Lytic Bacteriophage Screening Strategies for Multidrug-Resistant Bloodstream Infections in a Burn Intensive Care Unit

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Background: Increasing antibiotic resistance and multidrug resistance (MDR) in patients with bloodstream infection (BSI) has resulted in treatment using bacteriophage. This study aimed to identify Gram-negative bacilli and Gram-positive cocci and antibiotic resistance in patients with BSI in a burn intensive care unit (BICU). The environment, including sewage systems, were investigated for the presence of lytic bacteriophage.

Material/Methods: Between January 2011 to December 2017, 486 patients with BSI were admitted to the BICU. Blood culture identified the main infectious organisms. Bacterial screening tests for antibiotic resistance included the D test and the modified Hodge test (MHT). Lytic bacteriophage was isolated from the environment.

Results: In 486 patients with BSI, the main causative organisms were Gram-negative bacilli (64.6%), Gram-positive cocci (27.7%), and fungi (7.7%). The main pathogenic organisms that showed multidrug resistance (MDR) were *Acinetobacter baumannii* (26.0%), *Staphylococcus aureus* (16.8%), and *Pseudomonas aeruginosa* (14.2%). Bacteriophage was mainly isolated from Gram-negative bacilli. Screening of hospital and residential sewage systems identified increased levels of bacteriophage in hospital sewage.

Conclusions: The causative organisms of BSI and the presence of MDR in a hospital BICU were not typical, which supports the need for routine bacterial monitoring. Hospital sewage provides a potential source of bacteriophage for the treatment of MDR pathogenic bacteria.

MeSH Keywords: Antibiotics, Antitubercular • Bacterial Infections • Bacteriophages • Burn Units

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Background

Bloodstream infection (BSI) is one of the most dangerous complications in patients with severe burns [1]. Empirical treatment with broad-spectrum antibiotics is a standard treatment option available for early anti-infection therapy [2]. However, the uncontrolled and indiscriminate use of antibiotics has resulted in increased numbers cases of antibiotic drug resistance and multidrug resistance (MDR) [3], with almost half of the world's antibiotics currently prescribed in China [4]. The Global Antimicrobial Resistance Surveillance program conducted by the World Health Organisation (WHO) showed that antibiotic resistance resulted in high mortality rates [4]. *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, Enterococcus faecium* and *Klebsiella pneumoniae* (ESKAP) accounted for approximately 50% of the resistant infections against the most potent antibiotics, including third-generation beta-lactam antibiotics [4]. Therefore, there is an urgent need for new approaches to treat BSI due to MDR bacteria.

Bacteriophage, or phage, is a type of virus that infects and lyses bacteria. Bacteriophage was identified more than 100 years ago and can be screened from the environment, often from feces or sewage, where they are enriched and diverse [5]. Therapeutic use for bacteriophage was proposed when they were first identified. However, only with the recent increase in MDR has bacteriophage therapy gained interest. Several studies have described the lytic efficacy of bacteriophage against pathogens that include *Staphylococcus aureus* [6], *Acinetobacter baumannii* [7], *Pseudomonas aeruginosa* [8,9], *Klebsiella pneumoniae* [10], *Escherichia coli* [11], and *Salmonella* species [12]. Bacteriophage has lytic abilities against even MDR bacteria [13], and they have been used clinically without adverse reactions [7,14]. Also, bacteriophages are involved in immune modulation in addition to their lytic function [15]. Bacteriophages replicate within their specific host by taking over a set of host functions [16]. There is host specificity that includes particular species or strains of bacteria [17]. The successful use of bacteriophage against MDR bacteria relies on identifying the type of pathogen and screening the effects of the bacteriophage. When investigating the feasibility and practicality of using bacteriophage to treat MDR bacteria in patients with severe burns who suffer from BSI it is important that optimum bacteriophage screening is performed.

Therefore, this study aimed to identify Gram-negative bacilli and Gram-positive cocci and antibiotic resistance in patients with BSI in a burn intensive care unit (BICU). The environment, including sewage systems, were investigated for the presence of lytic bacteriophage.

Material and Methods

Collection of clinical data

Between January 2011 and December 2017, all patients with severe burns who developed bloodstream infection (BSI) in the Burn Intensive Care Unit (BICU) of the Southwest Hospital, Third Military Medical University, Chongqing, China were enrolled in this study. Clinical data were obtained from electronic medical records. Patient data were collected in accordance with the Declaration of Helsinki. The study was approved by the Local Ethics Committee, and informed consent was obtained from the patients. The demographic characteristics of the patients were recorded using medical and microbiology computerized laboratory records. Patients with severe burns were included according to the burn criteria from the American Burn Association [18]. Patients were classified as having severe burns with a total body surface area (TBSA) >50%, or third-degree burns >20%, and non-severe burns [19]. There were 486 patients with burns who were diagnosed with BSI, according to the 1996 Hospital Infection Diagnosis Criteria from the US Centers for Disease Control and Prevention (CDC) [20].

Isolation and identification of bacteria in patients with BSI

Blood samples were obtained from the patients during their treatment. Briefly, the blood was collected according to the procedures recommended by the Clinical and Laboratory Standards Institute (CLSI) in 2012 (standard M47) [21]. Briefly, 5–10 mL of venous blood was drawn from adult patients (3–5 mL from pediatric patients) during the early stage of chills and fever, or when patients had symptoms of infection. The blood samples were injected into a culture flask and transferred to a BacT/Alert 3D fully automated blood culture system (BioMérieux, Marcy-l'Étoile, France) for bacterial culture. Positive cultures underwent Gram's staining. Positive cultures were transferred to a culture dish to harvest the strain colonies. The identification of pathogenic bacteria was performed using the analytical profile index (API) bacterial identification panel (BioMérieux, Marcy-l'Étoile, France).

Antibiotic susceptibility testing

Antibiotic susceptibility testing of the main bacteria and fungi were performed and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) criteria [22]. The Epsilometer test (E-test) (BioMérieux, Marcy-l'Étoile, France) was performed to identify the minimum inhibitory concentration (MIC) and bacterial resistance to vancomycin. The Kirby-Bauer (K-B) disk diffusion method was used to detect the resistance of the pathogen to other antibiotics.
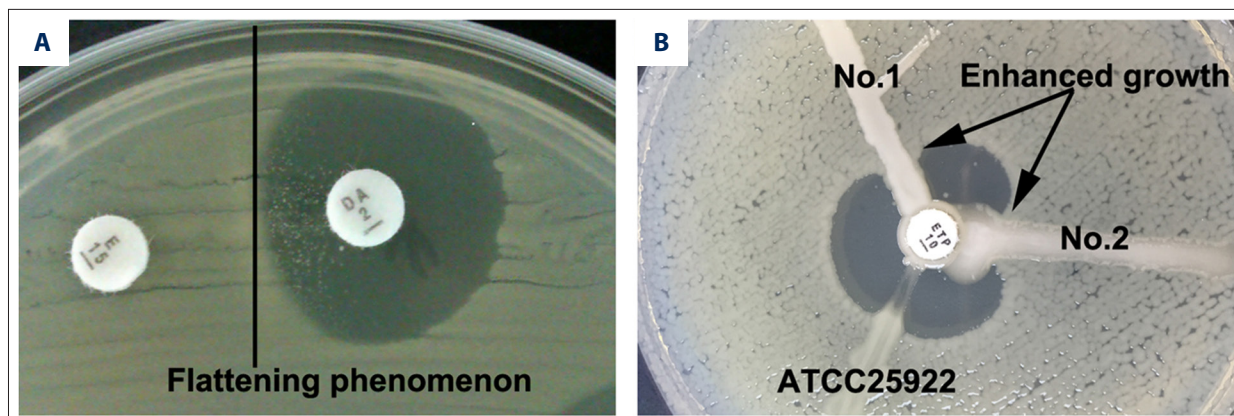
Figure 1. Demonstration of bacterial screening tests, the D test and the modified Hodge test (MHT). (**A**) The D test for inducible clindamycin resistance in *Staphylococcus aureus*. (**B**) The modified Hodge test (MHT), a phenotypic test that detects carbapenemase resistance in bacteria.

There were 37 antibiotics tested that included: ampicillin, piperacillin, cefoperazone, cefoperazone/sulbactam, ampicillin/sulbactam, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefuroxime, ceftazidime, ceftizoxime, cefepime, cefoxitin, aztreonam, imipenem, meropenem, amikacin, gentamicin, netilmicin, tobramycin, ciprofloxacin, levofloxacin, compound sulfamethoxazole, tetracycline, minocycline, polymyxin B, penicillin G, oxacillin, rifampicin, ofloxacin, clindamycin, erythromycin, linezolide, vancomycin, teicoplanin, chloramphenicol, nitrofurantoin, and high-dose gentamicin (120 μg). There were five anti-fungal agents tested that included: voriconazole, amphotericin B, fluconazol, itraconazole, and ketoconazole. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) were detected using cefoxitin.

The D test for inducible clindamycin resistance in *Staphylococcus aureus* was performed in bacterial isolates. *Staphylococcus aureus* at a concentration of 0.5 McFarland Standard (MCF) was evenly spread onto a Müller-Hinton (M-H) agar plate. Erythromycin strips (15 μg) and clindamycin strips (2 μg) were placed in the M-H agar plate. The center of the strips were maintained more than 15 mm from the edge of the plate and 20 mm apart from each other. A positive result was determined when a flattening phenomenon appeared at the inhibition ring on the clindamycin strip adjacent to the erythromycin strip **(**Figure 1A).

The modified Hodge test (MHT), a phenotypic test that detects carbapenemase resistance in bacteria, was performed to further confirm imipenem and meropenem resistance of *Klebsiella pneumoniae* strains. *Escherichia coli* (ATCC 25922) (Pangong Medical Device Co., Ltd., Chongqing, China) at a concentration of 0.5 MCF were diluted×10 with normal saline and then spread onto the M-H agar plate (Pangong Medical Device Co., Ltd., Chongqing, China). Then, the ertapenem strip (10 μg) was placed in the center of the plate. One or two *Klebsiella. pneumoniae* colonies and negative control strains (ATCC 25922) which had been grown in the agar plate overnight were selected using a 10 μg inoculating loup and spread from the area surrounding the strip to the edge of the plate (each line was equal to or longer than 20 mm). The plate was cultured for 16 to 20 hours at about 35°C. Carbapenemase resistance was determined once enhanced growth was found at the intersection between the sterile loup and the test organism (Figure 1B) [22].

Bacteriophage isolation protocol and sewage sampling

Colonies were collected from the selective media agar plates to isolate lytic bacteriophages against the predominant multidrug-resistant (MDR) bacteria, including *Acinetobacter baumannii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Enterococcus*. Untreated sewage water was collected at different locations, including from the sewage management center of Southwest Hospital, Xinqiao Hospital, Daping Hospital, and the sewer of the nearby residential area.

During the bacteriophage enrichment steps, unprocessed sewage samples were centrifuged at 6,000 x g in a 5702 R Eppendorf centrifuge for 10 min at 4°C. The sewage supernatant was filtrated through a 0.45 μm filter (Millipore, Billerica, MA, USA) to remove all remaining bacterial cells. Filtered sewage water was made up to 30–40 ml with lysogeny broth (LB) medium. The mixture was co-cultured with 1 ml of the target bacterial strain overnight at 37°C, shaken at 150 rpm to enrich (potential) bacteriophages in the sample. The sample was centrifuged at 6,000×g for 10 min at 4°C and filtered using a 0.45 μm filter to remove bacteria for a second enrichment.

A double-layer agar method was used to identify target bacteriophages [23]. The second enrichment sample was centrifuged

Table 1. Characteristics of patients admitted to the burn intensive care unit (BICU).

Characteristics	Total (n = 486 (%))	
Demographics		
Age (yrs) (mean ±SD)	35±21	
Gender (Male/Female)	331/155	
Type of burn (n)		
Scald burn	89	
Flame burn	285	
Electric burn	85	
Other	27	
Mortality (n, %)		
Severe burn	39 (10.9%)*#	Overall mortality rate 2011–2017: 56 (0.9%, among all 6325 burn patients)
Non-severe burn	5 (3.9%)#	
Infected pathogen		
Severe burn	3.1±1.6*	
Non-severe burn	1.5±0.9	

Significant difference compared with overall mortality rate during 2011 to 2017; * Significant difference compared with non-severe burn group.

at 13,000×g for 15 min at room temperature. A mixture with 10 µl supernatant combined with 100 µl of the target bacterial strain and 3 ml of 0.7% soft-agar was cultured on an LB agar plate. The plates were incubated overnight at 37°C. Colonies were sampled and transferred into 300 µl of LB medium. Incubation was performed three times to isolate a new lytic bacteriophage from the potentially non-homogeneous bacteriophage mixture. The range of bacteriophage isolated from the host was determined by a spot test based on the double-layer agar method. Lysed strains were excluded for the next round of bacteriophage isolation to avoid overlap with previously isolated bacteriophage [24].

Statistical analysis

Statistical analysis was performed using SPSS version 22.0 software (IBM, Armonk, NY, USA). Quantitative data were expressed as the mean ± standard deviation (SD). P<0.05 indicated that the difference was of statistical significance. The chi-squared test with Fisher's exact correction were applied to discrete variables. Student's t-test was used for parametric variables, and the Wilcoxon rank-sum test was used on non-parametric continuous variables. WHO Collaborating Centre for Surveillance of Antimicrobial Resistance (WHONET) software version 5.6 (Boston, MA, USA) was used to analyze the distribution of pathogens and antibiotic resistance of bacteria. GraphPad Prism version 5 (GraphPad Software, Inc., La Jolla, CA, USA) was used to convert the data to figures.

Results

Patients with severe burns had increased rates of bloodstream infection (BSI) and increased mortality rates

Among the 486 patients in the burn intensive care unit (BICU), 331 (68.1%) were male, and 155 (31.9%) were female. The mean age of the patients was 35 years (±21 years). Demographic characteristics of the study population are presented in Table 1. There were 107 patients with non-severe burns who self-discharged from hospital against medical advice who were excluded from the study. The severity of burn in all patients was classified according to current guidelines [25]. Of the 357 patients with severe burns, 39 died (10.9%). Of the 129 patients with non-severe burns, five died (3.9%). The mortality rate of patients with severe burns was significantly higher than that of patients with non-severe burns (Table 1, p<0.01). Between 2011 to 2017, the overall mortality rate among 6,325 hospitalized burn patients was previously reported to be 0.9% [26]. Also, the number of different types of pathogens increased with burn severity, from 1.5±0.9 in patients with non-severe burns to 3.1±1.6 in patients with severe burns, consistent with the significantly higher mortality and polymicrobial blood cultures (Z=–2.985; P<0.005) (Table 1).

Epidemiologic description of infections in patients in BICU between 2011 and 2017

From 1,824 samples analyzed, there were 703 pathogenic strains identified, excluding the same strain isolated a single patient.
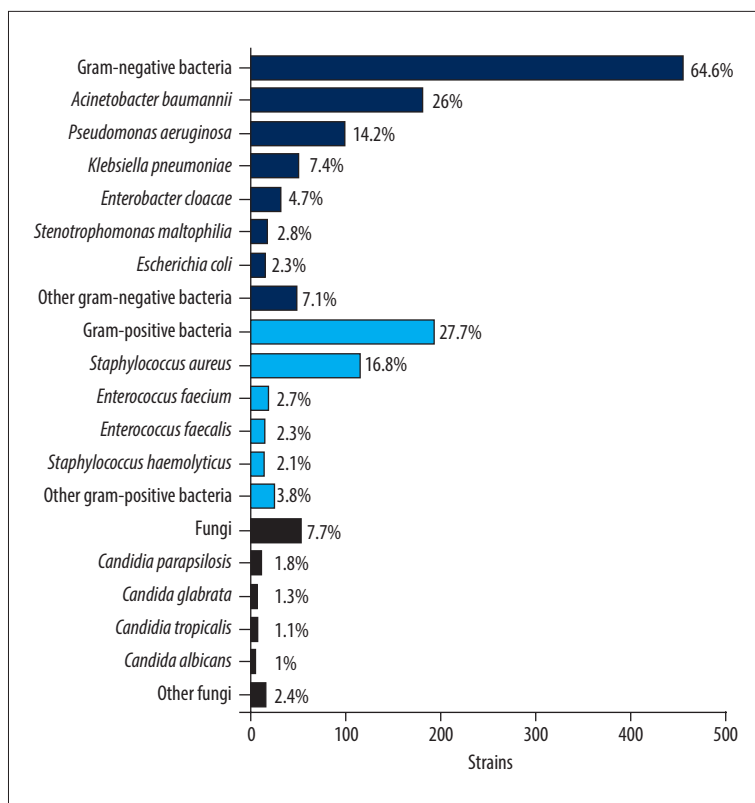
Figure 2. Distribution of the pathogens in patients with burns and bloodstream infection (BSI)

The positive blood culture rate was 38.5%, from which 454 (64.6%) strains were Gram-negative, consisting mainly of *Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter cloacae*, and *Klebsiella. pneumoniae*; 195 (27.7%) strains were Gram-positive, including *Staphylococcus aureus* and *Enterococcus*; and 54 (7.7%) strains were fungi, including *Candida parapsilosis, Candida glabrata, Candida tropicalis*, and *Candida albicans* (Figure 2). Of all the pathogens, the main species associated with bloodstream infection (BSI), according to the number of isolates, were *Acinetobacter baumannii, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter cloacae, K. pneumoniae* and *Enterococcus*.

There were 37 antibiotics and five antifungal agents selected to test against Gram-negative and Gram-positive bacteria and fungi, based on the availability and frequency of prescribing these drugs in the study population. As shown in Table 2, most antibiotics were resistant by the top isolated BSI pathogens. Only polymyxin B, vancomycin, teicoplanin, and linezolide showed adequate antibiotic sensitivity, indicating as significant degree of antibiotic resistance in BSI in patients with severe burns.

However, fungi showed no resistance to amphotericin B, while the resistance rate ranged only from 7.2–12.5% against voriconazole, fluconazole, itraconazole, fluorocytosine, and ketoconazole (Figure 3). Since most of the antifungal agents were

associated with less antimicrobial resistance, fungi were not included in further bacteriophage isolation studies.

Antibiotic resistance profiling identified the main BSI pathogens associated with MDR

Analysis of the antibiotic resistance profiles was based on each species of isolated bacteria. The results showed that six main causative pathogens for BSI of patients with severe burn were all MDR isolates, including *Acinetobacter baumannii, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter cloacae, Kkebsiella pneumoniae,* and *Enterococcus*, according to the definition of drug resistance [27]. Specifically, *Acinetobacter baumannii* showed minor resistance to polymyxin B and minocycline, with high resistance to all the other tested antibiotics, with the resistance rate ranging from 81.0–100.0% (Figure 4A). Also, *Pseudomonas aeruginosa* showed moderate resistance to cefoperazone/sulbactam, ceftazidime, imipenem, meropenem, ciprofloxacin, and levofloxacin, with the resistance rate ranging from 57.7–69.2%, with a high resistance to the other tested antibiotics with a resistance rate ranging from 80.3–100.0% (Figure 4B). *Klebsiella pneumoniae* showed very little resistance to cefoperazone/sulbactam, imipenem, and meropenem with a resistance rate that ranged from 5.9–15.6% (Figure 4C). The imipenem and meropenem resistant strain were further confirmed by the modified Hodge test, which showed that only two strains were positive. However, *Klebsiella pneumoniae* had

Table 2. Overall resistance of Gram-negative and Gram-positive strains*.

Gram negative		Gram positive	
Antibiotics	Resistance (%)	Antibiotics	Resistance (%)
Ampicillin	100.0	Penicillin G	100.0
Cefotaxime	98.4	Oxacillin	94.6
Piperacillin	96.6	Gentamycin	94.6
Tobramycin	95.5	Ofloxacin	89.3
Compound sulfamethoxazile	94.4	Tetracyclin	92.7
Ampicillin/sulbactam	94.2	Rifampin	92.2
Gentamycin	90.3	Levofloxacin	89.6
Tetracyclin	87.7	Ciprofloxacin	88.6
Cefuroxime	87.2	Chloramphenicol	63.3
Netilmicin	86.6	Ampicillin	60.0
Cefoperazone	85.6	Erythromycin	42.1
Amikacin	85.4	High unit gentamicin(120µg)	30.0
Ceftazidime	83.1	Minocycline	27.2
Cefepime	82.9	Nitrofurantoin	20.0
Piperacillin/tazobactam	82.7	Clarithromycin	28.0
Aztreonam	78.5	Compound sulfamethoxazile	17.4
Ciprofloxacin	76.9	Linezolide	2.4
Levofloxacin	75.1	Vancomycin	0.0
Amoxicillin/clavulanat	84.3	Teicoplanin	0.0
Cefoxitin	74.2		
Imipenem	67.4		
Meropenem	67.2		
Cefoperazone/sulbactam	66.1		
Minocycline	38.1		
PolymyxinB	0.0		

* Ampicillin, cefoperazone, amoxicillin/potassium clavulanate, cefuroxime, cefoxitin, and aztreonam were not tested in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Ampicillin/sulbactam, compound sulfamethoxazole, cefotaxime, tetracycline, and minocycline were not tested in *Pseudomonas aeruginosa*. Polymyxin B and minocycline were not tested in *Enterobacter cloacae* and *Klebsiella pneumoniae*. Penicillin G, oxacillin, cidomycin, levofloxacin, ofloxacin. Compound sulfamethoxazole, and clarithromycin were not tested in *Enterococcus*. Ampicillin, nitrofurantoin, and high-dose gentamicin (120 µg) were not tested in methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA). The resistance rates were calculated by excluding the above.
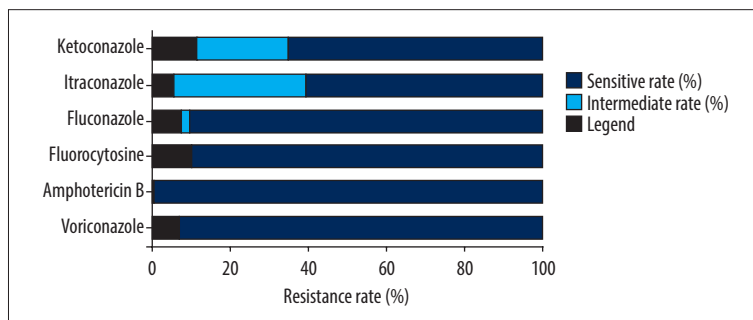

Figure 3. Tests for antimicrobial resistance of fungi. * Suggests that the antibiotic was not tested in the species.
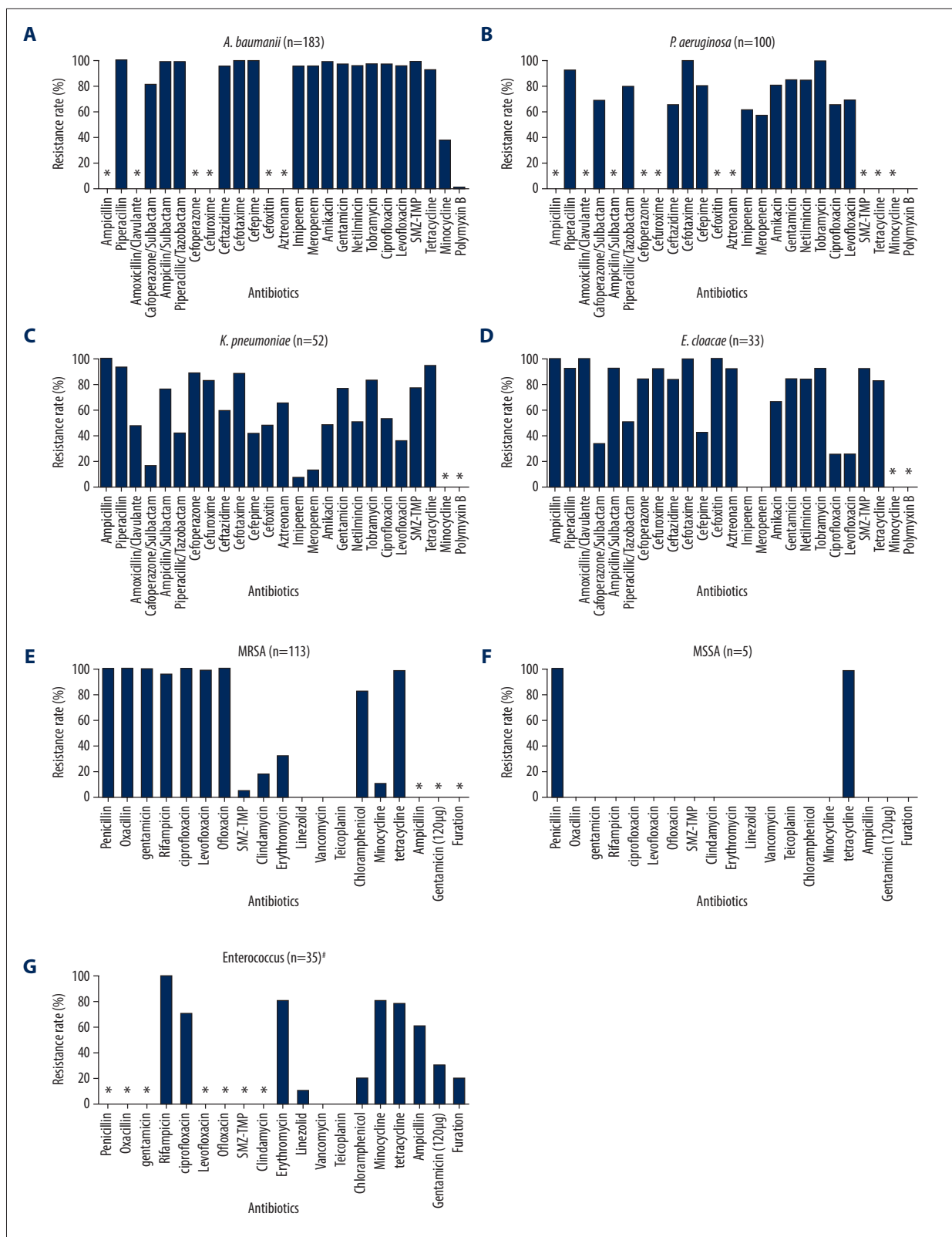
Figure 4. (**A–G**) Antibiotic resistance in the main pathogenic bacteria. * Suggests that the antibiotics were not tested in the bacterial species because these antibiotics were seldom used clinically.
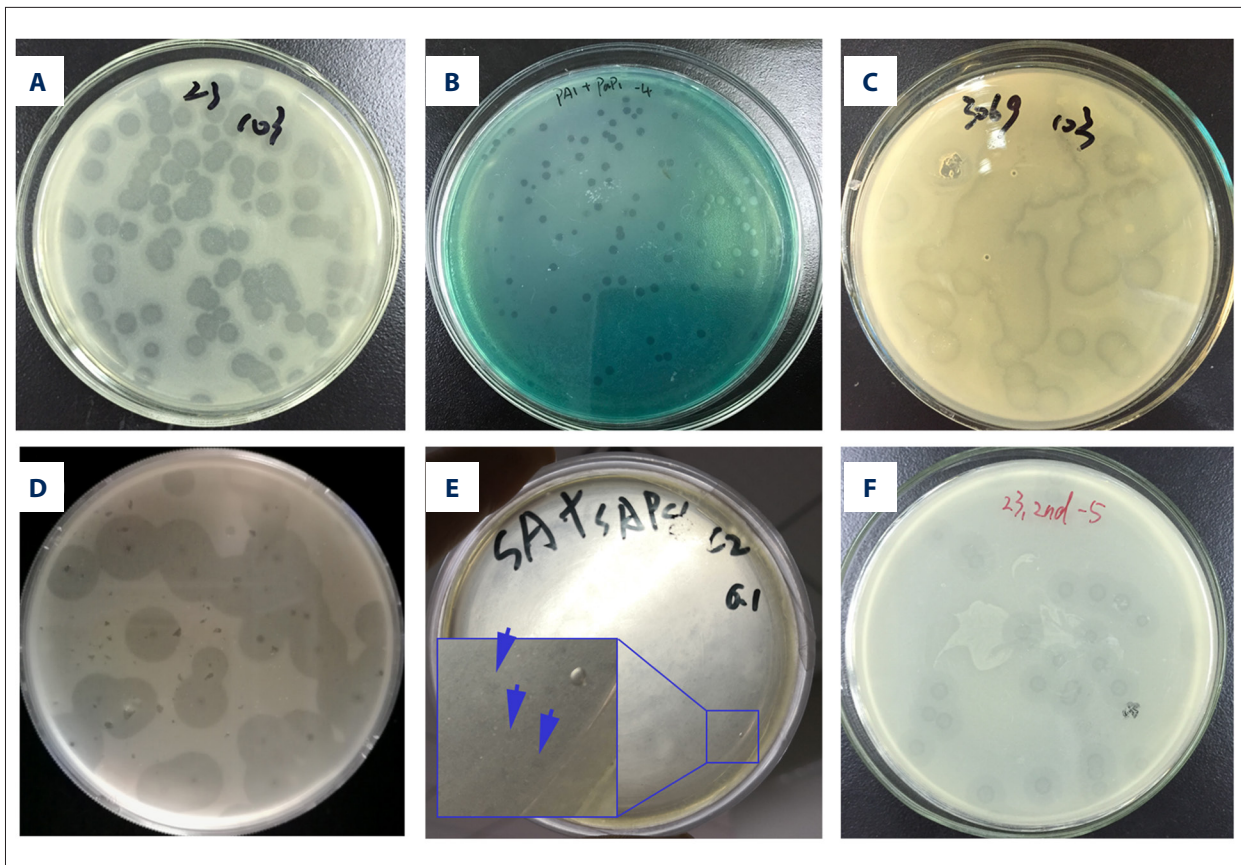
Figure 5. Representative images of bacteriophage lytic plaques on double-layer agar plates. (**A**) Bacteriophage lytic plaques for *Acinetobacter baumannii*. (**B**) Bacteriophage lytic plaques for *Pseudomonas aeruginosa*. (**C**) Bacteriophage lytic plaques for *Enterobacter cloacae*. (**D**) Bacteriophage lytic plaques for *Klebsiella pneumoniae*. (**E**) Bacteriophage lytic plaques for *Staphylococcus aureus*. (**F**) Bacteriophage lytic plaques for *Enterococcus*. The blue arrow indicates the small-sized plaque of the *Staphylococcus aureus* phage.

a high resistance to the other tested antibiotics, with the resistance rate ranging from 50.0–100.0%, except for amoxicillin/clavulanic acid, cefepime, piperacillin/tazobactam, cefoxitin, amikacin, and levofloxacin that had a resistance rate that ranged from 35.3–47.1% (Figure 4C). Also, *Enterobacter cloacae* showed high resistance to other tested antibiotics, ranging from 66.7–100.0%, and only a moderate resistance to ciprofloxacin, cefoperazone/sulbactam, levofloxacin, cefepime, and piperazine/tazobactam, ranging from 25.0–49.0%, but was sensitive to imipenem and meropenem (Figure 4D).

In patients with BSI associated with severe burn, MRSA accounted for 95.8% (113/118) of *Staphylococcus aureus*, while MSSA accounted for only 4.2% (5/118) (Figure 4E). Even if the MRSA strain had a higher resistance to most tested antibiotics than MSSA, MRSA still had no resistance to linezolid, vancomycin, and teicoplanin (Figure 4E). The resistance rate of MRSA to tested antibiotics was as high at between 81.6–100.0%, except for the compound sulfamethoxazole, clindamycin, erythromycin, and minocycline, which ranged from 5.3–31.6% (Figure 4E).

However, only five strains of MSSA still showed complete resistance to penicillin G and tetracycline, but was sensitive to other tested antibiotics (Figure 4F). Thirty cases of *Staphylococcus aureus* showed erythromycin-induced clindamycin resistance with a detection rate of 25.0% (30/118). *Enterococcus* showed high resistance to ciprofloxacin, erythromycin, minocycline, and ampicillin, with a resistance rate of 60.0–80.0%), but a relatively low resistance to linezolid, chloramphenicol, nitrofurantoin, and high-dose gentamicin that ranged from 10.0–30.0%), but was sensitive to vancomycin and teicoplanin (Figure 4G). No resistance to rifampicin was found in *Enterococcus* from patients with BSI (Figure 4G).

The probability of screening lytic bacteriophages against MDR isolates from patients with BSI

The main MDR species were *Acinetobacter baumannii, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella pneumoniae,* and *Enterococcus,* which were included to screen bacteriophage using sewage from

Table 3. The probability of screening lytic bacteriophages against MDR bacteria of bloodstream infections.

Pathogen species	Isolation attempts	Isolation success	Bacteriophage isolates	Coverage*
Acinetobacter baumannii	9	9	9	153/183 (83.6%)
Pseudomonas aeruginosa	7	7	7	81/100 (81%)
Enterobacter cloacae	3	3	3	27/33 (81.2%)
Klebsiella pneumoniae	2	2	2	41/52 (78.8%)
Staphylococcus aureus	8	4	4	113/118 (95.7%)
Enterococcus (faecium and faecalis)	5	3	3	27/35 (77.1%)

* Coverage means the isolated phage lytic spectrum against the host bacteria (n,%).

Table 4. Different source of sewage on phage isolation attempts against *Acinetobacter baumannii*.

Sewage source	Attempts	Successes	Bacteriophage isolates
Southwest Hospital sewage	9	9	9
Xinqiao Hospital sewage	9	7	7
Daping Hospital sewage	9	9	9
Residential sewage	9	1	1

Southwest Hospital. The representative images in Figure 5 show bacteriophage against MDR isolates of each species tested in this study shown by plaque formation on the double-layer agar plate. The bacteriophage plaque sizes ranged from small (Figure 5B, 5E), medium (Figure 3A, 3F), to large circles (Figure 5C, 5D). Bacteriophage for *Staphylococcus aureus* showed very small plaque sizes, as indicated by the blue arrow in the magnified box in Figure 5E, but had a very wide spectrum (data not shown), which resulted in further investigation.

To assess the probability of finding a lytic bacteriophage from hospital sewage for different host bacteria, we recorded and calculated the lytic spectrum until the bacteriophage lysed more than 80% of the bacterial species. The bacteriophage isolates against the Gram-negative bacilli, *Acinetobacter baumannii, P. aeruginosa, Enterobacter cloacae,* and *Klebsiella. pneumoniae*, showed successful lysis at every attempt (Table 3). However, bacteriophage screening for Gram-positive cocci had a relatively low success rate, especially for *Staphylococcus aureus* where only half of the attempts resulted in lysis.

Bacteriophage screening was more successful when hospital sewage was the source

The success rate for bacteriophage screening was higher in this study than previously reported, which might have been related to the sewage source. Therefore, we performed a further comparison between sewage from three different hospitals

that included Southwest Hospital, Xinqiao Hospital, and Daping Hospital, and sewage from a residential site. The bacteriophage against MDR *Acinetobacter baumannii* was successfully separated by screening from the sewage of the hospital during almost all of nine attempts, but there was only one successful bacteriophage isolate from the residential site (p<0.05) (Table 4). The success rate of using the hospital sewage to isolate bacteriophage was significantly higher than that from the residential sewage.

Discussion

The bacteriophage is a specific type of virus that infects and lyses bacteria, which has been recognized to have potential therapeutic applications for infectious disease since it was first identified [5]. Recently, bacteriophage therapy has attracted attention due to the increasing prevalence of antibiotic resistance and multidrug resistance (MDR). Studies have shown that bacteriophage therapy has advantages that include strong host specificity, effective lysis of bacteria, a different mechanism of action from antibiotics, and high therapeutic efficacy [28]. The characteristics of bacteriophage make it an ideal candidate for precision medicine [28], which has been supported by clinical trial data [24]. In the present study, the epidemiological characteristics of bacteria and antibiotic resistance in patients with bloodstream infection (BSI) in a hospital burn intensive care unit (BICU) were investigated,

and the environment, including sewage systems, were investigated for the presence of lytic bacteriophage.

Patients with severe burns often experience serious or even fatal systemic infections, due to the absence of skin barriers [29], humoral and cellular immunodeficiency, mechanical ventilation [30,31] and the use of invasive catheters [32]. Previously reported studies have shown that BSI is the most common cause of death in patients with severe burns [33,34]. The findings from this study are consistent with previous reports [35], which have shown that the patients with more severe burns also tend to develop a wider range of infections. This finding partially explains why severe burns result in higher mortality rates than mild burns. As the antibiotic resistance increases, epidemiological surveillance and new therapeutic approaches are needed to combat BSI in patients with severe burns.

To our knowledge, this is the first study that has identified the main pathogenic bacteria in cases of BSI in patients with burn, which included *Acinetobacter baumannii, Staphylococcus aureus*, and *Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella pneumoniae,* and *Enterococcus*. Also, between 2011 and 2017, these main bacterial pathogens were all MDR in patients with severe burns in our BICU. In this study, the positive rate of blood culture was higher than previously reported [36], which might be associated with the absence of larger skin areas in patients with severe burns. Also, Gram-negative bacilli were detected at a higher rate than Gram-positive cocci, which was consistent with the trend of pathogen previously reported in patients with BSI [37]. The top three pathogens were *Acinetobacter baumannii, Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and these three bacteria were also the main three pathogens in a non-acute burn department of our hospital, although the order of these main organisms was different [38]. Although it is considered to be a less virulent bacteria, the relationship between *Acinetobacter baumannii* and BSI requires further investigation. Also, the methicillin-resistant *Staphylococcus aureus* (MRSA) had a detection rate of 94.6% in all *Staphylococcus aureus*, which was even higher than the MRSA detection previously reported in patients with burns [39]. This findings from the present study, together with previous reports, indicate that antibiotic resistance in *Staphylococcus aureus* infections in patients with burns remains a serious threat [40]. Also, increased attention should be paid to new emerging pathogens such as *Klebsiella pneumoniae*. Recently, *Klebsiella pneumoniae* was reported to cause several deaths of patients with severe burns in the BICU (data not shown). The increasing rate of resistance of the main isolated pathogens to the most commonly used antibiotics, confirms the serious situation of MDR infection in patients with severe burns. The use of constant surveillance practiced routinely at our hospital is recommended for use elsewhere.

In this study, isolation bacteriophage was successful against Gram-negative bacilli at every attempt, while the isolated bacteriophage against Gram-positive cocci was successful in only half the cases. This finding might be attributed to the thickness of the bacterial cell wall or the isolation protocol [41]. Because no previous studies have focused on the bacteriophage isolation success rates between Gram-negative and Gram-positive bacteria, further studies are needed. Also, although bacteriophages can be isolated from any environment, sewage is considered to be the best source of [23]. In view of the process of isolation of bacteriophage, the attempts and hit ratio, the time and cost of isolating bacteriophages from sewage is acceptable. Previously studies reported an isolation rate of bacteriophage to *Escherichia coli*, *Salmonella*, and *Pseudomonas aeruginosa*, of more than 75% [42]. However, the isolation rate for *Acinetobacter baumannii* bacteriophage was significantly lower (38.9%) than that in our study, which suggested that the source of sewage affects the success rate of phage screening [42].

In the present study, we further performed a comparison of the success rate between sewage from the different sources in screening bacteriophage against *Acinetobacter baumannii*. Bacteriophage screening using hospital sewage was superior to the residential sewage sources, which indicates that hospital sewage might be a better source for bacteriophage screening against MDR pathogens. This finding might be due to the complexity of the hospital sewage environment where phages were able to evolve along with the bacterial hosts. Therefore, to avoid the lack of availability of specific bacteriophage when needed, we suggest that bacteriophage isolation and storage should be undertaken routinely in case of an outbreak of MDR pathogens in the hospital environment.

Conclusions

This study aimed to investigate the distribution of Gram-negative bacilli and Gram-positive cocci in 486 patients with bloodstream infection (BSI) and in the hospital environment of a burn intensive care unit (BICU). The infectious agents that caused BSIs in our BICU differed from the findings from previously reported studies. *Acinetobacter baumannii* was the most common cause of BSI, and the main isolated pathogens were all multidrug-resistant (MDR) strains. The method of screening and isolation of bacteriophage against bloodstream MDR bacterial isolates included those found in hospital sewage. These results may provide new insights into the isolation and therapeutic use of bacteriophage for the treatment of BSI in patients with severe burns.

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Conflict of interest

None declared.

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