmental sanitation. J Hosp Infect. 2016; 94(2):193-4.
33. Caselli E, D'Accolti M, Vandini A, *et al*. Impact of a probiotic-based cleaning intervention on the microbiota ecosystem of the hospital surfaces: focus on the resistome remodulation. PLoS One. 2016;11(2): e0148857.
34. Caselli E. Testing antibacterial activity of bacteriophages on hard surfaces. http://www.protocols.io. 2018; Available from: dx.doi.org/10.17504/protocols.io.nwmdfc6. Accessed October 10, 2018.
35. Green J, Wright PA, Gallimore CI, *et al.* The role of environmental contamination with small round structured viruses in a hospital outbreak investigated by reverse-transcriptase polymerase chain reaction assay. J. Hosp. Infect. 1998; 39:39-45.
36. Greer GG. Bacteriophage control of foodborne bacteriat. J Food Prot. 2005; 68(5):1102-11.
37. Jamal M, Hussain T, Rajanna Das C, *et al.* Isolation and characterization of a myoviridae MJ1 bacteriophage against multi-drug resistant *Escherichia coli* 3. Jundishapur J Microbiol. 2015; 8(11):e25917.
38. Kudva, IT, Jelacic S, Tarr PI, *et al.* Biocontrol of Escherichia coli O157 with O157-specific bacteriophages. Appl. Environ. Microbiol. 1999; 65:3767-73.
39. Sulakvelidze A. Phage therapy: an attractive option for dealing with antibiotic-resistant bacterial infections. Drug Discov Today. 2005; 10(12):807–9.
40. Tomat D, Quiberoni A, Mercanti D, *et al.* Hard surfaces decontamination of enteropathogenic and Shiga toxin-producing Escherichia coli using bacteriophages. Food Res Int. 2014; 57:123-9.
41. Vandini A, Temmerman R, Frabetti A, *et al*. Hard surface biocontrol in hospitals using microbial-based cleaning products. PLoS One. 2014; 9(9):e108598.