JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Impact of wound microbiology on limb preservation in patients with diabetic foot infection

Shih-Yuan Hung[1], Cheng-Hsun Chiu[2], Chung-Huei Huang[1], Cheng-Wei Lin[1], Jiun-Ting Yeh[3], Hui-Mei Yang[1], Yu-Yao Huang[1,4,5,*]

[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung Memorial Hospital, Taoyuan City, Taiwan, [2]Division of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan City, Taiwan, [3]Division of Trauma Plastic Surgery, Department of Plastic and Reconstructive Surgery, Chang Gung Memorial Hospital, Chang Gung University, Taoyuan City, Taiwan, [4]College of Medicine, Chang Gung University, Taoyuan City, Taiwan, and [5]Department of Medical Nutrition Therapy, Chang Gung Memorial Hospital, Taoyuan City, Taiwan

Keywords
Diabetic foot, Gram-negative bacteria, Lower-extremities amputation

***Correspondence**
Yu-Yao Huang
Tel.: +886-3-328-1200
Fax: +886-3-328-8257
E-mail address:
yyh@cgmh.org.tw

J Diabetes Investig 2022; 13: 336–343

doi: 10.1111/jdi.13649

ABSTRACT

Aims/Introduction:: To investigate the association between specific bacterial pathogens and treatment outcome in patients with limb-threatening diabetic foot infection (LT-DFI).

Materials and Methods:: Consecutive patients treated for LT-DFI in a major diabetic foot center in Taiwan were analyzed between the years 2014 and 2017. Patients with positive wound culture results at first aid were enrolled. Clinical factors, laboratory data, and wound culture results were compared. Lower-extremity amputations and in-hospital mortality were defined as a poor outcome.

Results:: Among the 558 patients, 272 (48.7%) patients had lower extremity amputation and 22 (3.9%) patients had in-hospital mortality. Gram-negative bacterial (GNB) infection was the independent factor following factors adjustment. When all the 31 microorganisms were analyzed, only *E. coli* (adjusted odds ratio [aOR], 3.01; 95% CI, 1.60–5.65), *Proteus* spp. (aOR, 2.99; 95% CI, 1.69–5.29), and *Pseudomonas aeruginosa* (aOR, 2.00; 95% CI 1.20–3.32) were associated with poor outcome. The analysis of specific GNB species in association with major- or minor- amputation have been reported. No specific pathogen was associated with cause of death in patients with mortality within 30 days. The antimicrobial-resistant strains were not associated with a poor treatment outcome.

Conclusions:: The presence of GNB was associated with limb amputations. This study provides insight into more timely and appropriate management of the diabetic foot infection.

INTRODUCTION

Diabetic foot ulcer (DFU) is a major complication of diabetes and contributes to most causes of non-traumatic lower-extremity amputation (LEA)[1–4]. Among factors such as perfusion, wound depth and width, infection and neuropathy that might affect wound severity, diabetic foot infection (DFI) is one of the major factors attributed to limb loss while treating DFU[5–8].

According to the Infectious Diseases Society of America (IDSA) and the International Working Group on Diabetic Foot (IWGDF) guidelines, DFI has been classified into four grades[9]. Patients with severe foot infection (grade 4) and those with moderate infection (grade 3) plus relevant morbidities are under limb-threatening DFI (LT-DFI) status and usually need interdisciplinary limb management[9,10]. In our recent study[7], major adverse cardiovascular event (MACE), poor peripheral circulation as well as the grade of DFI were associated with these poor prognoses (LEA and in-hospital mortality).

Previously, gram-positive aerobic bacteria have been reported to be the dominant pathogen in mild to moderate DFI by tissue curettage[11]. *Staphylococcus aureus* is common in patients with acute DFI, whereas gram-negative bacteria and obligatory anaerobic bacteria are more present in chronic foot infection[12]. *Pseudomonas aeruginosa* causes DFI more often in hot and

Received 1 December 2020; revised 26 July 2021; accepted 18 August 2021

humid areas[13]. However, to our best knowledge, the impact of microorganisms in patients with LT-DFI has not been well recognized. This study aimed to further analyze the bacteria in patients with LT-DFI (grades 3 and 4) in order to research the association between specific pathogens and treatment outcome.

METHODS

Data source and study population

This study used an anonymous public use data set without identifiable information about individuals in the study. It was approved by the Institutional Review Board of Chang Gung Memorial Hospital (No. NMRPG3K0391), and follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

From January 1, 2014 to December 31, 2017, 722 type 2 diabetic patients with LT-DFI (infection grade ≥ 3) were consecutively reviewed and treated in the diabetic foot care unit at Chang Gung Memorial Hospital, an interdisciplinary diabetic foot care center accredited by the International Diabetes Federation West-Pacific Region in Taiwan [14]. Every patient received wound culture on admission. Patients with a negative culture result (n = 164) were excluded, while 558 patients with documented culture results were enrolled in this study.

Wound recording

Wound classification was recorded as PEDIS describing the perfusion, extent size, depth/tissue loss, infection, and sensation of the wounds [15]. LT-DFI was defined by an infection score greater than 3. Patients with grade 3 infection presented with either erythema >2 cm around the wound or the structures deeper than skin and subcutaneous tissues were involved, but with no systemic inflammatory response syndrome (SIRS). The definition of SIRS was according to matching two or more of the four criteria including abnormal body temperature >38°C or <36°C; tachycardia with pulse >90 beat per minute; abnormal respiratory rate with >20 breaths per minute; and an abnormal leukocyte count >12,000 or <4,000 /cu mm.

The perfusion status was categorized into three grades, with grade 3 perfusion status representing critical limb ischemia as defined by the presence of gangrene or ulcers with an ankle pressure <70 mmHg[16], or monophasic wave form of distal segment of the posterior tibial artery and dorsalis pedis artery[17]. Adjunct angiography was performed for confirmation.

Wound culture

For clinically infected ulcers, we obtained specimens by cotton swabs (Transystem, COPAN, Italia) for both bacteria and fungus culture after necrotic tissue and surrounding callus debridement at first aid was performed to determine the causative pathogens. Ninety-five percent of specimens in this study were obtained from deep ulcers (penetrating to fascia, muscle, tendon, and/or bone). Culture media included chocolate agar, sheep blood agar, and thioglycolate broth at 37°C. Mycosel agar plates were also obtained and maintained at 25°C to enhance fungal growth. Positive microbial cultures were defined as growth of the same pathogen on two or more culture media with positive fungal culture defined on morphology. Parenteral broad-spectrum antibiotic therapy was initiated empirically for common gram-positive and gram-negative bacteria, and obligatory anaerobic pathogens with antibiotic regimen were adjusted based on both the clinical response to empirical therapy and sensitivity results. Cultures were repeated for patients who were not responding to appropriate therapy.

Antibiotics strategy and consensus of managements

Broad-spectrum antibiotics against gram-negative and anaerobic pathogens, including third-generation cephalosporin (43%), extended-spectrum penicillin (31% with aminopenicillins and 5% with ureidopenicillins), fluoroquinolones (6%), carbapenems (5%), and metronidazole (19%) were prescribed promptly for these patients initially. Glycopeptide against methicillin-resistant *Staphylococcus aureus* (MRSA) was also prescribed in 26% of patients. Empiric antibiotics were subsequently modified according to the results of cultures. Surgical interventions, endovascular treatments, and foot amputations were scheduled in a timely manner after the diabetic foot team reached consensus.

Outcomes definitions

Those patients receiving minor (below the ankle) or major (above the ankle) amputations or expiring during the hospital treatment course were defined as poor outcomes.

Since various causes of death including nosocomial infection of patients with DFU were noted for a longer stay of hospitalization[14], only those patients who died within 30 days of hospitalization were analyzed to find the association between pathogens and mortality.

Statistical analysis

Clinical demographics, associated comorbidities (such as hypertension, history of MACE, and dialysis), and factors of PEDIS wound-grading were recorded from the patient's first visit at admission, with laboratory data of routine hematology tests and chemistry profile at enrolment being analyzed. Categorical variables were reported as numbers with percentages, and continuous variables were reported as means and standard deviations. Comparisons between patients with preserved limbs or poor outcome were performed using the Mann–Whitney test for continuous variables and Pearson's chi-square test for categorical variables. Each factor odds ratio to poor outcome was calculated via an adjusted model of logistic regression, while the same statistical method was used in comparing the two groups with different treatment outcomes. The significant risk factors in the univariate analysis found above were then entered into a multivariate logistic regression model to identify independent risk factors to adverse outcome among these patients. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for Mac, version 26.0, IBM Corp., Armonk, NY, USA) data analysis software.

RESULTS

Baseline characteristics and clinical factors associated with poor outcome

Among the 558 patients with LT-DFI, 272 (48.7%) patients had LEA (217 and 55 patients have minor and major LEA respectively) and 22 (3.9%) patients had in-hospital mortality. The comparison of baseline characteristics, PEDIS classification, laboratory data, and wound culture between the two groups is shown in Table 1. Patients prone to a poor outcome had a higher incidence of MACE (42.2% vs 22.7%, $P < 0.001$) and end-stage renal disease (ESRD) (28.6% vs 11.0%, $P < 0.001$). A worse glomerular filtration rate was noted in the poor outcome group (48.5 ± 40.9 vs 58.7 ± 51.0 ml/min/1.73 m², $P = 0.002$). There were no statistical differences in age, gender, and diabetes duration.

Compared with patients with preserved limbs, those with poor outcomes had worse perfusion (63.9% vs 22.0% of grade 3 perfusion, $P < 0.001$), larger wound size (40.3 cm^2 vs 33.4 cm^2, $P < 0.001$), deeper wound (95.2% vs 54.5% of grade 3 depth, $P < 0.001$), more severe infection (40.1% vs 27.7% of grade 4 infection, $P = 0.001$), and worse sensation (62.2% vs 22.7% of grade 2 sensation, $P < 0.001$). The incidence of osteomyelitis was higher in patients with poor outcome (51.4% vs 40.0%, $P = 0.017$).

A higher leukocyte count (15.2 vs 12.3 10^3/mL, $P < 0.001$) and c-reactive protein level (131.3 ± 99.9 vs 90.3 ± 89.3mg/dL, $P < 0.001$) were both found in the poor outcome group, indicating the severity of infection status, while lower hemoglobin, serum albumin, and high-density lipoprotein were also revealed. There were no statistical differences in HbA1c. Of note, higher incidences of gram-negative and obligatory anaerobic bacteria were documented in the poor outcome group.

Table 1| | Demographics, wound grading and associated bacteria in patients with limb-threatening diabetic foot infection

	Limb preserved (n = 264)	Poor outcome[a] (n = 294)	P value
Baseline characteristics			
Age (years)	62.9 ± 14.0	63.5 ± 12.1	0.540
Male (%)	60.2	65.0	0.143
Diabetes duration (years)	13.7 ± 9.2	14.6 ± 10.0	0.400
Hypertension (%)	68.9	69.7	0.456
Major adverse cardiovascular disease (%)	22.7	42.2	<0.001
Coronary artery disease (%)	15.5	31.0	<0.001
Stroke (%)	9.5	16.0	0.015
ESRD (%)	11.0	28.6	<0.001
eGFR (mL/min/1.73 m²)	58.7 ± 51.0	48.5 ± 40.9	0.002
Wound assessment			
Wound duration (days)	37.2 ± 47.5	44.2 ± 55.0	0.106
Perfusion, Grade 3 (%)	22.0	63.9	<0.001
Toe involvement (%)	82.4	91.8	0.545
Extension (cm^2)	33.4 ± 109.9	40.3 ± 75.4	<0.001
Depth, Grade 3 (%)	54.5	95.2	<0.001
Infection, Grade 4 (%)	27.7	40.1	0.001
Sensation, Grade 2 (%)	22.7	62.2	<0.001
Laboratory data			
WBC (10^3/mL)	12.3 ± 6.0	15.2 ± 7.0	<0.001
CRP (mg/dL)	90.3 ± 89.3	131.3 ± 99.9	<0.001
Hemoglobin (mg/dL)	11.2 ± 2.2	10.7 ± 4.3	<0.001
HbA1c (%)	9.1 ± 2.7	9.1 ± 2.5	0.482
Serum albumin (mg/dL)	3.2 ± 0.6	3.0 ± 0.5	0.002
LDL (mmol/L)	84.7 ± 34.4	83.4 ± 33.5	0.699
HDL (mmol/L)	31.2 ± 9.5	29.5 ± 12.3	0.033
Osteomyelitis (%)	42.0	51.4	0.017
Wound culture[b]			
Gram-positive bacteria (%)	84.5	78.9	0.057
Gram-negative bacteria (%)	52.3	73.8	<0.001
Anaerobic bacteria (%)	40.2	57.8	<0.001
Wound Management			
Endovascular therapy (%)	48.3	44.7	0.370
NPWT (%)	2.0	4.1	0.485
HBO (%)	5.9	6.1	0.642

ESRD, end stage renal disease; eGFR, estimated glomerular filtration rate; WBC, leukocyte count; CRP, C-reactive protein; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein; HDL, high-density lipoprotein; NPWT, negative pressure wound therapy; HBO, hyperbaric oxygen therapy. [a]Including minor amputation, major amputation, and mortality. [b]Bacterial infection was defined as the isolation of one or more than one type of bacteria from a wound culture.

Microorganisms associated with poor outcome

The most common pathogens in gram-positive strains are *Streptococcus* spp. (25.8%), methicillin-sensitive *Staphylococcus aureus* (21.7%), and methicillin-resistant *Staphylococcus aureus* (12.5%). The most common gram-negative pathogens are *Proteus* spp. (16.3%), *E. coli* (11.1%), and *Pseudomonas* spp. (8.8%). The most common anaerobic pathogens are *Peptostreptococcus* spp. (22.8%) and *Bacteroides* spp. (21.1%). When putting baseline characteristics with statistical significance into the multivariate logistic regression model, gram-negative aerobic bacteria (odds ratio 2.59, 95% confidence interval 1.77–3.79) and obligatory anaerobic bacteria (OR 2.28, 95% CI 1.58–3.29) infection could predict poor outcome. We further adjusted wound assessment, laboratory data with statistical significance, and the incidence of osteomyelitis, but gram-negative bacteria remained the independent factor for poor outcome in these groups (Table 2).

Forest plot analysis and unadjusted odds ratio of specific pathogens revealed poor outcome in patients with LT-DFI as demonstrated in Figure 1. *E. coli* (OR: 3.01, 95% CI 1.60–5.65), *Proteus* spp. (OR: 2.99, 95% CI 1.69–5.29), and *Pseudomonas aeruginosa* (OR: 2.00, 95% CI 1.20–3.32) had a more predictive value of poor outcome among all bacteria.

Table 2| | Gram negative bacteria as an independent factor for poor outcomes following various multivariate multinomial logistic regression analyses

Variables	Odds Ratio (95% CI)			
	a	b	c	d
Gram-positive bacteria	0.83 (0.52–1.33)	0.94 (0.53–1.68)	1.08 (0.50–2.32)	1.30 (0.57–2.96)
Gram-negative bacteria	2.59 (1.77–3.79)*	1.71 (1.08–2.69)*	2.20 (1.18–4.10)*	**2.06 (1.06–3.99)***
Anaerobic bacteria	2.28 (1.58–3.29)*	1.52 (0.98–2.37)	1.73 (0.94–3.19)	1.49 (0.78–2.86)

a: Adjusted for MACE, ESRD, and eGFR. b: Adjusted for MACE, ESRD, eGFR, and PEDIS classification. c: Adjusted for MACE, ESRD, eGFR, PEDIS classification, WBC, C-reactive protein, hemoglobin, and HDL. d: Adjusted for MACE, ESRD, eGFR, PEDIS classification, WBC, C-reactive protein, hemoglobin, HDL, and osteomyelitis. *Significance: P value < 0.05.

Gram-negative pathogens associated with the risk for individual component of poor outcome

Because the presence of gram-negative bacteria was the most predictive factor of poor outcome after multivariate logistic regression analysis, the specific gram-negative pathogens associated with each treatment outcome were further analyzed (Figure 2). *Proteus* spp. (OR 3.19, 95% CI 1.89–5.39, $P < 0.001$) and *E. coli* (OR 2.55, 95% CI 1.35–4.83, $P = 0.004$) were associated with minor amputation, whereas *Klebsiella* spp. (OR 4.10, 95% CI 1.47–11.44, $P = 0.007$), *E. coli* (OR 5.13, 95% CI 2.17–12.09, $P < 0.001$), and *Morganella morganii* (OR 3.28, 95% CI 1.32–8.18, $P = 0.011$) were associated with major amputation. There was no specific pathogen with clinical significance in patients with in-hospital mortality within 30 days. After adjustment of baseline characteristics and laboratory data with statistical significance and wound assessment, *Proteus* spp. and *Klebsiella* spp. remained statistically significant in minor and major amputations, respectively.

Prevalence of antimicrobial resistance strains

In order to investigate the role of antimicrobial-resistant strains, the cultured results of the causative pathogens in poor treatment outcome were further analyzed (Table 3). Ceftriaxone- or ceftazidime-resistant strains accounted for 2.7% (1/36) in *Klebsiella* spp., while low percentages of carbapenem-resistant strains (5.6% and 1.6% in *Klebsiella* spp. and *E. coli*, respectively) were found.

DISCUSSION

In addition to the well-known factors of poor outcome (e.g. MACE, ESRD, PAD, large wound size, deep wound, impaired sensation, severe infection, and the presence of osteomyelitis), our results demonstrated the association between gram-negative bacteria and poor in-hospital treatment outcome. After multivariate multinomial logistic regression analyses of these clinical factors with statistical significance, gram-negative bacteria remained an independent factor for poor outcome in our referral center. Among these, *Proteus* spp. and *Klebsiella* spp. could predict minor and major amputations, respectively, even with low percentages of antimicrobial resistance strain.

DFI is a heterogenous group of infection in a diverse patient population[18], with the causative pathogens of DFI varying by geographic, demographic, and clinical situations. Gram-positive bacteria, especially *S. aureus*, are reported to be the predominant and virulent pathogens in mild or moderate DFI[11,19]; however, the incidence of gram-negative bacteria infection has become more common in warmer climates[20] and chronic wounds[21]. Compared with gram-positive infection, gram-negative bacteria induce a greater magnitude of inflammatory response[22], leading to LEA[23] and high in-hospital mortality[24]. In the diabetic foot, gram-negative bacterial infection has been reported to induce ischemic tissue necrosis and thereby progressive infection and poor outcome[25,26]. Thus, infections with gram-negative bacteria might generally require more frequent surgical debridement, endovascular therapy, and longer hospitalization than those with gram-positive bacteria.

The predominance of the *Enterobacteriaceae* family (*E. coli*, *Klebsiella* spp., *Morganella morganii*, and *Proteus* spp.) has recently been reported as the largest group of aerobic gram-negative bacteria in DFI[27]. Extraintestinal pathogenic *E. coli* is well-known for its virulence potential to invade host tissue and to be transmitted via the bloodstream[28]. *Klebsiella* spp. can cause severe infection with a high mortality rate[29], is a common pathogen of pyogenic liver abscess[30], and potentially fatal necrotizing fasciitis in Taiwan[31]. *Proteus* spp. exhibits a characteristic swarming activity, which enables colonization, tissue invasion, and biofilm formation in chronic wounds[32], and is one of the most common pathogens of osteomyelitis[33], and diabetic patients with osteomyelitis are prone to limb loss if this is accompanied by exposed bone, the presence of ischemia, and necrotizing soft tissue infection[34]. Major LEA is indicated in these patients when accompanied by severe sepsis, extensive tissue loss, and poor-healing wound(s). This study demonstrates the importance of specific pathogens in the *Enterobacteriaceae* family and their role in amputations and in-hospital mortality.

In the past few years, antibiotic-resistant pathogens, particularly MRSA and extended-spectrum β-lactamase- (ESBL) or carbapenemase-producing gram-negative bacteria have become a major problem in treating DFI[35]. DFIs caused by MRSA have been thought to have worse outcomes; however, a recent review

found that they did not differ from those of other pathogens[36]. Similarly, Henig *et al.* reported that there was no association between MRSA and poor outcome, but there was a trend toward a significantly higher likelihood of having recurrent DFI within 1 year[37]. In our study, ceftriaxone- or ceftazidime-resistant strains were low in *Klebsiella spp*. and *E. coli,* indicating that a poor outcome was not associated with antibiotic-resistant strains.

The main limitation of this study is its retrospective nature. In addition, wound culture samples in patients with LT-DFI were obtained by swab, the most common real-world practice. Previous broad-spectrum antibiotic treatment before patient referral might influence the culture result on admission. Although new advances obtained from DNA- and RNA-based techniques for bacterial identification could improve therapeutic approaches[27], gram-negative bacteria (especially *Enterobacteriaceae* group) have

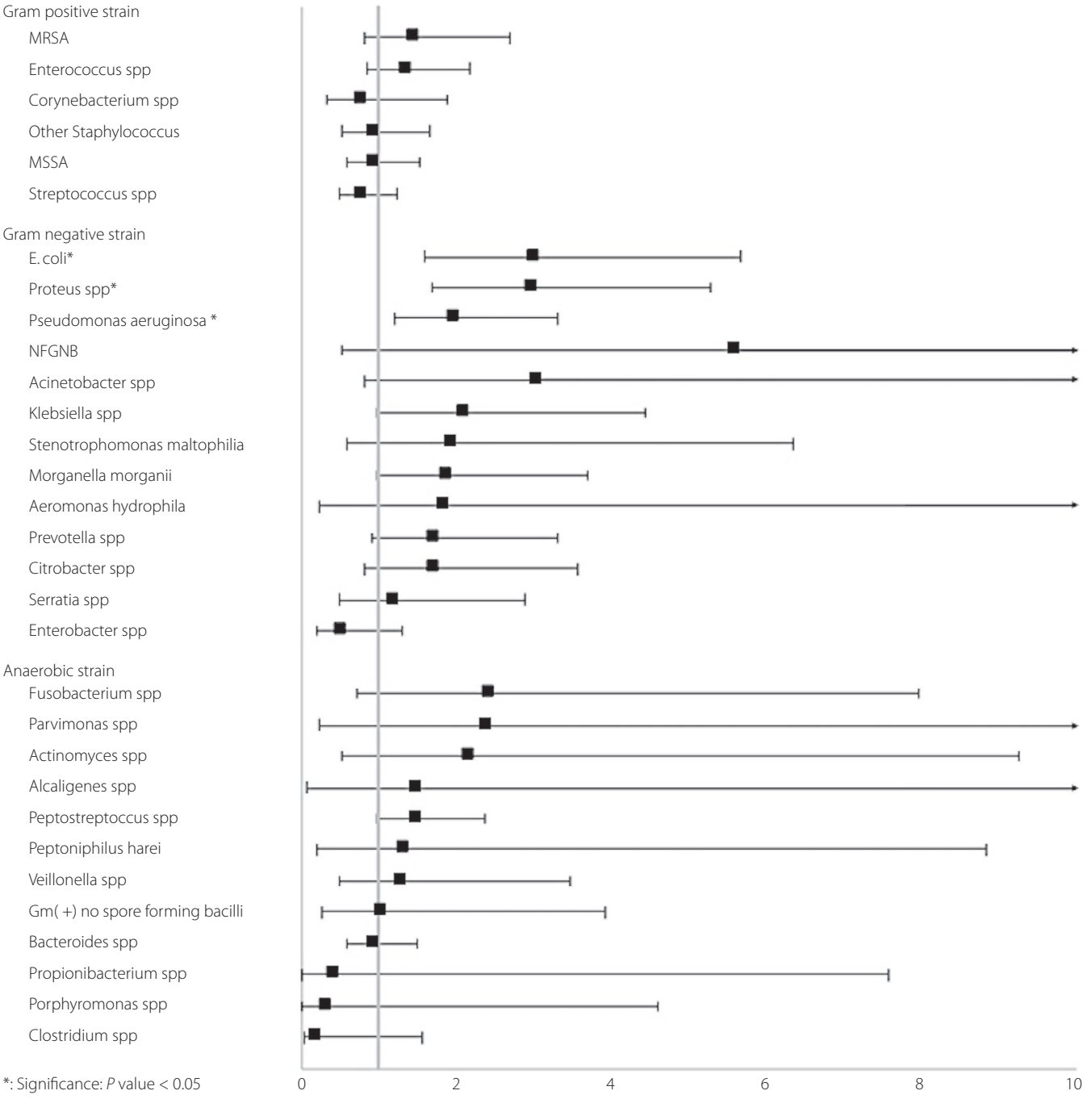
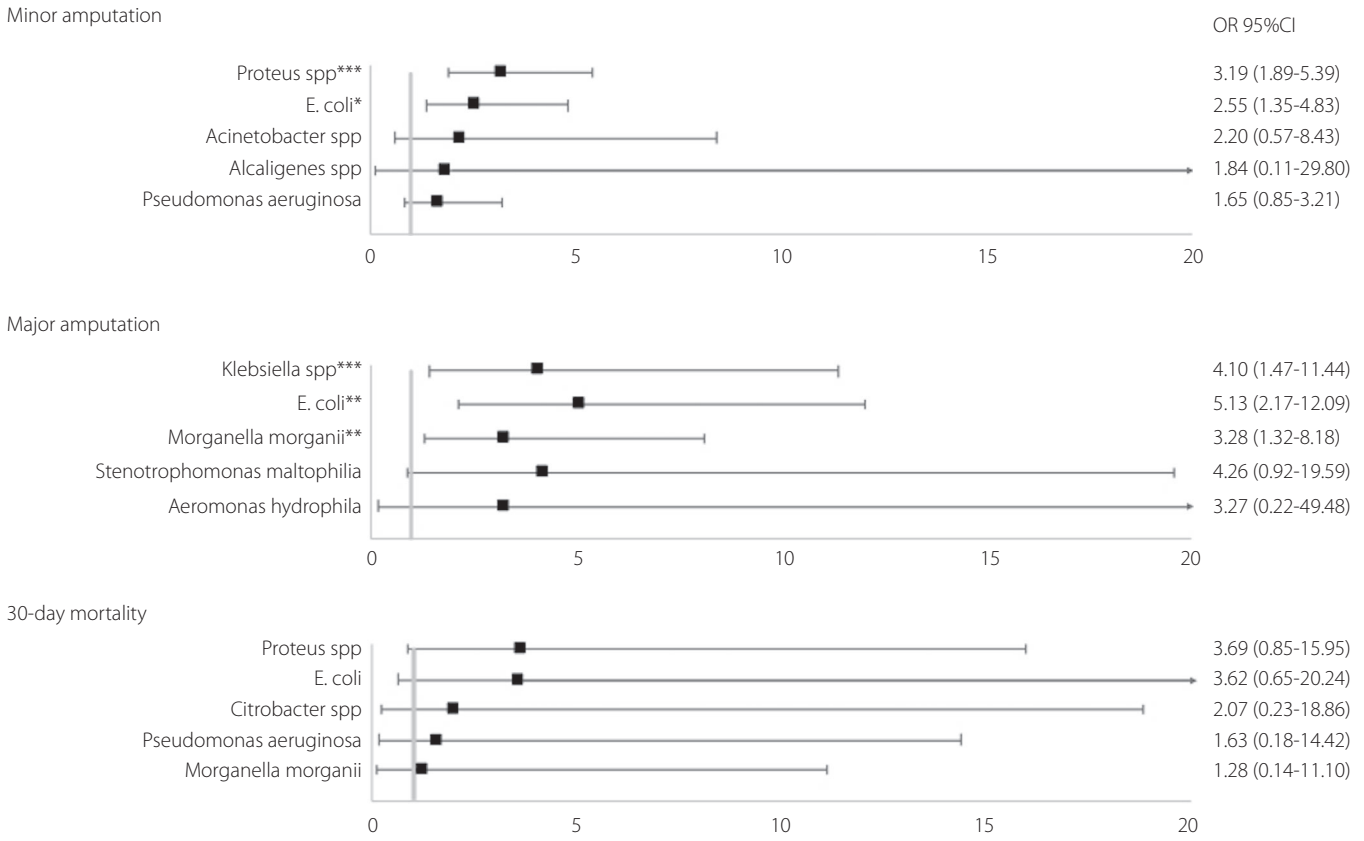


Figure 1| | The odds ratios of poor outcome in individual microorganisms.



*: Significance: *P* value < 0.05
**: Significance: *P* value < 0.05, after adjust for MACE, ESRD, and eGFR
***: Significance: *P* value < 0.05, after adjust for MACE, ESRD, eGFR, Grade 3 perfusion, Extension, Grade 3 depth, Grade 4 infection, and Grade 2 sensation
Factors for this multivariate logistic regression were determined by significant factors in univariate analysis in Table 1.

Figure 2| | The five most common microorganisms associated with each individual component of poor outcome.

Table 3| | Antimicrobial resistance rate of microorganisms and its association with poor outcomes

	Patient number	Minor LEA	Major LEA	Mortality
***Klebsiella* spp.**	36	13	7	2
Carbapenem-resistant strains	2	1	-	-
Ceftriaxone- or ceftazidime-resistant strains	1	-	1	-
E. coli	62	28	12	3
Carbapenem-resistant strains	1	0	0	0
Ceftriaxone- or ceftazidime-resistant strains	0	-	-	-

still shown their importance by different culturing methodology[21] and identification technique[38]. A larger sample investigation is needed to clarify the microbiological impact.

CONCLUSION

This study demonstrated that the presence of gram-negative bacteria ought to raise awareness in clinicians treating patients with LT-DFI. Further evaluation of these specific species might provide further insight for the management of DFI.

ACKNOWLEDGMENTS

None

FUNDING SOURCES

The study was supported by project grant from the Ministry of Science and Technology, Taiwan (MOST 109-2314-B-182A-128).

DISCLOSURE

The author declares no conflict of interest.

Approval of the research protocol: N/A.
Informed Consent: N/A.
Approval date of Registry and the Registration No. of the study/trial: N/A.
Animal Studies: N/A.

REFERENCES

1. Lin CW, Armstrong DG, Lin CH, *et al*. Nationwide trends in the epidemiology of diabetic foot complications and lower-extremity amputation over an 8-year period. *BMJ Open Diabetes Res Care* 2019; 7: e000795.
2. Huang YY, Lin CW, Yang HM, *et al*. Survival and associated risk factors in patients with diabetes and amputations caused by infectious foot gangrene. *J Foot Ankle Res* 2018; 11: 1.
3. Zhang Y, Lazzarini PA, McPhail SM, *et al*. Global disability burdens of diabetes-related lower-extremity complications in 1990 and 2016. *Diabetes Care* 2020; 43: 964–974.
4. Lipsky BA, Apelqvist J, Bakker K, *et al*. Diabetic foot disease: moving from roadmap to journey. *Lancet Diabetes Endocrinol* 2015; 3: 674–675.
5. Monteiro-Soares M, Boyko EJ, Jeffcoate W, *et al*. Diabetic foot ulcer classifications: a critical review. *Diabetes Metab Res Rev* 2020; 36(Suppl 1): e3272.
6. Beckman JA, Duncan MS, Damrauer SM, *et al*. Microvascular disease, peripheral artery disease, and amputation. *Circulation* 2019; 140: 449–458.
7. Lin CW, Hung SY, Huang CH, *et al*. Diabetic foot infection presenting systemic inflammatory response syndrome: a unique disorder of systemic reaction from infection of the most distal body. *J Clin Med* 2019; 8: 1538.
8. Lavery LA, Armstrong DG, Wunderlich RP, *et al*. Risk factors for foot infections in individuals with diabetes. *Diabetes Care* 2006; 29: 1288–1293.
9. Lipsky BA, Senneville E, Abbas ZG, *et al*. Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). *Diabetes Metab Res Rev* 2020; 36 (Suppl 1): e3280.
10. Lipsky BA, Weigelt JA, Sun X, *et al*. Developing and validating a risk score for lower-extremity amputation in patients hospitalized for a diabetic foot infection. *Diabetes Care* 2011; 34: 1695–1700.
11. Ge Y, MacDonald D, Hait H, *et al*. Microbiological profile of infected diabetic foot ulcers. *Diabet Med* 2002; 19: 1032–1034.
12. Lipsky BA, Berendt AR, Cornia PB, *et al*. Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 2012; 2012: e132–173.
13. Bansal E, Garg A, Bhatia S, *et al*. Spectrum of microbial flora in diabetic foot ulcers. *Indian J Pathol Microbiol* 2008; 51: 204–208.
14. Hung S-Y, Huang Y-Y, Hsu L-A, *et al*. Treatment for diabetic foot ulcers complicated by major cardiac events. *Can J Diabetes* 2015; 39: 183–187.
15. Schaper NC. Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. *Diabetes Metab Res Rev* 2004; 20(Suppl 1): S90–95.
16. Uccioli L, Meloni M, Izzo V, *et al*. Critical limb ischemia: current challenges and future prospects. *Vasc Health Risk Manag* 2018; 14: 63–74.
17. Tsai CY, Chu SY, Wen YW, *et al*. The value of Doppler waveform analysis in predicting major lower extremity amputation among dialysis patients treated for diabetic foot ulcers. *Diabetes Res Clin Pract* 2013; 100: 181–188.
18. Uckay I, Aragon-Sanchez J, Lew D, *et al*. Diabetic foot infections: what have we learned in the last 30 years? *Int J Infect Dis* 2015; 40: 81–91.
19. Lipsky BA, Berendt AR, Deery HG, *et al*. Diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 2004; 39: 885–910.
20. Schwab F, Gastmeier P, Meyer E. The warmer the weather, the more gram-negative bacteria – impact of temperature on clinical isolates in intensive care units. *PLoS One* 2014; 9: e91105.
21. Citron DM, Goldstein EJ, Merriam CV, *et al*. Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. *J Clin Microbiol* 2007; 45: 2819–2828.
22. Abe R, Oda S, Sadahiro T, *et al*. Gram-negative bacteremia induces greater magnitude of inflammatory response than Gram-positive bacteremia. *Crit Care* 2010; 14: R27.
23. Barshes NR, Mindru C, Ashong C, *et al*. Treatment failure and leg amputation among patients with foot osteomyelitis. *Int J Low Extrem Wounds* 2016; 15: 303–312.
24. Gaynes R, Edwards JR. National Nosocomial Infections Surveillance System. Overview of nosocomial infections caused by gram-negative bacilli. *Clin Infect Dis* 2005; 41: 848–854.
25. Maharaj D, Bahadursingh S, Shah D, *et al*. Sepsis and the scalpel: anatomic compartments and the diabetic foot. *Vasc Endovascular Surg* 2005; 39: 421–423.
26. Benavent E, Murillo O, Grau I, *et al*. The impact of gram-negative bacilli in bacteremic skin and soft tissue infections among patients with diabetes. *Diabetes Care* 2019; 42: e110–e112.
27. Sadeghpour Heravi F, Zakrzewski M, Vickery K, *et al*. Bacterial diversity of diabetic foot ulcers: current status and future prospectives. *J Clin Med* 2019; 8: 1935.
28. Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, *et al*. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. *Gut Pathog* 2019; 11: 10.
29. Huang CH, Tsai JS, Chen IW, *et al*. Risk factors for in-hospital mortality in patients with type 2 diabetes complicated by community-acquired *Klebsiella pneumoniae* bacteremia. *J Formos Med Assoc* 2015; 114: 916–922.
30. Huang CH, Chiu CH, Chen IW, *et al*. Antimicrobial resistance and outcomes of community-onset bacterial bloodstream

infections in patients with type 2 diabetes. *J Glob Antimicrob Resist* 2018; 15: 271–276.
31. Cheng NC, Yu YC, Tai HC, *et al*. Recent trend of necrotizing fasciitis in Taiwan: focus on monomicrobial *Klebsiella pneumoniae* necrotizing fasciitis. *Clin Infect Dis* 2012; 55: 930–939.
32. Allison C, Emody L, Coleman N, *et al*. The role of swarm cell differentiation and multicellular migration in the uropathogenicity of *Proteus mirabilis*. *J Infect Dis* 1994; 169: 1155–1158.
33. Lipsky BA, Berendt AR, Deery HG, *et al*. Infectious Diseases Society of America. Diagnosis and treatment of diabetic foot infections. *Plast Reconstr Surg* 2006; 117: 212S–238S.
34. Aragon-Sanchez FJ, Cabrera-Galvan JJ, Quintana-Marrero Y, *et al*. Outcomes of surgical treatment of diabetic foot osteomyelitis: a series of 185 patients with histopathological confirmation of bone involvement. *Diabetologia* 2008; 51: 1962–1970.
35. Kwon KT, Armstrong DG. Microbiology and antimicrobial therapy for diabetic foot infections. *Infect Chemother* 2018; 50: 11–20.
36. Zenelaj B, Bouvet C, Lipsky BA, *et al*. Do diabetic foot infections with methicillin-resistant *Staphylococcus aureus* differ from those with other pathogens? *Int J Low Extrem Wounds* 2014; 13: 263–272.
37. Henig O, Pogue JM, Martin E, *et al*. The impact of multidrug-resistant organisms on outcomes in patients with diabetic foot infections. *Open Forum Infect Dis* 2020; 7: ofaa161.
38. van Asten SA, La Fontaine J, Peters EJ, *et al*. The microbiome of diabetic foot osteomyelitis. *Eur J Clin Microbiol Infect Dis* 2016; 35: 293–298.