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Data Availability Statement: Due to the management system, if you need to access the data, you should contact the corresponding authors of this study or Bining Yang, email: 18262031927@163.com, the data manager of the Institute of Critical Care Medicine of Soochow University to make a request and explain the reason for obtaining the data. The research institutions where the corresponding authors work will gradually improve the development of data RESEARCH ARTICLE

Distribution of pathogens and risk factors for post-replantation wound infection in patients with traumatic major limb mutilation

Chang Gao^{1,2,3}, Haiyan Wang³, Jihui Ju⁴, Keran Zhang⁵, Ye Gao⁶, Shiqi Guo³, Di Yin³, Ruixing Hou⁴, Qiang Guo^{1,2,7}

1 Department of Emergency and Critical Care Medicine, The Fourth Affiliated Hospital of Soochow University (Suzhou Dushu Lake Hospital), Suzhou, Jiangsu, China, 2 Medical Center of Soochow University, Suzhou, Jiangsu, China, 3 Medical College of Soochow University, Suzhou, Jiangsu, China, 4 Department of Orthopaedic, Suzhou Ruihua Orthopaedic Hospital (Suzhou Ruixing Medical Group), Suzhou, Jiangsu, China, 5 Department of Critical Care Medicine, Suzhou Ruihua Orthopaedic Hospital (Suzhou Ruixing Medical Group), Suzhou, Jiangsu, China, 6 Department of Critical Care Medicine, Taicang Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China, 7 The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China

• These authors contributed equally to this work.

* guojiang@suda.edu.cn (QG); hrx2020@suda.edu.cn (RH)

Abstract

Purpose

Even though replantation of limb mutilation is increasing, postoperative wound infection can result in increasing the financial and psychological burden of patients. Here, we sought to explore the distribution of pathogens and identify risk factors for postoperative wound infection to help early identification and managements of high-risk patients.

Methods

Adult inpatients with severed traumatic major limb mutilation who underwent replantation from Suzhou Ruixing Medical Group between November 09, 2014, and September 6, 2022 were included in this retrospective study. Demographic, and clinical characteristics, treatments, and outcomes were collected. Data were used to analyze risk factors for postoperative wound infection.

Results

Among the 249 patients, 185 (74.3%) were males, the median age was 47.0 years old. Postoperative wound infection in 74 (29.7%) patients, of whom 51 (20.5%) had infection with multi-drug resistant bacteria. Ischemia time (OR 1.31, 95% CI 1.13–1.53, P = 0.001), wound contamination (OR 6.01, 95% CI 2.38–15.19, P <0.001), and stress hyperglycemia (OR 23.37, 95% CI 2.30–236.93, P = 0.008) were independent risk factors, while the albumin level after surgery (OR 0.94, 95% CI 0.89–0.99, P = 0.031) was significant associated with the decrease of postoperative wound infection. Ischemia time (OR 1.21, 95% CI 1.05–1.40, P = 0.010), wound contamination (OR 8.63, 95% CI 2.91–25.57, P <0.001), and MESS (OR information system to ensure long-term data storage and further development of cooperation.

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1.32, 95% CI 1.02–1.71, P = 0.037 were independent risk factors for multi-drug resistant bacteria infection.

Conclusions

Post-replantation wound infection was common in patients with severe traumatic major limb mutilation, and most were multi-drug resistant bacteria. Ischemia time and wound contamination were associated with the increase of postoperative wound infection, including caused by multi-drug resistant. Positive correction of hypoproteinemia and control of stress hyper-glycemia may be beneficial.

Introduction

High energy trauma such as traumatic mutilation of major limbs was the leading cause of amputation and can be life-threatening [1-3]. With the development of surgical techniques, the limbs saving rate of patients gradually increased [1, 4], however, with the aggravation of trauma severity, the incidence of postoperative complications also gradually increased [2, 5, 6]. Postoperative wound infection is one of the most common complications after surgery [7, 8]. Studies showed that Gustilo-Anderson (GA) Classification type III was positively associated with the increased of infection and other complications [8, 9]. Most of the major traumatic limb mutilations were GA type III, nevertheless, the incidence of wound infection after limb salvage surgery was limited.

In patients infected after traumatic fractures, reported strains include but are not limited to Aeromonas, Enterobacter Pseudomonas Enterococcus, Staphylococcus Salmonella, Cutibacterium Proteus, Coagulase negative Staphylococci, and others [10, 11]. The distribution of pathogenic microorganisms varies according to the center, the type of surgery, and even the season [12]. Prophylactic anti-infective measures for GA type III fractures are recommended to cover both gram-negative and gram-positive bacteria, but evidence is limited [13, 14]. With the spread of drug-resistant bacteria in communities and hospitals [15], for patients after replantation, relevant epidemiological characteristics are urgently needed to provide a basis for targeted anti-infection.

In addition, wound infection after replantation can lead to limb loss and increase the psychological and economic burden of patients, with serious adverse consequences [2, 16]. Risk factors for surgical site infection included laboratory findings, trauma grade, and obesity [5, 17, 18], the causes of infection after major limb replantation are poorly understood. Identifying the underlying causes of infections, especially multidrug-resistant bacteria, and intervening early may benefit more patients. Here we sought to identify risk factors for postoperative wound infection to help early identification of high-risk patients.

Methods

This multicenter retrospective cohort study included all adult patients (age \geq 18 years) who had traumatic mutilation of major limbs (defined as an amputation between the trunk and the wrist or ankle) and underwent replantation [19, 20] between November 09, 2014, and September 6, 2022 from the hospitals in the Suzhou Ruixing Medical Group (Suzhou Ruihua Orthopaedic Hospital and Ruixing Hospital). Data were accessed for research purposes on May 1, 2021, data on patients admitted after this date were still collected retrospectively. Ruixing medical group includes Level III specialized hospitals, rehabilitation hospitals, and an institute of applied technology in hand surgery [21]. Orthopedic trauma, amputated limbs (fingers and toes) replantation, and rehabilitation are the focus medical programs of the group, with an average annual operation volume of more than 10,000, which increasing yearly. All of the mutilation limbs were accompanied by discontinuous vessels, nerves, muscles, and bone structures to varying degrees. Patients with severe limb damage that could not be replanted or had first-stage amputations were excluded, which were detailed in our previous study [21]. Identify patients who receive reimplantation by reviewing and analyzing admission logs and histories from all available electronic medical records and patient care resources.

Medical records were reviewed, entered and verified independently by two trained physicians, blinded to each other. Demographic and clinical characteristics including traumatic conditions, laboratory findings, MESS, treatments, and outcomes of the patients were collected. Examinations and treatments during hospitalization were performed by clinicians according to the conditions. Following-up of the patients was from admission to hospital discharge. The primary outcome was the postoperative wound infection rate during hospitalization.

Indications for replantation of severed limb: (1) Relatively complete distal limb and mild skin contusion, (2) The tissue structure of the proximal limb is relatively complete, and the bone and joint injury does not seriously affect the appearance and function of the limb, (3) No avulsive nerve injury or only minor local contusion, (4) Patients could tolerate microsurgery with stable physical signs and without serious complications[21]. Gustilo-Anderson classification of open fractures were defined as follows [22]: type I, open fracture with a wound less than 1 cm long, low energy, without gross contamination; type II, open fracture with a wound 1-10 cm long, low energy, without gross contamination or extensive soft-tissue damage, flaps, or avulsions; type III A, open fracture with a wound greater than 10 cm with adequate soft-tissue coverage, or any open fracture due to high-energy trauma or with gross contamination, regardless of the size of the wound; type III B, open fracture with extensive soft-tissue injury or loss, with periosteal stripping and bone exposure that requires soft-tissue coverage in the form of muscle rotation or transfer; type III C, open fracture associated with arterial injury requiring repair. Stress hyperglycemia was defined as fasting glucose >6.9 mmol/L or random glucose >11.1 mmol/L without evidence of previous diabetes, and preexisting diabetes with deterioration of pre-existing glycemic control [23]. Postoperative wound infection was defined as purulent discharge, erythema, and/or surgical wound dehiscence exposing underlying hardware following definitive fixation and wound closure, necessitating a return to the operating theatre for irrigation and debridement [24]. During surgical irrigation and debridement, at least two separate deep tissue/implant specimens must be collected, returning a phenotypically indistinguishable pathogen, following the consensus statement of an international expert group [24]. Multidrug-resistant was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [25].

The secondary outcomes were the length of ICU and hospital stay, partial/total necrosis, and delayed amputations. Delayed amputations were defined as amputations performed within the same hospitalization period after replantation [26]. Frequency data were expressed as proportions. Continuous data are presented as median (interquartile range [IQR]) if they showed skewed distribution. Shapiro-wilk test was used to determine normal/skewed distribution of the data. Differences in categorical variables were assessed using the χ^2 test, while comparisons of continuous variables were made using the Mann-Whitney U test, as appropriate. According to whether the data were normally distributed, correlation analysis was performed using Pearson or Spearman, respectively.

To determine the independent risk factors for postoperative wound infection, multivariate logistic regression models were used. Results of the logistic regression were presented as (odds ratio [OR], 95% confidence interval [CI]). Variables with P < 0.1 in univariate logistic regression were included in the multivariate analysis. The probabilities of entering and removing variables in a stepwise manner in the multivariate model were 0.05 and 0.10, respectively.

SPSS (version 25.0; IBM, Chicago, IL, USA) was used to analysis data. GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA) and StataMP 16 (StataCorp, College Station, Texas, USA) were used to generate the statistical charts. A two-tailed P value of <0.05 was considered statistically significant.

This study was approved by the Institutional Review Boards of the Suzhou Ruixing Medical Group (2021023). Due to the retrospective nature of the study, no informed consent was required.

Results

A total of 283 patients were admitted to the hospital after experiencing traumatic major limb mutilation during eight years study period. 34 patients who had either severe limb damage that could not be replanted or first-stage amputations were excluded. 249 patients who underwent replantation were included in this study. The median age of the patients was 47.0 (IQR, 36.0–54.0) years and the majority were males (n = 185, 74.3%). Most patients experienced moderate-to-severe contamination, 91 (36.5%) had lower limb trauma, 181 (72.7%) had blunt trauma, and 100 (40.2%) had total mutilation. The median MESS was 10.0 (IQR, 8.5–12.0) and median ischemia time was 7.5 (IQR, 5.7–9.6) hours. The upper limb salvage rate was 97.5% (154 in 158 cases), the lower limb salvage rate was 95.6% (87 in 91 cases). According to the Gustilo-Anderson Classification for open fractures, all the injuries were type IIIC. All patients received prophylactic antibiotics. The severed part of 249 patients was shown in S1 Table.

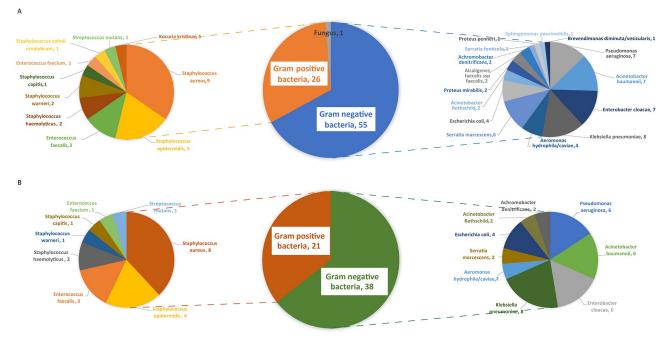
Pathogenic characteristics

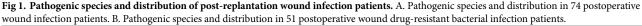
Postoperative wound infection in 74 (29.7%) patients, of whom 51 (20.5%) had infection with multi-drug resistant bacteria. Of the 74 patients with postoperative wound infection, 45 had only gram-negative bacterial infection, 23 had only Gram-positive bacterial infection, and 6 had mixed infection (Table 1). A total of 82 pathogens were detected, including 26 Gram-positive bacteria were: Staphylococcus aureus, Staphylococcus epidermidis, and Enterococcus; the top three Gram-negative bacteria were: Klebsiella pneumoniae, Enterobacter cloacae, Acinetobacter baumannii, and Pseudomonas aeruginosa (Fig 1A). Among the 82 pathogens, 59 were multi-drug resistant bacteria, including 21 Gram-positive bacteria and 38 Gram-negative bacteria. Among the resistant bacteria, the top three Gram-positive bacteria and the top three

Table 1.	Pathogenic	species of	of infection.
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Pathogenic species of infection	Number of patients
Gram-negative bacteria (G ⁻) only	45
Gram-positive bacteria (G^+) only	23
Mixed infection	6
$G^- + G^+$	2
G ⁻ + G ⁻	2
G ⁻ + G ⁻ + fungus	1
$G^{+} + G^{-} + G^{-}$	1

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Gram-negative bacteria were the same as in all pathogens (Fig 1B). The median time from trauma to infection was 8.0 (IQR, 5.0–13.0) days, median duration of infection was 34.0 (IQR, 23.8–52.0) days (Fig 2A and 2B).

Clinical characteristics

Compare with the non-infection group, patients with postoperative wound infection presented with a higher rate of stress hyperglycemia after surgery (9.5% vs. 0.6%, P <0.001), partial/total necrosis (91.9% vs. 50.9%, P <0.001), and delayed amputations (8.1% vs. 1.1%, P = 0.014) (Table 2). The infection group presented higher median values of MESS (12.0 [IQR, 9.0–12.0] vs. 10.0 [IQR, 8.0–11.0], P <0.001), ischemia time (9.0 [IQR, 7.1–11.7] vs. 6.7 [IQR, 5.3–8.5] hours, P <0.001), length of ICU stays (5.0 [IQR, 0.0–8.3] vs. 2.0 [IQR, 0.0–5.0] days, P = 0.002), and length of hospital stays (57.5 [IQR, 41.8–73.3] vs. 36.0 [IQR, 22.0–54.0] days, P <0.001) (Table 2). Patients in postoperative wound infection group had lower median values of red blood cell (RBC) count (3.0 [IQR, 2.3–3.8] vs. 3.3 [IQR, 2.8–3.9] × 10¹²/L, P = 0.027) and platelet count (118.0 [IQR, 69.0–170.0] vs. 147.5 [IQR, 95.0–219.3] × 10⁹/L, P = 0.005) measured immediately after surgery (Table 2).

Furthermore, in a correlation analysis, we found that the duration of infection was significantly associated with the length of hospital stay ($r_s = 0.625$, P < 0.001) (Fig 2C). Patients who suffered postoperative wound infection needed more times of operations than non-infection ones (2.0 [IQR, 2.0–3.0] vs. 1.0 [IQR, 1.0–2.0], P < 0.001) (Fig 2D).

Risk factors for postoperative wound infection and multi-drug resistant bacteria infection

Univariate logistic regression analysis showed that age, wound contamination, ischemia time, MESS, lactic acid on admission, RBC count immediately after surgery, platelet count after

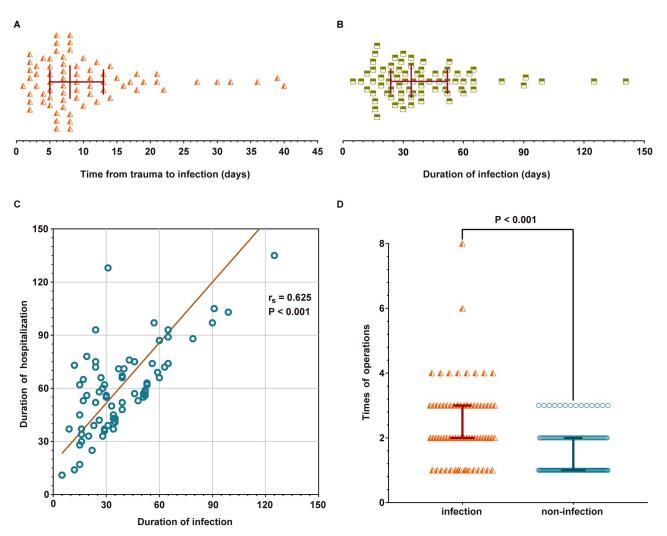


Fig 2. Postoperative wound infection characteristics. A. Time from trauma to infection. B. Duration of postoperative wound infection. C. Linear regression analysis of duration of infection and length of hospital stay. D. Comparison of operation times between infected group and non-infected group.

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surgery, albumin level after surgery, D-dimer level after surgery, and stress hyperglycemia were significantly associated with Postoperative wound infection (S2 Table). Multivariable logistic regression analysis found that ischemia time (OR 1.31, 95% CI 1.13–1.53, P = 0.001), wound contamination (OR 6.01, 95% CI 2.38–15.19, P <0.001), D-dimer after surgery (OR 1.16, 95% CI 1.01–1.35, P = 0.042), and stress hyperglycemia (OR 23.37, 95% CI 2.30–236.93, P = 0.008) were independent risk factors, while the albumin level after surgery (OR 0.94, 95% CI 0.89–0.99, P = 0.031) was significant associated with the decrease of postoperative wound infection (Fig 3A).

In addition, through univariate logistic regression analysis, results showed that wound contamination, ischemia time, MESS, lactic acid on admission, platelet count after surgery, albumin level after surgery, ALT level after surgery, and D-dimer level after surgery were significantly associated with multi-drug resistant bacteria infection (S3 Table). By multivariable logistic regression analysis, ischemia time (OR 1.21, 95% CI 1.05–1.40, P = 0.010), wound contamination (OR 8.63, 95% CI 2.91–25.57, P <0.001), MESS (OR 1.32, 95% CI 1.02–1.71,

Characteristics	All patients	Infection	Non-infection	Р
	(n = 249)	(n = 74)	(n = 175)	
Age, median (IQR), yr	47.0 (36.0-54.0)	50.0 (40.0-57.0)	46.0 (32.0-53.0)	0.030
Sex, male patients, n (%)	185 (74.3)	58 (78.4)	127 (72.6)	0.338
Current smokers, n (%)	35 (14.1)	7 (9.5)	28 (16.0)	0.175
Alcohol preference, n (%)	5 (2.0)	1 (1.4)	4 (2.3)	0.631
Pre-existing hypertension, n (%)	17 (6.8)	6 (8.1)	11 (6.3)	0.602
Pre-existing diabetes, n (%)	16 (6.4)	8 (10.8)	8 (4.6)	0.067
Pre-existing cardiovascular disease, n (%)	8 (3.2)	0 (0.0)	8 (4.6)	0.062
Heart rate, median (IQR), beats per minute	80.0 (76.0-89.0)	80.0 (77.5-89.0)	80.0 (76.0-89.0)	0.889
Fraumatic condition, n (%)				
Lower limb	91 (36.5)	32 (43.2)	59 (33.7)	0.154
Blunt mutilation	181 (72.7)	59 (79.7)	122 (69.7)	0.105
Total mutilation	100 (40.2)	37 (50.0)	63 (36.0)	0.039
5, n (%)				< 0.001
Mild	1 (0.4)	0 (0.0)	1 (0.6)	
Moderate	113 (45.4)	15 (20.3)	98 (56.0)	
Severe	135 (54.2)	59 (79.7)	76 (43.4)	
Time from trauma to admission, median (IQR), hr.	2.0 (1.0-3.0)	1.3 (1.0-3.5)	2.0 (1.0-3.0)	0.152
Time from trauma to operation started, median (IQR), hr.	3.3 (2.5–4.5)	3.0 (2.3-4.7)	3.4 (2.5–4.5)	0.458
MESS, median (IQR)	10.0 (8.5–12.0)	12.0 (9.0–12.0)	10.0 (8.0-11.0)	< 0.001
Duration of operation, median (IQR), hr.	5.5 (3.5-7.5)	5.9 (3.5-7.8)	5.1 (3.5–7.5)	0.447
schemia time, median (IQR), hr. ^a	7.5 (5.7–9.6)	9.0 (7.1–11.7)	6.7 (5.3–8.5)	< 0.001
irst laboratory findings after surgery, median (IQR)				
WBC count, $\times 10^9$ /L	10.3 (8.1–12.7)	10.0 (8.3–12.4)	10.3 (8.0–12.9)	0.992
Platelet count, $\times 10^{9}/L$	138.0	118.0	147.5	0.005
	(93.0-204.0)	(69.0-170.0)	(95.0-219.3)	
RBC count, $\times 10^{12}/L$	3.2 (2.6–3.8)	3.0 (2.3–3.8)	3.3 (2.8–3.9)	0.027
Albumin, g/L	32.1 (26.4–36.1)	31.4 (23.5–35.8)	32.4 (28.3–36.2)	0.078
ALT, U/L	19.3 (13.6–28.1)	18.0 (13.0-29.3)	19.5 (13.6–27.9)	0.965
TBiL, μmol/L	15.0 (9.8-20.0)	15.1 (10.6–20.2)	15.0 (9.4–19.7)	0.521
BUN, mmol/L	4.4 (3.6–5.7)	4.5 (3.8-6.0)	4.4 (3.4–5.2)	0.064
Creatinine, µmol/L	60.9 (52.1-71.1)	61.1 (51.9–75.2)	60.7 (52.3–69.8)	0.369
D-dimer, µg/ml	1.0 (0.4–2.2)	1.2 (0.4–3.4)	0.9 (0.4–2.0)	0.149
Stress hyperglycemia after surgery, n (%)	8 (3.2)	7 (9.5)	1 (0.6)	< 0.001
Freatment after surgery, n (%)				
ICU admission	136 (54.6)	45 (60.8)	91 (52.0)	0.202
Anticoagulant therapy	156 (62.7)	53 (71.6)	103 (58.9)	0.057
Antiplatelet therapy	211 (84.7)	65 (87.8)	146 (83.4)	0.377
Dutcomes				
Length of ICU stay, day, median (IQR)	2.0 (0.0-6.0)	5.0 (0.0-8.3)	2.0 (0.0-5.0)	0.002
Length of hospital stay, day, median (IQR)	43.0 (27.0–59.5)	57.5 (41.8-73.3)	36.0 (22.0–54.0)	< 0.001
Partial/total necrosis, n (%)	157 (63.1)	68 (91.9)	89 (50.9)	< 0.001
Delayed amputations, n (%)	8 (3.2)	6 (8.1)	2 (1.1)	0.014

Table 2. Characteristics of 249 patients with replantation of severed limb.

^{*a*} Ischemia time was defined as the time from mutilation to recovery of blood circulation.

^b Yates's correction was used.

Abbreviations: IQR, interquartile range; WBC, white blood cell; RBC, red blood cell; ALT, alanine aminotransferase; TBiL, total bilirubin; BUN, blood urea nitrogen; MESS, mangled extremity severity score; PT, prothrombin time; ICU, intensive care unit.

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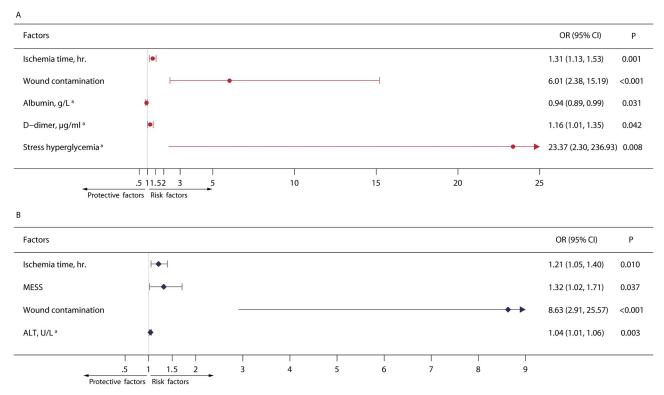


Fig 3. Multivariate logistic analysis of factors associated with post-replantation wound infection. A. Multivariate logistic analysis of factors associated with post-replantation wound infection. B. Multivariate logistic analysis of factors associated with post-replantation wound drug-resistant bacterial infection. ^a Conditions or first laboratory findings after surgery.

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P = 0.037), and ALT level after surgery (OR 1.04, 95% CI 1.01–1.06, P = 0.003) were independent risk factors (Fig 3B).

Discussion

Traumatic major limb mutilation is one of the main causes of limb loss. Even though replantation of limb mutilation is increasing gradually with the development of microsurgery and surgical techniques [1, 4, 27], postoperative wound infection can result in multiple operations and even delayed amputation, which may increase the financial and psychological burden of patients [2, 4, 16]. This study found that postoperative wound infection was common in patients with severe traumatic major limb mutilation, and most of the pathogenic bacteria were multi-drug resistant bacteria, which was associated with ischemic time, degrees of wound contamination, stress hyperglycemia, and MESS.

Surgical site infections, the most common health care-acquired infections, are associated with the emergence of postoperative infected with multidrug-resistant bacteria [7, 28–32]. Previous studies reported that the postoperative wound infection rate of GA classification type III can be even as high as 50% or more [8, 11, 33], which is itself associated with higher postoperative wound infection of fractures [17]. The high incidence of postoperative wound infection in our research may be explained by the fact that the patients are all GA IIIC and had high damage contamination, which leaded to a greater risk of multi-microbial infection [34]. The median time for postoperative wound infection was 8.0 days after trauma. Consistent with previous reports, a strong positive correlation was shown between the duration of the infection

and the length of hospital stay, and the number of operations in patients with infection increased significantly, which may lead to an increased chance of acquiring nosocomial infections [35, 36]. Special attention should be paid to the healing or infection status of the wound during this duration after surgery.

The etiological distribution of postoperative wound infection varies according to different surgical reasons, sites and even seasons [9, 11, 12, 37]. Reports on etiological characteristics after regrafting of severe traumatic mutilation of major limbs were limited. For patients with type III open fractures, gram-negative bacilli should be covered in addition to gram-positive bacilli, for which the evidence of evidence-based medicine was insufficient [7, 13, 14, 22]. We here found the main pathogen distribution characteristics, which may provide targeted basis for the use of prophylactic antibiotics in patients in these regional centers.

This study showed that with the increase of wound contamination degree and the prolongation of ischemia time, the incidence of multidrug-resistant bacterial infection increased significantly. The fracture types included in this study were all IIIc types, with large soft tissue, severe vascular injury, and a wide range of exposure, which had high probability of contact with pathogens. Contamination levels and postoperative deep infections have been demonstrated [38], as well as the transmission of multidrug-resistant in communities has become increasingly common [15], which partly explained the high rate of multidrug resistance. Severe open limb trauma had very high risks of tissue ischemia, necrosis and infection. The invasion of pathogenic microorganisms into the damaged skin barrier is easy to cause infection. Tissue ischemia weakens the body's ability to clear microorganisms, and the necrotic tissue becomes a hotbed for nourishing microorganisms and accelerates the occurrence and development of infection [39]. Therefore, shortening the ischemic time is particularly important for preventing postoperative wound infection [40].

Laboratory markers such as serum albumin [41] and fasting blood glucose [42] were independent risk factors for surgical site infection [18]. In this study, the first postoperative albumin level was collected, which was associated with multi-drug-resistant bacterial infection. Albumin has anti-inflammatory and anti-apoptotic effects [43, 44], and active correction of perioperative hypoproteinemia may be beneficial. Postoperative stress hyperglycemia, not diabetes, was found as a risk factor of infection here. Hyperglycemia can lead to endothelial dysfunction [45], platelet aggregation and thrombosis [46]. A recent study [47] showed that glycemia on arrival in the emergency room was significantly higher in patients with surgical site infection within 1 year after open leg fractures surgery than in patients without infection, and was an independent risk factor for infection. Almost all guidelines recommend the prevention of hyperglycemia to prevent surgical site infections [29, 48]. Treatment of stress hyperglycemia is necessary throughout the treatment course, including in the emergency room and postoperative period. In addition, other scores and laboratory indicators may be used as early warning indicators to help early detection of postoperative wound infection, especially in patients at high risk of multi-drug resistant infection.

Due to the limitations of retrospective study, this study could not fully obtain more detailed details of post-traumatic infection, and did not conduct subgroup analysis of upper and lower limbs or deep and superficial soft tissue infections respectively. It is not possible to explore the resistance genes of multidrug-resistant bacteria and whether there is a synergistic effect when pathogens are co-infected. The specific details of antimicrobial use in all patients were not fully available in the risk factor analysis, and thus could not be used to adjust the risk factors for infection, however, the obtained bacterial distribution characteristics may provide some reference value for future prophylactic antimicrobial use programs. Prospective studies with larger sample sizes and more details of perioperative care are needed to identify potential risk factors.

Conclusion

Wound infection was common in patients with severe traumatic major limb mutilation after replantation, Gram-negative bacteria were the main pathogenic bacteria, and most of the pathogenic bacteria were multi-drug resistant, which need to be focused on. Ischemia time and wound contamination were associated with the increase of postoperative wound infection, including caused by multi-drug resistant. Positive correction of hypoproteinemia and control of stress hyperglycemia may be beneficial.

Supporting information

S1 Table. The severed part of 249 patients with replantation of severed limb. (DOCX)

S2 Table. Univariate logistic analysis of factors associated with postoperative wound infection.

(DOCX)

S3 Table. Univariate logistic analysis of factors associated with postoperative wound drugresistant bacterial infection. (DOCX)

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Author Contributions

Conceptualization: Ruixing Hou, Qiang Guo.

Formal analysis: Chang Gao, Haiyan Wang, Ye Gao.

Funding acquisition: Qiang Guo.

Investigation: Ye Gao, Shiqi Guo, Di Yin.

Methodology: Chang Gao, Jihui Ju, Keran Zhang.

Resources: Jihui Ju, Keran Zhang, Ruixing Hou.

Visualization: Chang Gao, Haiyan Wang, Jihui Ju.

Writing - original draft: Chang Gao, Haiyan Wang, Qiang Guo.

References

- 1. Battiston B, Tos P, Pontini I, Ferrero S. Lower limb replantations: Indications and a new scoring system. Microsurgery. 2002; 22(5):187–92. https://doi.org/10.1002/micr.22505 PMID: 12210963
- Harris AM, Althausen PL, Kellam J, Bosse MJ, Castillo R. Complications Following Limb-Threatening Lower Extremity Trauma. Journal of Orthopaedic Trauma. 2009; 23(1):1–6. <u>https://doi.org/10.1097/</u> BOT.0b013e31818e43dd PMID: 19104297
- Fufa D, Lin CH, Lin YT, Hsu CC, Chuang CC, Lin CH. Secondary Reconstructive Surgery following Major Upper Extremity Replantation. Plastic and Reconstructive Surgery. 2014; 134(4):713–20. <u>https://doi.org/10.1097/PRS.00000000000538</u> PMID: 24945950
- Toro G, Cecere AB, Braile A, Cicco AD, Liguori S, Tarantino U, et al. New insights in lower limb reconstruction strategies. Therapeutic Advances in Musculoskeletal Disease. 2023; 15. https://doi.org/10. 1177/1759720X231189008 PMID: 37529331

- Ukai T, Hamahashi K, Uchiyama Y, Kobayashi Y, Watanabe M. Retrospective analysis of risk factors for deep infection in lower limb Gustilo–Anderson type III fractures. Journal of Orthopaedics and Traumatology. 2020; 21(1). https://doi.org/10.1186/s10195-020-00549-5 PMID: 32683562
- Li J, Wang Q, Lu Y, Feng Q, He X, Li MDZ, et al. Relationship Between Time to Surgical Debridement and the Incidence of Infection in Patients with Open Tibial Fractures. Orthopaedic Surgery. 2020; 12 (2):524–32. https://doi.org/10.1111/os.12653 PMID: 32202051
- Suzuki T, Inui T, Sakai M, Ishii K, Kurozumi T, Watanabe Y. Type III Gustilo–Anderson open fracture does not justify routine prophylactic Gram-negative antibiotic coverage. Scientific Reports. 2023; 13 (1).
- Thakore RV, Francois EL, Nwosu SK, Attum B, Whiting PS, Siuta MA, et al. The Gustilo–Anderson classification system as predictor of nonunion and infection in open tibia fractures. European Journal of Trauma and Emergency Surgery. 2016; 43(5):651–6. <u>https://doi.org/10.1007/s00068-016-0725-y</u> PMID: 27658943
- Le J, Dong Z, Liang J, Zhang K, Li Y, Cheng M, et al. Surgical site infection following traumatic orthopaedic surgeries in geriatric patients: Incidence and prognostic risk factors. International Wound Journal. 2019; 17(1):206–13. https://doi.org/10.1111/iwj.13258 PMID: 31730274
- Lowen RM, Rodgers CM, Ketch LL, Phelps DB. Aeromonas hydrophila infection complicating digital replantation and revascularization. The Journal of Hand Surgery. 1989; 14(4):714–8. <u>https://doi.org/10.1016/0363-5023(89)90197-4 PMID: 2490149</u>
- Otchwemah R, Grams V, Tjardes T, Shafizadeh S, Bäthis H, Maegele M, et al. Bacterial contamination of open fractures–pathogens, antibiotic resistances and therapeutic regimes in four hospitals of the trauma network Cologne, Germany. Injury. 2015; 46:S104–S8. https://doi.org/10.1016/S0020-1383(15) 30027-9 PMID: 26542854
- Sagi HC, Cooper S, Donahue D, Marberry S, Steverson B. Seasonal variations in posttraumatic wound infections after open extremity fractures. Journal of Trauma and Acute Care Surgery. 2015; 79 (6):1073–8. https://doi.org/10.1097/TA.000000000000705 PMID: 26317814
- Keating JF, Blachut PA, O'Brien PJ, Court-Brown CM. Reamed nailing of Gustilo grade-IIIB tibial fractures. The Journal of bone and joint surgery British volume. 2000; 82(8):1113–6. https://doi.org/10. 1302/0301-620x.82b8.10566 PMID: 11132268
- Mody RM, Zapor M, Hartzell JD, Robben PM, Waterman P, Wood-Morris R, et al. Infectious complications of damage control orthopedics in war trauma. The Journal of trauma. 2009; 67(4):758–61. https://doi.org/10.1097/TA.0b013e3181af6aa6 PMID: 19820582
- van Duin D, Paterson DL. Multidrug-Resistant Bacteria in the Community. Infectious Disease Clinics of North America. 2020; 34(4):709–22.
- **16.** Woffenden H, Yasen Z, Burden E, Douthwaite A, Elcock SB, McLean L, et al. Fracture-related infection: Analysis of healthcare utilisation and associated costs. Injury. 2023; 54(12):111109. <u>https://doi.org/10.1016/j.injury.2023.111109</u> PMID: 37871348
- Zhang J, Lu V, Zhou AK, Stevenson A, Thahir A, Krkovic M. Predictors for infection severity for open tibial fractures: major trauma centre perspective. Archives of Orthopaedic and Trauma Surgery. 2023; 143 (11):6579–87. https://doi.org/10.1007/s00402-023-04956-1 PMID: 37418004
- Hu Q, Zhao Y, Sun B, Qi W, Shi P. Surgical site infection following operative treatment of open fracture: Incidence and prognostic risk factors. International Wound Journal. 2020; 17(3):708–15. <u>https://doi.org/10.1111/iwj.13330</u> PMID: 32068337
- Daigle JP, Kleinert JM. Major limb replantation in children. Microsurgery. 1991; 12(3):221–31. <u>https://</u> doi.org/10.1002/micr.1920120312 PMID: 1865816
- Larson JV, Kung TA, Cederna PS, Sears ED, Urbanchek MG, Langhals NB. Clinical factors associated with replantation after traumatic major upper extremity amputation. Plast Reconstr Surg. 2013; 132 (4):911–9. https://doi.org/10.1097/PRS.0b013e31829f4a49 PMID: 24076683
- Gao C, Yang L, Ju J, Gao Y, Zhang K, Wu M, et al. Risk and prognostic factors of replantation failure in patients with severe traumatic major limb mutilation. European Journal of Trauma and Emergency Surgery. 2022; 48(4):3203–10. https://doi.org/10.1007/s00068-021-01876-w PMID: 35050386
- Garner MR, Sethuraman SA, Schade MA, Boateng H. Antibiotic Prophylaxis in Open Fractures: Evidence, Evolving Issues, and Recommendations. Journal of the American Academy of Orthopaedic Surgeons. 2020; 28(8):309–15. https://doi.org/10.5435/JAAOS-D-18-00193 PMID: 31851021
- Dungan KM, Braithwaite SS, Preiser J-C. Stress hyperglycaemia. The Lancet. 2009; 373(9677):1798– 807. https://doi.org/10.1016/S0140-6736(09)60553-5 PMID: 19465235
- Metsemakers WJ, Morgenstern M, McNally MA, Moriarty TF, McFadyen I, Scarborough M, et al. Fracture-related infection: A consensus on definition from an international expert group. Injury. 2018; 49 (3):505–10. https://doi.org/10.1016/j.injury.2017.08.040 PMID: 28867644

- 25. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2012; 18(3):268–81. https://doi.org/10.1111/j.1469-0691.2011.03570.x PMID: 21793988
- 26. Hafez HM, Woolgar J, Robbs JV. Lower extremity arterial injury: results of 550 cases and review of risk factors associated with limb loss. J Vasc Surg. 2001; 33(6):1212–9. https://doi.org/10.1067/mva.2001. 113982 PMID: 11389420
- Levin LS. From replantation to transplantation: The evolution of orthoplastic extremity reconstruction. Journal of orthopaedic research: official publication of the Orthopaedic Research Society. 2023; 41 (7):1587–99. https://doi.org/10.1002/jor.25488 PMID: 36413095
- Poulakou G, Giamarellou H. Investigational treatments for postoperative surgical site infections. Expert Opinion on Investigational Drugs. 2007; 16(2):137–55. https://doi.org/10.1517/13543784.16.2.137 PMID: 17243935
- 29. Seidelman JL, Mantyh CR, Anderson DJ. Surgical Site Infection Prevention. Jama. 2023; 329(3).
- Zimlichman E, Henderson D, Tamir O, Franz C, Song P, Yamin CK, et al. Health care-associated infections: a meta-analysis of costs and financial impact on the US health care system. JAMA internal medicine. 2013; 173(22):2039–46. https://doi.org/10.1001/jamainternmed.2013.9763 PMID: 23999949
- Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, et al. Multistate point-prevalence survey of health care-associated infections. The New England journal of medicine. 2014; 370 (13):1198–208. https://doi.org/10.1056/NEJMoa1306801 PMID: 24670166
- Gantz O, Zagadailov P, Merchant AM. The Cost of Surgical Site Infections after Colorectal Surgery in the United States from 2001 to 2012: A Longitudinal Analysis. The American surgeon. 2019; 85(2):142– 9. PMID: 30819289
- Khatod M, Botte MJ, Hoyt DB, Meyer RS, Smith JM, Akeson WH. Outcomes in open tibia fractures: relationship between delay in treatment and infection. The Journal of trauma. 2003; 55(5):949–54. https://doi.org/10.1097/01.TA.0000092685.80435.63 PMID: 14608171
- Tennent DJ, Shiels SM, Jennings JA, Haggard WO, Wenke JC. Local control of polymicrobial infections via a dual antibiotic delivery system. J Orthop Surg Res. 2018; 13(1):53. <u>https://doi.org/10.1186/</u> s13018-018-0760-y PMID: 29544509
- 35. Kay HF, Sathiyakumar V, Yoneda ZT, Lee YM, Jahangir AA, Ehrenfeld JM, et al. The effects of American Society of Anesthesiologists physical status on length of stay and inpatient cost in the surgical treatment of isolated orthopaedic fractures. J Orthop Trauma. 2014; 28(7):e153–9. https://doi.org/10.1097/ 01.bot.0000437568.84322.cd PMID: 24149446
- Kurtz SM, Lau EC, Son MS, Chang ET, Zimmerli W, Parvizi J. Are We Winning or Losing the Battle With Periprosthetic Joint Infection: Trends in Periprosthetic Joint Infection and Mortality Risk for the Medicare Population. The Journal of arthroplasty. 2018; 33(10):3238–45. <u>https://doi.org/10.1016/j.arth.</u> 2018.05.042 PMID: 29914821
- Ma T, Lyu J, Ma J, Huang X, Chen K, Wang S, et al. Comparative analysis of pathogen distribution in patients with fracture-related infection and periprosthetic joint infection: a retrospective study. BMC Musculoskeletal Disorders. 2023; 24(1). https://doi.org/10.1186/s12891-023-06210-6 PMID: 36782133
- Hull PD, Johnson SC, Stephen DJG, Kreder HJ, Jenkinson RJ. Delayed debridement of severe open fractures is associated with a higher rate of deep infection. The Bone & Joint Journal. 2014; 96-B (3):379–84. https://doi.org/10.1302/0301-620X.96B3.32380 PMID: 24589795
- Li Q LW, Zhu L, Zheng X, Wu P, Zhong G, Huang X, et al. Expert consensus on early treatment of severe open limb trauma. Chinese Journal of Microsurgery. 2023; 46, No.1.
- Liang NL, Alarcon LH, Jeyabalan G, Avgerinos ED, Makaroun MS, Chaer RA. Contemporary outcomes of civilian lower extremity arterial trauma. Journal of vascular surgery. 2016; 64(3):731–6. <u>https://doi.org/10.1016/j.jvs.2016.04.052</u> PMID: 27444360
- Zhu Y, Liu S, Zhang X, Chen W, Zhang Y. Incidence and risks for surgical site infection after adult tibial plateau fractures treated by ORIF: a prospective multicentre study. Int Wound J. 2017; 14(6):982–8. https://doi.org/10.1111/iwj.12743 PMID: 28303656
- 42. Ma T, Lu K, Song L, Wang D, Ning S, Chen Z, et al. Modifiable Factors as Current Smoking, Hypoalbumin, and Elevated Fasting Blood Glucose Level Increased the SSI Risk Following Elderly Hip Fracture Surgery. Journal of investigative surgery: the official journal of the Academy of Surgical Research. 2020; 33(8):750–8. https://doi.org/10.1080/08941939.2018.1556364 PMID: 30885013
- **43.** Vincent JL, Russell JA, Jacob M, Martin G, Guidet B, Wernerman J, et al. Albumin administration in the acutely ill: what is new and where next? Critical care (London, England). 2014; 18(4):231. https://doi.org/10.1186/cc13991 PMID: 25042164

- Vincent JL. Relevance of albumin in modern critical care medicine. Best practice & research Clinical anaesthesiology. 2009; 23(2):183–91. https://doi.org/10.1016/j.bpa.2008.11.004 PMID: 19653438
- 45. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. Jama. 2006; 295(14):1681–7. https://doi.org/10.1001/jama.295.14.1681 PMID: 16609090
- 46. Gresele P, Guglielmini G, De Angelis M, Ciferri S, Ciofetta M, Falcinelli E, et al. Acute, short-term hyperglycemia enhances shear stress-induced platelet activation in patients with type II diabetes mellitus. Journal of the American College of Cardiology. 2003; 41(6):1013–20. https://doi.org/10.1016/s0735-1097(02)02972-8 PMID: 12651051
- 47. Cianni L, Caredda M, De Fazio A, Basilico M, Greco T, Cazzato G, et al. Stress-Induced Hyperglycemia is a Risk Factor for Surgical-Site Infections in Nondiabetic Patients with Open Leg Fractures. Advances in Orthopedics. 2023; 2023:1–8. https://doi.org/10.1155/2023/6695648 PMID: 37920443
- Calderwood MS, Anderson DJ, Bratzler DW, Dellinger EP, Garcia-Houchins S, Maragakis LL, et al. Strategies to prevent surgical site infections in acute-care hospitals: 2022 Update. Infection Control & Hospital Epidemiology. 2023; 44(5):695–720. https://doi.org/10.1017/ice.2023.67 PMID: 37137483