Effect of broad-spectrum antibiotics on bacterial translocation in burned or septic rats

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Abstract

Background: Antibiotics are frequently used to treat critically ill patients, and its use is often accompanied by intestinal dysbiosis that might further lead to bacterial translocation (BT). Nevertheless, studies on the relationship between antibiotic therapy and BT are rare. In the present study, we investigated the effect of broad-spectrum antibiotics on BT in an experimental rat model of burn or sepsis injury.

Methods: The septic rat model was simulated by a second insult with lipopolysaccharides after burn injury. Ninety-two male Sprague-Dawley rats were randomly divided into control, burn, and sepsis groups ($n = 8$ or 9, each group), and the latter two groups were then treated with imipenem or ceftriaxone for 3 or 9 days. The mesenteric lymph nodes, liver, lungs, and blood were collected at each time point under sterile conditions for quantitative bacterial culture and strain identification. The differences between the groups were compared by Fisher exact test or Mann-Whitney U test.

Results: Only minimal *Escherichia coli* translocation to the mesenteric lymph nodes was observed in the normal control group, in which the BT rate was 12.5%. Burn injury did not affect the BT rate (Burn group *vs.* Control group, 12.5% *vs.* 12.5%, $P = 1.000$), whereas the BT rate showed an increased trend after the second insult with lipopolysaccharide (Sepsis group *vs.* Control group, 44.4% *vs.* 12.5%, $P = 0.294$), and many strains of *Enterobacteria spp*. were detected in distant organs (liver, lung, and blood) [Sepsis group *vs.* Control group, 0 (0,3) *vs.* 0 (0,0), $U = 20$, $P = 0.045$]. After the antibiotic treatment, BT to the distant organs was increased in burned rats [Burn IT3 group *vs.* Burn group, 0 (0,2) *vs.* 0 (0,0); Burn IT9 group *vs.* Burn group, 0 (0,1) *vs.* 0 (0,0); Burn CT9 group *vs.* Burn group, 0 (0,2) *vs.* 0 (0,0); all $U = 20$ and $P = 0.076$] but decreased in septic rats [Sepsis CT3 group *vs.* Sepsis group, 0 (0,0) *vs.* 0 (0,3), $U = 20$, $P = 0.045$]. The total amount of translocated bacteria, regardless of which antibiotic was used, was increased in burned rats [Burn IT9 group *vs.* Burn group, 2.389 (0,2.845) *vs.* 0 (0,2.301) Log_{10} colony-forming units (CFU)/g, $U = 14$, $P = 0.034$; Burn CT3 group *vs.* Burn group, 2.602 (0,3.633) *vs.* 0 (0,2.301) Log_{10} CFU/g, $U = 10.5$, $P = 0.009$], but there was a slightly decreased trend in septic rats [Sepsis IT9 group *vs.* Sepsis group, 2.301 (2,3.146) *vs.* 0 (0,4.185) Log_{10} CFU/g, $U = 36$, $P = 0.721$; Sepsis CT9 group *vs.* Sepsis group, 2 (0,3.279) *vs.* 0 (0,4.185) Log_{10} CFU/g, $U = 32.5$, $P = 0.760$]. Remarkably, the quantity of *Enterococci spp*. dramatically increased after broad-spectrum antibiotic treatment in both the burned and septic groups [Burn IT3 group *vs.* Burn group, 1 (0,5.164) *vs.* 0 (0,0) Log_{10} CFU/g, $U = 16$; Burn IT9 group *vs.* Burn group, 1 (0,2.845) *vs.* 0 (0,0) Log_{10} CFU/g, $U = 16$; Burn CT3 group *vs.* Burn group, 2.602 (0,3.633) *vs.* 0 (0,0) Log_{10} CFU/g, $U = 8$; Burn CT9 group *vs.* Burn group, 1 (0,4.326) *vs.* 0 (0,0) Log_{10} CFU/g, $U = 16$; Sepsis IT3 group *vs.* Sepsis group, 2.477 (0,2.903) *vs.* 0 (0,0) Log_{10} CFU/g, $U = 4.5$; Sepsis IT9 group *vs.* Sepsis group, 2 (0,3.146) *vs.* 0 (0,0) Log_{10} CFU/g, $U = 9$; Sepsis CT3 group *vs.* Sepsis group, 1.151 (0,2.477) *vs.* 0 (0,0) Log_{10} CFU/g, $U = 18$; Sepsis CT9 group *vs.* Sepsis group, 2 (0,3) *vs.* 0 (0,0) Log_{10} CFU/g, $U = 13.5$; all $P < 0.05$].

Conclusions: Broad-spectrum antibiotics promote BT in burned rats but prevent BT in septic rats, especially preventing BT to distant organs, such as the liver and lung. Moreover, *Enterococci spp*. with high drug resistance and high pathogenicity translocated most after antibiotic treatment.

Keywords: Antibiotic; Bacterial translocation; *Enterococci*; *Enterobacteria*; Sepsis

Introduction

The development and application of antibiotics have significantly reduced the mortality of infection-related diseases, but the widespread use of broad-spectrum antibiotics has made microbial resistance to antibiotics increasingly serious and has become a global public health problem.[1] Previous studies have shown that broad-spectrum antibiotics lead to an imbalance in the intestinal micro-ecological environment in the body.[2,3] The gastrointestinal

tract is the largest bacterial reservoir in the human body, and intestinal flora imbalance caused by broad-spectrum antibiotics has outstanding clinical significance. Under the pressure of broad-spectrum antibiotics, the intestinal microbial barrier is destroyed. Exogenous conditions, especially resistant bacteria, can easily colonize the intestine. In addition, intestinal pathogenic bacteria selected by broad-spectrum antibiotics may cause gut-driven infections, especially drug-resistant and even multidrug-resistant strains, which have become an intractable clinical problem.[4,5]

It has been reported that the infection rate of intensive care unit patients reaches up to 51% and that around three-quarters of these patients receive daily antimicrobial treatment.[6] Sepsis is a critical disease with high morbidity and mortality in the intensive care unit, and many highly effective broad-spectrum antibiotics are used in patients with severe sepsis.[7] Sepsis is a complicated and dynamic condition that is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.[8] A clinical epidemiology study has shown that sepsis is a common complication and important death cause of critical illnesses such as serious burns, trauma, shock, and major surgery.[7] Numerous studies have shown that critical illness including sepsis can lead to rapid and obvious intestinal flora disturbance[9-11] inducing intestinal barrier impairment.[12,13] Overgrowth of opportunistic pathogens, gut barrier dysfunction, and damaged host defence will result in bacterial translocation (BT).[14] The translocation of bacteria and toxins not only elevates the infection rate in patients with critical illnesses[15] but also causes systemic inflammation and even multiple organ dysfunction syndrome.[16,17] However, studies on the relationship between antibiotic therapy and BT in septic patients are scarce.

In the present study, we used a septic rat model established through lipopolysaccharide (LPS) stimulation after burn to observe the effect of broad-spectrum antibiotics frequently used in the clinic (imipenem and ceftriaxone) on BT and to provide a basis for the rational choice and clinical use of antibiotics.

Methods

Ethical approval

The study was approved by the Medical Ethics Committee of Second Military Medical University and was in compliance with *The Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health.

Animal model generation

Adult male Sprague-Dawley rats (220–250 g) were obtained from B&K Universal Group Limited (Shanghai, China; SCXK No. [Shanghai]2018-0006). The rats were acclimatized in the animal quarters for 1 week and food and water were withdrawn for 12 h before experiments. The septic rat model was previously described.[2,3,18]

Briefly, the rats were anesthetized with an intraperitoneal injection of 1% sodium pentobarbital (Notlas, Ltd, Beijing, China) with a dose of 40 mg/kg of body weight, and the area (dorsum) to be burned was shaved with a clipper to ensure an even burn. The rats were then fixed in a place and immersed in boiling water (100°C) for 15 s, which delivered a full-thickness cutaneous burn indicated by histological sections. Subsequently, the rats were resuscitated with saline (0.9% NaCl, Baxter International, Inc, Deerfield, IL, USA) with an intraperitoneally administered dose of 100 mL/kg of body weight. For the next 24 h, the animals were placed in individual sterile cages. The rats those were randomly assigned to the sepsis model were intraperitoneally administered endotoxin (*Escherichia coli* O111B4 LPS; Sigma Company, St. Louis, MO, USA) with a dose of 10 mg/kg of body weight 1 day after the initial burn injury. The rats those were randomized to the burn model were treated with an identical volume of saline. The normal control rats received only depilation and saline resuscitation without burn injury or endotoxin treatment. The animals were all fed regular chow and provided with clean water throughout the study period.

Study protocol and grouping

Ninety-two male Sprague-Dawley rats were randomly divided into 11 groups of eight to nine animals each. The groups were defined by the experimental conditions (burn injury only or burn injury followed by endotoxin challenge) and the treatment [imipenem, namely, imipenem-cilastatin (Merck Sharp & Dohme Ltd, Hertfordshire, UK); ceftriaxone (Roche, Basel, Switzerland); or saline] [Figure 1] as follows: (1) normal control group, which was named control ($n = 8$); (2) burn control group, which was named burn ($n = 8$); (3) sepsis control group which was named sepsis ($n = 9$); (4) burn rats treated with imipenem for 3 days, which was named burn IT3 ($n = 8$); (5) burn rats treated with imipenem for 9 days, which was named burn IT9 ($n = 8$); (6) sepsis rats treated with imipenem for 3 days, which was named sepsis IT3 ($n = 9$); (7) sepsis rats treated with imipenem for 9 days, which was named sepsis IT9 ($n = 9$); (8) burn rats treated with ceftriaxone for 3 days, which was named burn CT3 ($n = 9$); (9) burn rats treated with ceftriaxone for 9 days, which was named burn CT9 ($n = 8$); (10) sepsis rats treated with ceftriaxone for 3 days, which was named sepsis CT3 ($n = 8$); and (11) sepsis rats treated with ceftriaxone for 9 days, which was named sepsis CT9 ($n = 8$). Imipenem and ceftriaxone were both injected into the abdominal cavity of the rats at a dose of 60 mg/kg twice a day. The doses of imipenem and ceftriaxone were used as previously described.[2,3]

Specimen collection and quantitative bacterial culture

The rats were sacrificed by an overdose of sodium pentobarbital at each time point. The chest and abdomen were shaved and sterilized. Midline laparotomy and thoracotomy were performed to exsanguinate the mice through a cardiac puncture and to remove the right lobe of the liver, left inferior pulmonary lobe, and mesenteric lymph nodes (MLNs). To avoid cross-contamination, the instruments were not reused in each sampling. Next, 0.5 mL of blood samples and 4.5 mL of normal saline were

Experimental protocol and grouping
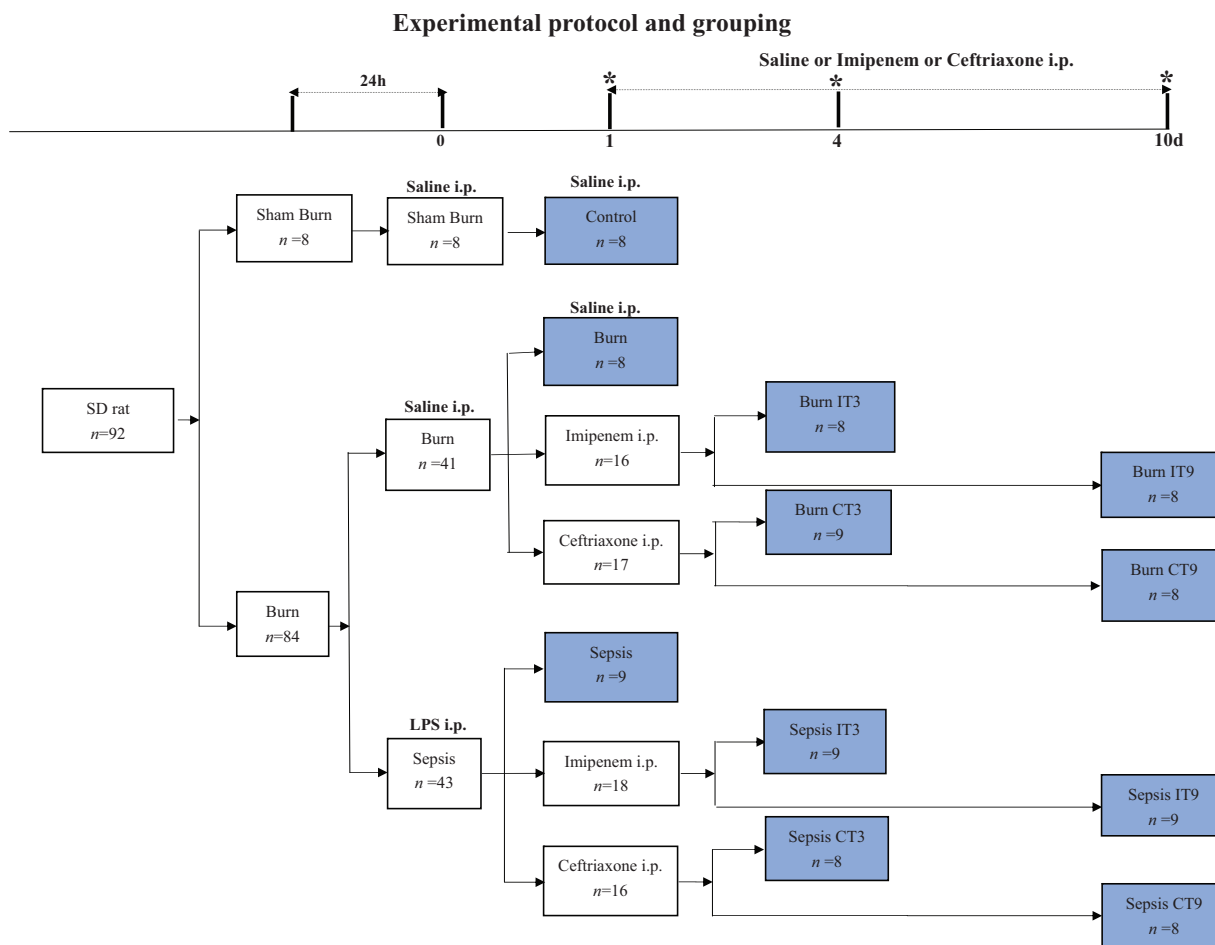
Figure 1: Experimental protocol and grouping. Time zero on the timeline represents the day of sepsis model generation, and the symbols indicate the preset sampling times. CT3: Treated with ceftriaxone for 3 days; CT9: Treated with ceftriaxone for 9 days. d: days; i.p.: Intraperitoneal; IT3: Treated with imipenem for 3 days; IT9: Treated with imipenem for 9 days; SD: Sprague-Dawley.

collected, 100 mg liver samples were harvested, and lung/MLN samples in 0.9 mL of normal saline were collected in sterile glass tissue grinders at low temperature. Subsequently, the samples were homogenized and diluted 10 or 100 times with normal saline. All diluted specimens (100 μL) were cultured on MacConkey agar plates and *Enterococci* plates (Kemajia Microbe Technology Ltd., Shanghai, China) and incubated for 24 h at 37°C for evaluation of *Enterobacteria spp.* and *Enterococci spp.* The culture method used in the experiment was consistent with the clinical microbiology laboratory protocols, so it could culture the bacilli and cocci that can be seen in the clinic, such as *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Proteus mirabilis*, *Staphylococcus spp.*, *Enterococci spp.*, and so on. However, it could not cultivate *anaerobic bacteria* and *fungi*. Each of the diluted specimens was seeded in duplicate. Strains were then preliminarily identified through Gram staining and colony morphology and verified by an automatic bacteria identification system (BioMerieux, Marcy, France). Bacterial counts were determined by counting colony-forming units (CFU). Bacterial quantitation was calculated according to the formula: colony number in a sample (CFU/g) = average number of colonies of effective plate × dilution ratio/

quantity of sample, and the quantity of bacteria was presented as Log_{10} CFU/g.

Statistical analysis

GraphPad Prism 7.00 (San Diego, CA, USA) was applied to generate graphs and statistical analysis. Categorical variables were expressed as frequency or percentage (%), whereas continuous variables were expressed as the median (minimum − maximum). Categorical variables were compared by Fisher exact test. For continuous variables, differences between the groups were assessed with a Mann-Whitney U test. All P values were two-tailed, and a $P < 0.05$ was considered statistically significant.

Results

Effect of broad-spectrum antibiotics on the BT rate in burned or septic rats

To assess the effect of broad-spectrum antibiotics on the BT rate in burned or septic rats, the animal model we generated above was used. As shown in Tables 1 and 2, BT occurred in only one case in the normal control group, and the BT rate was 12.5%. Simple burn injury did not affect

the BT rate (Burn group *vs.* Control group, 12.5% *vs.* 12.5%, $P = 1.000$), whereas the BT rate increased to 44.4% (Sepsis group *vs.* Control group, 44.4% *vs.* 12.5%, $P = 0.294$) after a second insult with LPS. The BT rate of burned rats increased from 12.5% to 50.0% after 3 days of imipenem administration (Burn IT3 group *vs.* Burn group, 50.0% *vs.* 12.5%, $P = 0.282$) and to 62.5% after 9 days (Burn IT9 group *vs.* Burn group, 62.5% *vs.* 12.5%, $P = 0.119$). Furthermore, the BT rate of septic rats increased to 100.0% after 9 days of imipenem treatment (Sepsis IT9 group *vs.* Sepsis group, 100% *vs.* 44.4%, $P = 0.029$). As with imipenem treatment, the BT rates of burned and septic rats both showed an increasing trend after ceftriaxone treatment (Burn CT3 group *vs.* Burn group, 77.8% *vs.* 12.5%, $P = 0.015$; Burn CT9 group *vs.* Burn group, 50.0% *vs.* 12.5%, $P = 0.282$; Sepsis CT3 group *vs.* Sepsis group, 62.5% *vs.* 44.4%, $P = 0.637$; Sepsis CT9 group *vs.* Sepsis group, 62.5% *vs.* 44.4%, $P = 0.637$).

Effect of broad-spectrum antibiotics on BT to different organs in burned or septic rats

To evaluate the effect of broad-spectrum antibiotics on BT to different organs in burned or septic rats, the BT to different tissues was analyzed. As shown in the heatmap of Figure 2A, only a single case of BT in MLNs was observed in the normal control and burn control groups. Nevertheless, all organs were invaded by translocated bacteria in the sepsis control group, and many strains of *Enterobacteria spp.* were detected in distant organs [Sepsis group *vs.* Control group, 0 (0,3) *vs.* 0 (0,0), $U = 20$, $P = 0.045$] [Figure 3A and 3B], indicating that sepsis could aggravate enterogenic infection. From box-and-whiskers plots [Figure 2B] we could clearly see that more BT was observed in distant organs in burned rats after either antibiotic treatment [Burn IT3 group *vs.* Burn group, 0 (0,2) *vs.* 0 (0,0); Burn IT9 group *vs.* Burn group, 0 (0,1) *vs.* 0 (0,0); Burn CT9 group *vs.* Burn group, 0 (0,2) *vs.* 0 (0,0); all $U = 20$ and $P = 0.076$, near statistical

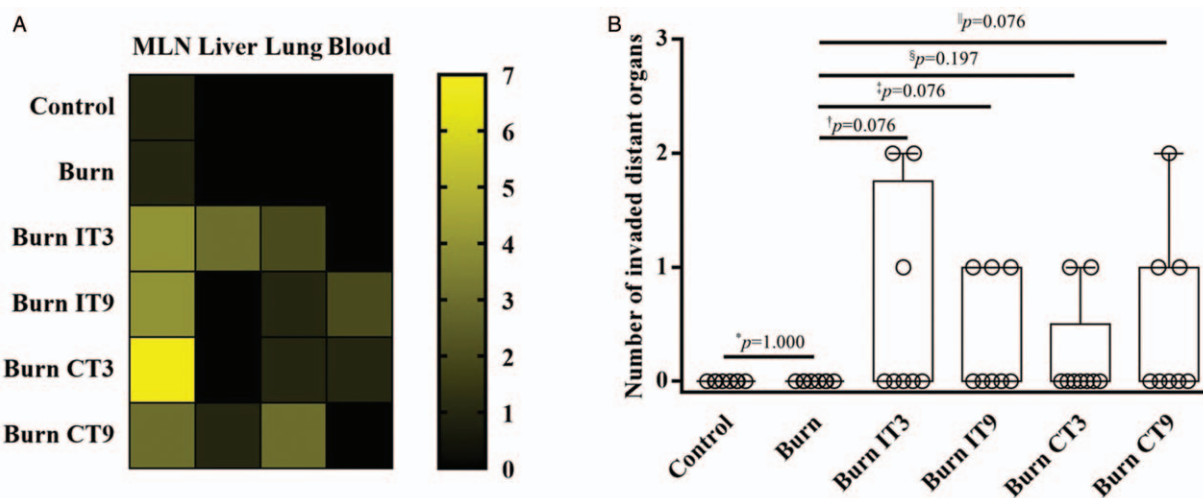**Table 1: Effect of broad-spectrum antibiotics on BT rate in burned rats.**

Group	Non-BT case	BT case	P
Control	7 (87.5)	1 (12.5)	
Burn	7 (87.5)	1 (12.5)	1.000[*]
Burn IT3	4 (50.0)	4 (50.0)	0.282[†]
Burn IT9	3 (37.5)	5 (62.5)	0.119[†]
Burn CT3	2 (22.2)	7 (77.8)	0.015[†]
Burn CT9	4 (50.0)	4 (50.0)	0.282[†]

Values are presented as n (%); $n = 9$ for the Burn CT3 group, $n = 8$ each for the other groups. [*]*vs.* the Control group. [†]*vs.* the Burn group. Significance between the two groups was determined by Fisher exact test. BT: Bacterial translocation; CT3: Treated with ceftriaxone for 3 days; CT9: Treated with ceftriaxone for 9 days; IT3: Treated with imipenem for 3 days; IT9: Treated with imipenem for 9 days.

Table 2: Effect of broad-spectrum antibiotics on BT rate in septic rats.

Group	Non-BT case	BT case	P
Control	7 (87.5)	1 (12.5)	
Sepsis	5 (55.6)	4 (44.4)	0.294[*]
Sepsis IT3	1 (11.1)	8 (88.9)	0.131[†]
Sepsis IT9	0 (0.0)	9 (100.0)	0.029[†]
Sepsis CT3	3 (37.5)	5 (62.5)	0.637[†]
Sepsis CT9	3 (37.5)	5 (62.5)	0.637[†]

Values are presented as n (%); $n = 9$ for the Sepsis group, Sepsis IT3 group, and Sepsis IT9 group; $n = 8$ each for the other groups. [*]*vs.* the Control group. [†]*vs.* the Sepsis group. Significance between the two groups was determined by Fisher exact test. BT: Bacterial translocation; CT3: Treated with ceftriaxone for 3 days; CT9: Treated with ceftriaxone for 9 days; IT3: Treated with imipenem for 3 days; IT9: Treated with imipenem for 9 days.
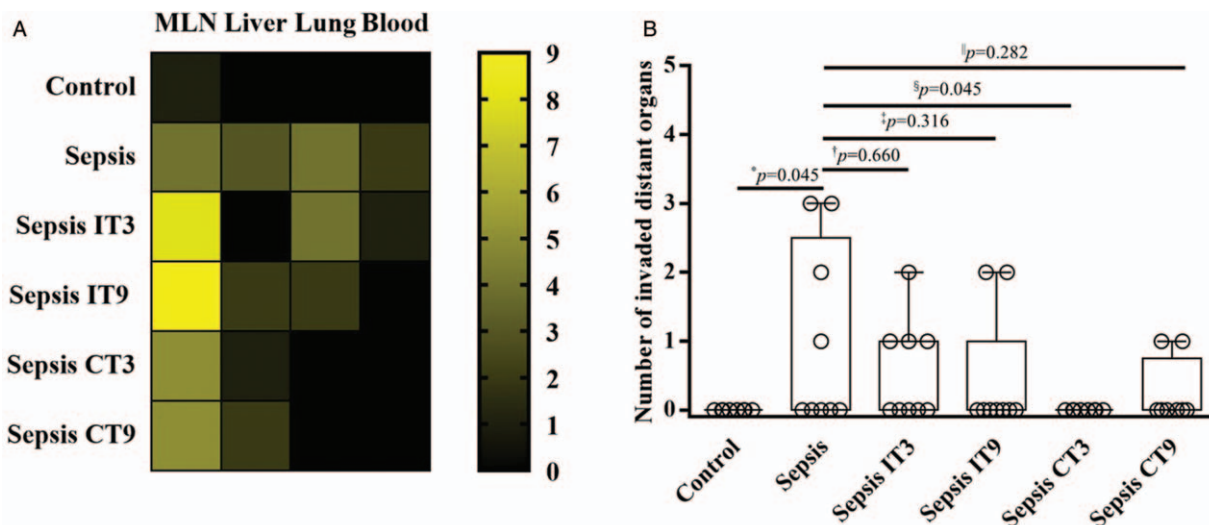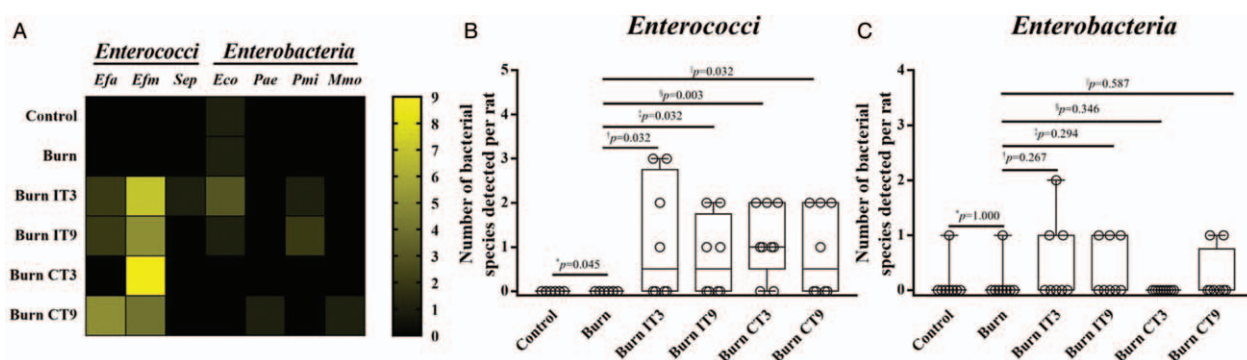

Figure 2: Effect of broad-spectrum antibiotics on BT to different organs in burned rats. (A) Heat map of the numerical change in invaded organs for burned rats with or without antibiotics. (B) Box-and-whiskers plot showing the effect of antibiotics on BT to distant organs (liver, lung, and blood) in burned rats. The circle represents the number of distant organs in which BT occurred in each rat. $n = 9$ for the Burn CT3 group, $n = 8$ each for the other groups. Significance between the two groups was determined by Mann-Whitney U test. [*]$U = 32$; [†]$U = 20$; [‡]$U = 20$; [§]$U = 28$; [||]$U = 20$. BT: Bacterial translocation; CT3: Treated with ceftriaxone for 3 days; CT9: Treated with ceftriaxone for 9 days; IT3: Treated with imipenem for 3 days; IT9: Treated with imipenem for 9 days.

Figure 3: Effect of broad-spectrum antibiotics on BT to different organs in septic rats. (A) Heat map of the numerical change in invaded organs for septic rats with or without antibiotics. (B) Box-and-whiskers plot showing the effect of antibiotics on BT to distant organs in septic rats. The circle represents the number of distant organs in which BT occurred in each rat. $n = 9$ for the Sepsis group, Sepsis IT3 group, and Sepsis IT9 group; $n = 8$ each for the other groups. Significance between the two groups was determined by Mann-Whitney U test. [*]$U = 20$; [†]$U = 35.5$; [‡]$U = 30.5$; [§]$U = 20$; [∥]$U = 26$. BT: Bacterial translocation; CT3: Treated with ceftriaxone for 3 days; CT9: Treated with ceftriaxone for 9 days; IT3: Treated with imipenem for 3 days; IT9: Treated with imipenem for 9 days.
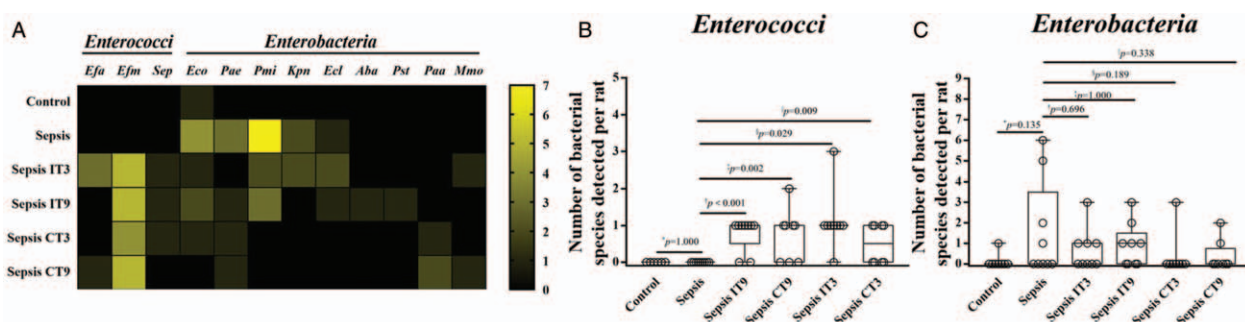

Figure 4: Effect of broad-spectrum antibiotics on translocated bacterial strains in burned rats. (A) Heat map of the total number of translocated bacterial strains detected in all organs of burned rats with or without antibiotics. (B) Box-and-whiskers plot showing the effect of antibiotics on *Enterococci spp.* translocation in burned rats. The circle represents the number of *Enterococci spp.* translocated in all organs of each rat. Significance between the two groups was determined by the Mann-Whitney U test. [*]$U = 32$; [†]$U = 16$; [‡]$U = 16$; [§]$U = 8$; [∥]$U = 16$. (C) Box-and-whiskers plot showing the effect of antibiotics on *Enterobacteria spp.* translocation in burned rats. The circle represents the number of *Enterobacteria spp.* translocated in all organs of each rat. $n = 9$ for the Burn CT3 group, $n = 8$ each for the other groups. Significance between the two groups was determined by Mann-Whitney U test. [*]$U = 32$; [†]$U = 23.4$; [‡]$U = 24$; [§]$U = 31.5$; [∥]$U = 28$. *Eco*: *Escherichia coli*; *Efa*: *Enterococci faecalis*; *Efm*: *Enterococci faecium*; *Mmo*: *Morganella morganii*; *Pae*: *Pseudomonas aeruginosa*; *Pmi*: *Proteus mirabilis*; *Sep*: *Staphylococcus epidermis*. CT3: Treated with ceftriaxone for 3 days; CT9: Treated with ceftriaxone for 9 days; IT3: Treated with imipenem for 3 days; IT9: Treated with imipenem for 9 days.

significance]. For septic rats, however, more BT was observed in MLNs but less in distant organs such as liver, lung, and blood after antibiotic treatment than in the absence of antibiotics [Figure 3A], especially for ceftriaxone treatment [Sepsis CT3 group *vs.* Sepsis group, 0 (0,0) *vs.* 0 (0,3), $U = 20$, $P = 0.045$] [Figure 3B]. These results imply that rational use of antibiotics can effectively control enterogenic infection in septic rats, but there is no benefit for burned rats.

Effect of broad-spectrum antibiotics on translocated bacterial strains in burned or septic rats

Next, we asked if broad-spectrum antibiotics affect translocated bacterial strains in burned or septic rats. To this end, the translocated bacterial strains were detected. As shown in Figures 4A and 5A, seven strains

of bacteria were identified in burned rats and 12 strains in septic rats. Strikingly, only the predominant bacteria *E. coli* (*Eco*) were cultured from MLNs in the normal control and burn control groups, but there were more conditional pathogenic bacteria isolated after the second insult with LPS. *Enterococci spp.*, especially *Enterococci faecium* (*Efa*), dramatically increased after antibiotic treatment in both the burn and the sepsis groups [Burn IT3 group *vs.* Burn group, 0.5 (0,3) *vs.* 0 (0,0), $U = 16$; Burn IT9 group *vs.* Burn group, 0.5 (0,2) *vs.* 0 (0,0), $U = 16$; Burn CT3 group *vs.* Burn group, 1 (0,2) *vs.* 0 (0,0), $U = 8$; Burn CT9 group *vs.* Burn group, 0.5 (0,2) *vs.* 0 (0,0), $U = 16$; Sepsis IT3 group *vs.* Sepsis group, 1 (0,3) *vs.* 0 (0,0), $U = 4.5$; Sepsis IT9 group *vs.* Sepsis group, 1 (0,1) *vs.* 0 (0,0), $U = 9$; Sepsis CT3 group *vs.* Sepsis group, 0.5 (0,1) *vs.* 0 (0,0), $U = 18$; Sepsis CT9 group *vs.* Sepsis group, 1 (0,2) *vs.* 0 (0,0), $U = 13.5$; all $P < 0.05$] [Figures 4A, 5A, 4B, and 5B].

Figure 5: Effect of broad-spectrum antibiotics on translocated bacterial strains in septic rats. (A) Heat map of the total number of translocated bacterial strains detected in all organs of septic rats with or without antibiotics. (B) Box-and-whiskers plot showing the effect of antibiotics on *Enterococci spp.* translocation in septic rats. The circle represents the number of *Enterococci spp.* translocated in all organs of each rat. Significance between the two groups was determined by the Mann-Whitney *U* test. $^*U = 32$; $^†U = 4.5$; $^‡U = 9$; $^§U = 18$; $^{||}U = 13.5$. (C) Box-and-whiskers plot showing the effect of antibiotics on *Enterobacteria spp.* translocation in septic rats. The circle represents the number of *Enterobacteria spp.* translocated in all organs of each rat. $n = 9$ for the Sepsis group, Sepsis IT3 group, and Sepsis IT9 group; $n = 8$ each for the other groups. Significance between the two groups was determined by Mann-Whitney *U* test. $^*U = 23$; $^†U = 36$; $^‡U = 40$; $^§U = 24.5$; $^{||}U = 27$. *Aba*: *Acinetobacter baumannii*; *Ecl*: *Enterobacter cloacae*; *Eco*: *Escherichia coli*; *Efa*: *Enterococci faecalis*; *Efm*: *Enterococci faecium*; *Kpn*: *Klebsiella pneumoniae*; *Mmo*: *Morganella morganii*; *Paa*: *Pseudomonas aeruginosa*; *Pae*: *Pseudomonas aeruginosa*; *Pmi*: *Proteus mirabilis*; *Pst*: *Providencia stuartii*; *Sep*: *Staphylococcus epidermidis*. CT3: Treated with ceftriaxone for 3 days; CT9: Treated with ceftriaxone for 9 days; IT3: Treated with imipenem for 3 days; IT9: Treated with imipenem for 9 days.

However, there were no significant changes in *Enterobacteria spp*. [Burn IT3 group *vs*. Burn group, 0 (0,2) *vs*. 0 (0,1), $U = 23.4$; Burn IT9 group *vs*. Burn group, 0 (0,1) *vs*. 0 (0,1), $U = 24$; Burn CT3 group *vs*. Burn group, 0 (0,0) *vs*. 0 (0,1), $U = 31.5$; Burn CT9 group *vs*. Burn group, 0 (0,1) *vs*. 0 (0,1), $U = 28$; Sepsis IT3 group *vs*. Sepsis group, 0 (0,3) *vs*. 0 (0,6), $U = 36$; Sepsis IT9 group *vs*. Sepsis group, 1 (0,3) *vs*. 0 (0,6), $U = 40$; Sepsis CT3 group *vs*. Sepsis group, 0 (0,3) *vs*. 0 (0,6), $U = 24.5$; Sepsis CT9 group *vs*. Sepsis group, 0 (0,2) *vs*. 0 (0,6), $U = 27$; all $P > 0.05$] [Figures 4A, 4C,5A, and 5C]. This result suggests that multidrug-resistant gram-positive pathogens should be focused on when treating translocation of intestinal bacteria in the clinic.
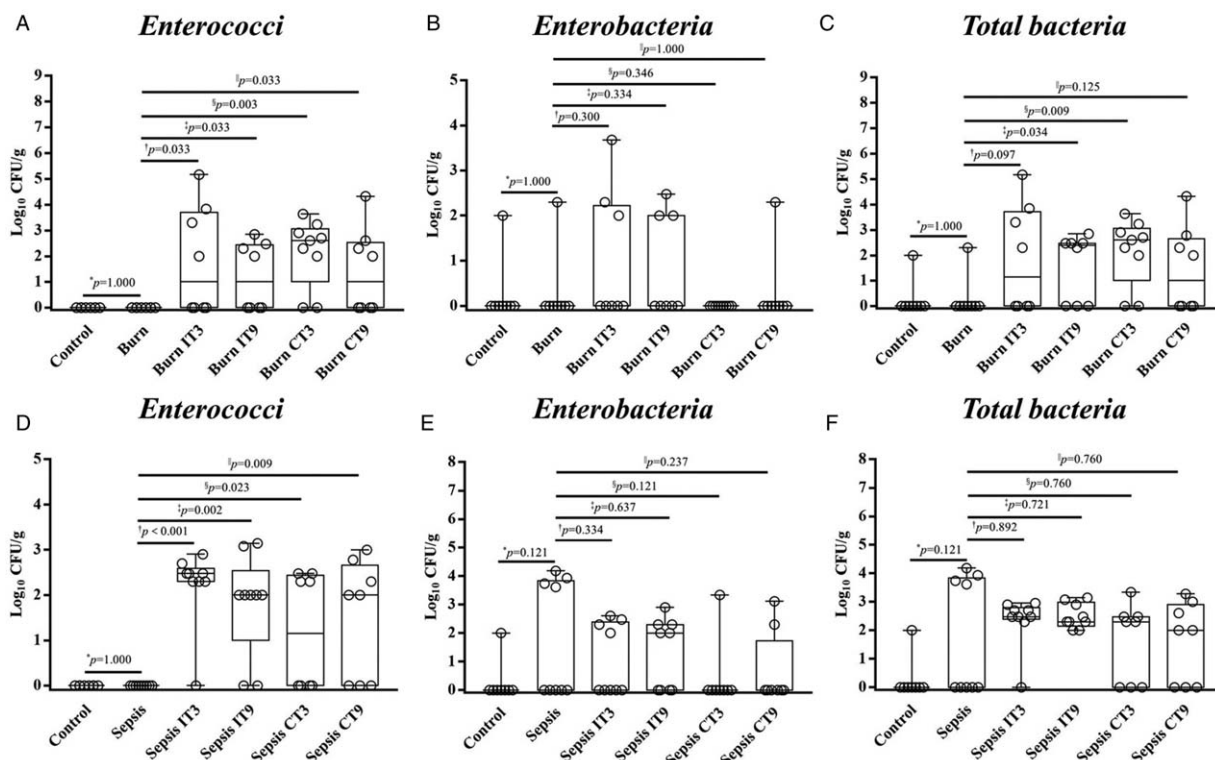
Effect of broad-spectrum antibiotics on the quantity of translocated bacteria in burned or septic rats

To determine the effect of broad-spectrum antibiotics on the quantity of translocated bacteria in burned or septic rats, we performed a quantitative analysis for BT in those rats. As shown in Figure 6A, 6B, and 6C, only a few *Enterobacteria spp*. but not *Enterococci spp*. were quantitated in the normal and burn control group. The quantity of translocated bacteria was increased after the second insult with LPS, but the species recovered were still all *Enterobacteria spp*. [Figure 6D, 6E, and 6F]. However, the quantity of *Enterococci spp*. was significantly increased after treatment with either antibiotic in both the burned and septic rats [Burn IT3 group *vs*. Burn group, 1 (0,5.164) *vs*. 0 (0,0) Log$_{10}$ CFU/g, $U = 16$; Burn IT9 group *vs*. Burn group, 1 (0,2.845) *vs*. 0 (0,0) Log$_{10}$ CFU/g, $U = 16$; Burn CT3 group *vs*. Burn group, 2.602 (0,3.633) *vs*. 0 (0,0) Log$_{10}$ CFU/g, $U = 8$; Burn CT9 group *vs*. Burn group, 1 (0,4.326) *vs*. 0 (0,0) Log$_{10}$ CFU/g, $U = 16$; Sepsis IT3 group *vs*. Sepsis group, 2.477 (0,2.903) *vs*. 0 (0,0) Log$_{10}$ CFU/g, $U = 4.5$; Sepsis IT9 group *vs*. Sepsis group, 2 (0,3.146) *vs*. 0 (0,0) Log$_{10}$ CFU/g, $U = 9$; Sepsis CT3 group *vs*. Sepsis group, 1.151 (0,2.477) *vs*. 0 (0,0) Log$_{10}$ CFU/g, $U = 18$; Sepsis CT9 group *vs*. Sepsis group, 2 (0,3) *vs*. 0 (0,0) Log$_{10}$ CFU/g, $U = 13.5$; all $P < 0.05$] [Figure 6A and 6D]. The total amount of translocated bacteria, regardless

of which antibiotic was used, was increased in burned rats [Burn IT3 group *vs*. Burn group, 1.51 (0,5.178) *vs*. 0 (0,2.301) Log$_{10}$ CFU/g, $U = 18.5$, $P = 0.097$; Burn IT9 group *vs*. Burn group, 2.389 (0,2.845) *vs*. 0 (0,2.301) Log$_{10}$ CFU/g, $U = 14$, $P = 0.034$; Burn CT3 group *vs*. Burn group, 2.602 (0,3.633) *vs*. 0 (0,2.301) Log$_{10}$ CFU/g, $U = 10.5$, $P = 0.009$; Burn CT9 group *vs*. Burn group, 1 (0,4.326) *vs*. 0 (0,2.301) Log$_{10}$ CFU/g, $U = 19.5$, $P = 0.125$] [Figure 6C] but showed a slightly decreased trend in septic rats [Sepsis IT3 group *vs*. Sepsis group, 2.477 (0,2.954) *vs*. 0 (0,4.185) Log$_{10}$ CFU/g, $U = 38.5$, $P = 0.892$; Sepsis IT9 group *vs*. Sepsis group, 2.301 (2,3.146) *vs*. 0 (0,4.185) Log$_{10}$ CFU/g, $U = 36$, $P = 0.721$; Sepsis CT3 group *vs*. Sepsis group, 2.301 (0,3.342) *vs*. 0 (0,4.185) Log$_{10}$ CFU/g, $U = 32.5$, $P = 0.760$; Sepsis CT9 group *vs*. Sepsis group, 2 (0,3.279) *vs*. 0 (0,4.185) Log$_{10}$ CFU/g, $U = 32.5$, $P = 0.760$] [Figure 6F].

Discussion

BT mainly refers to the transfer of intestinal bacteria and/ or their products from the gut to extraintestinal sites.[19] Intestinal ischemia–reperfusion is an adaptive reaction of patients who are critically ill. Studies have shown that sepsis causes intestinal epithelial cell apoptosis or autophagy[20,21] and intestinal vascular endothelial cells injury,[22] which further results in microcirculation disturbance of the intestinal mucosa, increasing gut permeability and leading to the breakdown of intestinal barriers. At steady state, there was only a small amount of BT to the MLNs. Once pathogenic bacteria overgrowth occurs in the gut, the intestinal mucosal barrier and host defences are impaired and BT is significantly increased.[12,23,24] To better study the relationship between antibiotics and BT, we chose a sepsis model because this model can cause intestinal barrier destruction and requires antibiotic treatment. Endotoxin, bacterial infusion, cecal ligation and puncture, and colon ascendens stent peritonitis models are the current commonly practiced methods to induce sepsis.[25] Although LPS-induced sepsis mainly reflects the early stage of sepsis and has a certain gap with the clinical course, considering that we needed to collect

Figure 6: Effect of broad-spectrum antibiotics on the quantity of translocated bacteria in burned or septic rats. $n = 9$ for the Burn CT3 group, $n = 8$ each for the other groups; $n = 9$ for the Sepsis group, Sepsis IT3 group, and Sepsis IT9 group; $n = 8$ each for the other groups. (A) Box-and-whiskers plot showing the effect of broad-spectrum antibiotics on the quantity of translocated *Enterococci spp.* in burned rats; the circle represents the quantity of translocated *Enterococci spp.* in all organs of each rat. Significance between the two groups was determined by the Mann-Whitney U test. $^*U = 32$; $^†U = 16$; $^‡U = 16$; $^§U = 8$; $^{||}U = 16$. (B) Box-and-whiskers plot showing the effect of broad-spectrum antibiotics on the quantity of translocated *Enterobacteria spp.* in burned rats; the circle represents the quantity of translocated *Enterobacteria spp.* in all organs of each rat. Significance between the two groups was determined by the Mann-Whitney U test. $^*U = 31.5$; $^†U = 24$; $^‡U = 24.5$; $^§U = 31.5$; $^{||}U = 32$. (C) Box-and-whiskers plot showing the effect of broad-spectrum antibiotics on the total quantity of translocated bacteria in burned rats; the circle represents the total quantity of translocated bacteria in all organs of each rat. Significance between the two groups was determined by the Mann-Whitney U test. $^*U = 31.5$; $^†U = 18.5$; $^‡U = 14$; $^§U = 10.5$; $^{||}U = 19.5$. (D) Box-and-whiskers plot showing the effect of broad-spectrum antibiotics on the quantity of translocated *Enterococci spp.* in septic rats; the circle represents the quantity of translocated *Enterococci spp.* in all organs of each rat. Significance between the two groups was determined by the Mann-Whitney U test. $^*U = 32$; $^†U = 4.5$; $^‡U = 9$; $^§U = 18$; $^{||}U = 13.5$. (E) Box-and-whiskers plot showing the effect of broad-spectrum antibiotics on the quantity of translocated *Enterobacteria spp.* in septic rats; the circle represents the quantity of translocated *Enterobacteria spp.* in all organs of each rat. Significance between the two groups was determined by the Mann-Whitney U test. $^*U = 22.5$; $^†U = 32.5$; $^‡U = 35$; $^§U = 22.5$; $^{||}U = 25$. (F) Box-and-whiskers plot showing the effect of broad-spectrum antibiotics on the total quantity of translocated bacteria in septic rats. The circle represents the total quantity of translocated bacteria in all organs of each rat. Significance between the two groups was determined by the Mann-Whitney U test. $^*U = 22.5$; $^†U = 38.5$; $^‡U = 36$; $^§U = 32.5$; $^{||}U = 32.5$. CFU: Colony-forming units; CT3: Treated with ceftriaxone for 3 days; CT9: Treated with ceftriaxone for 9 days; IT3: Treated with imipenem for 3 days; IT9: Treated with imipenem for 9 days.

the MLNs and liver under sterile conditions for bacterial culture, we could not choose other kinds of sepsis models with bacterial contamination of the abdominal cavity. The results in the present study showed that only a few predominant bacteria, *Eco*, were translocated to MLNs in the normal control group. Simple burn injury did not affect the BT rate, but many strains of *Enterobacteria spp.* were widely translocated to the MLNs, blood, liver, and lung after a second insult with LPS, indicating that critical illness itself could lead to BT.

Both imipenem and ceftriaxone are broad-spectrum antibiotics but have different metabolic pathways; they represent the majority of drugs commonly used in clinical practice, so they were used in this study. They have powerful antibacterial activity against most of the gram-negative bacteria and relatively few gram-positive micro-organisms. However, the quantity of translocated bacteria and the number of involved organs were both increased in burned rats after the application of those two antibiotics. Meanwhile, we found a distinct change in the strains of

translocated bacteria, in which *Enterococci spp.* accounted for a larger proportion after antibiotic treatment than before treatment. Unlike the burned group, we found a decreased quantity of translocated bacteria and fewer involved organs, especially remote organs such as the lung, liver, and blood, in septic rats after antibiotic treatment than in those before treatment. However, the percentage of translocated *Enterococci spp.* was increased as it was in the burned group. This result indicates that broad-spectrum antibiotics promote BT in burned rats but suppress it in septic rats, especially inhibiting BT to the distant organs. Why is this effect happening? We believe that the effect of scalding on the intestinal flora and intestinal mucosa of rats is small, so the displacement is also small, and the antibiotics aggravate the dysbiosis of the intestinal flora, which, in turn, leads to an increase in displacement. In the septic rats, the intestinal bacterial abundance increased sharply, and the quantitative advantage caused the shift. At this time, the intervention of antibiotics controlled the number of bacteria to some extent and reduced the bacillary shift. Meanwhile, because the antibiotics used are

mainly against *bacilli* and their antibacterial activity against *Enterococci spp*. is poor, the *Enterococci spp*. are growing uninhibited, and the resulting quantitative advantage has made them the main force for BT, which could also explain the phenomenon that *Enterococci spp*. are common pathogens of hospital-acquired infection.[4]

Interestingly, our results showed that the rate of BT in the imipenem treatment group was slightly higher than that in the ceftriaxone treatment group under the same conditions, although the antiseptic spectrum of imipenem is more extensive than that of ceftriaxone. We speculated that the difference of effective concentration in the intestine between the two antibiotics might contribute to this observation. Approximately 10% to 20% of ceftriaxone in the body is excreted into the gut through the enterohepatic circulation, resulting in a high antibiotic concentration and a strong sterilization activity in the intestinal tract.[26] Most intestinal indigenous bacteria, that is, *E. coli*, along with conditional pathogens that are susceptible to ceftriaxone are killed under the high concentration of ceftriaxone in the intestinal tract, leading to decreased BT. However, almost all imipenem is rapidly excreted via the kidneys[27] and unable to reach a high concentration in the gut, which weakens its bactericidal activity to intestinal bacteria and leads to more BT than occurs with ceftriaxone.

Our study had some limitations, including limited sample, failure to detect all bacteria with the traditional plate cultivation method, and no testing of anticoccidial drugs. If anticoccidial drugs are used, according to the results of this experiment, we think that their use will reduce the translocation of *Enterococci spp*., which is a perfect idea to further verify the conclusion of this experiment.

In summary, our results reveal that broad-spectrum antibiotics promote BT in burned rats but prevent BT in septic rats, especially inhibiting BT to distant organs, although there are still some limitations in the current study. Our findings also uncovere that the BT of *Enterococci spp*. is dramatically increased after treatment with broad-spectrum antibiotics. These data provide a basis for selecting antibiotics reasonably and reducing risks of enterogenic infection in patients with critical illness.

Funding

Conflicts of interest

None.

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