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ORIGINAL RESEARCH

Cigarette Smoking and Peripheral Vascular Disease are Associated with Increasing Risk of ESKAPE Pathogen Infection in Diabetic Foot Ulcers

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Objective: Diabetic foot ulcers (DFUs) and ESKAPE pathogens have attracted attention globally, but the role of ESKAPE pathogens in diabetic foot infection is not well described. The purpose of this study was to evaluate the clinical features, antimicrobial resistance, and risk factors for ESKAPE infection in patients with DFUs.

Methods: A retrospective study was conducted on 180 patients with diabetic foot infection admitted to The Affiliated Hospital of Southwest Medical University (Luzhou, China), from January 2017 to April 2021. Antimicrobial susceptibilities of all isolates were determined. Multivariate logistic regression analysis was performed to analyze the independent risk factors for ESKAPE infection, multidrug-resistant (MDR)-ESKAPE infection, MDR-pathogen infection, and severe group in patients with DFUs.

Results: A total of 206 isolates were collected, of which 42.2% were ESKAPE pathogens. The independent risk factors for ESKAPE infection were cigarette smoking (OR = 1.958; 95% CI, 1.015–3.777) and peripheral vascular disease (OR = 2.096; 95% CI, 1.100–3.992), while alcohol consumption (OR = 2.172; 95% CI, 1.104–4.272) was the independent risk factor for MDR-pathogen infection. Additionally, the independent risk factors for severe DFU group were invasive treatment (OR = 326.642; 95% CI, 76.644–1392.08), the duration of systemic antibiotic treatment (OR = 0.918; 95% CI, 0.849–0.992), and length of hospital stay (OR = 1.145; 95% CI, 1.043–1.256). No independent risk factors for MDR-ESKAPE infection were found.

Conclusion: Our data established the microbiological features of ESKAPE pathogens and clinical manifestations of diabetic foot infection, and provide support for monitoring and management of ESKAPE infection in patients with DFUs in southwest China.

Keywords: diabetic foot ulcers, ESKAPE pathogens, infection, antimicrobial resistance, risk factors

Introduction

A recent study of adults between the ages of 20 and 79 worldwide suggested that there was a total of 537 million patients with diabetes in 2021, and the number is expected to increase to 643 million and 784 million by 2030 and 2045.[1] Diabetic foot ulcers (DFUs) are caused mainly by a combination of the following three factors: an extrinsic mechanical factor, such as high plantar pressures or local trauma; an intrinsic factor, such as peripheral neuropathy or micro-vascular disease; impaired host immune response.[2] At present, DFUs have become one of the most common, expensive, and serious complications of diabetes.[3] In China, approximately 25% hospitalization among patients with diabetes are related to infection or ischemic diabetic foot, of which infection is more common. Diabetic foot infections can manifest as local signs of inflammation, including erythema, swelling, increased skin temperature, pain, and purulent discharge formation, and also display systemic inflammatory symptoms, such as fever, hemodynamic instability, and metabolic disorders.[4,5] Patients with DFUs not only have a high amputation rate, accounting for 60% of non-traumatic amputees,[6] but their risk of death is 2.5 times higher than that of patients without DFUs,[6,7] which places a huge psychological and financial burden

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on patients, their families, and public health system. Currently, the main treatment strategies for diabetic foot infections are wound care, antibiotics, and amputation.[8] However, their limitations result in unsatisfactory treatment outcomes. Moreover, no empirical antimicrobial therapy has been shown to be effective in the treatment of diabetic foot infection.[9] Therefore, strengthening early diagnosis is an essential part of the effective treatment of DFUs.

Diabetic foot infections are caused by a myriad of microorganisms, but *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp., which are particularly prevalent nosocomial pathogens and are often referred to by the mnemonic acronym "ESKAPE" originally defined by the Infectious Diseases Society of America.[10] *S. aureus* is the most common pathogen isolated from DFUs globally, and *K. pneumoniae and P. aeruginosa* are the most frequent isolates among Gram-negative bacteria other than *Escherichia coli*. Moreover, chronic infection and relapse are tightly associated with *Enterococcus* and *Acinetobacter*.[11–14] The clinical treatment of ESKAPE pathogens is receiving increasing attention in recent years, not only due to the high pathogenicity, but also the rapid dissemination of antimicrobial resistance.[15] In February 2017, the World Health Organization released a list of pathogens for which there is an urgent need to develop new antibiotics to treat them. The ESKAPE group was selected as the "priority state".[16]

Although many studies have reported the risk factors of diabetic foot infection,[17,18] the characteristics of patients with diabetic foot infection caused by specific ESKAPE pathogens are still unknown. Therefore, the purpose of this study is to investigate the clinical manifestations, antimicrobial resistance, and risk factors for ESKAPE infection in patients with DFUs in southwest China.

Materials and Methods

Study Design

The retrospective data of all patients hospitalized with DFUs from January 2017 to April 2021 in The Affiliated Hospital of Southwest Medical University (Luzhou, China) were obtained and used in this study. A total of 180 patients were included in this study and were selected according to the following criteria: (1) met the clinical diagnostic criteria of DFUs; (2) had at least one hospitalization record from January 2017 to April 2021; (3) had complete clinical data. The exclusion criteria were the following: (1) missing wound culture during hospitalization; (2) no screening for diabetic complications; (3) incomplete or omitted medical record information. The list of patients included in the study is shown in Figure 1.

Data Collection and Definitions

The information of all qualified patients with DFUs was collected from medical records, including basic demographic data (age, sex, duration of diabetes, length of hospital stay, etc.), characteristics of DFUs (ulcer area, depth, ischemic necrosis, etc.), underlying diseases (hypertension, hyperlipidemia, coronary heart disease, etc.), clinical treatment (blood glucose control, foot wound care, toe resection, surgery, antimicrobial therapy, etc.), and laboratory results (white blood cell count, neutrophil ratio, etc.).

Identification and Antimicrobial Susceptibility Testing

Identification and antimicrobial susceptibility testing of all clinical isolates that were collected from patients with DFUs were performed using the MicroScan Walkaway 96 Plus system (Beckman Coulter, Brea, CA, USA) and the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) Biotyper Microflex LT system (Bruker Daltonics, Fremont, CA, USA). Gram-positive bacteria were tested for susceptibility to 14 antimicrobial agents, including penicillin, ampicillin, oxacillin, ciprofloxacin, levofloxacin, daptomycin, gentamicin, erythromycin, tetracycline, nitrofurantoin, vancomycin, rifampicin, quinopristine-dalfopristin, and linezolid. In addition, 16 antimicrobial agents, namely imipenem, meropenem, gentamicin, tobramycin, amikacin, piperacillin-tazobactam, ampicillin-sulbactam, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefepime, aztreonam, ciprofloxacin, levofloxacin, and cotrimoxazole, were selected to detect gram-negative bacteria. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI-2020).

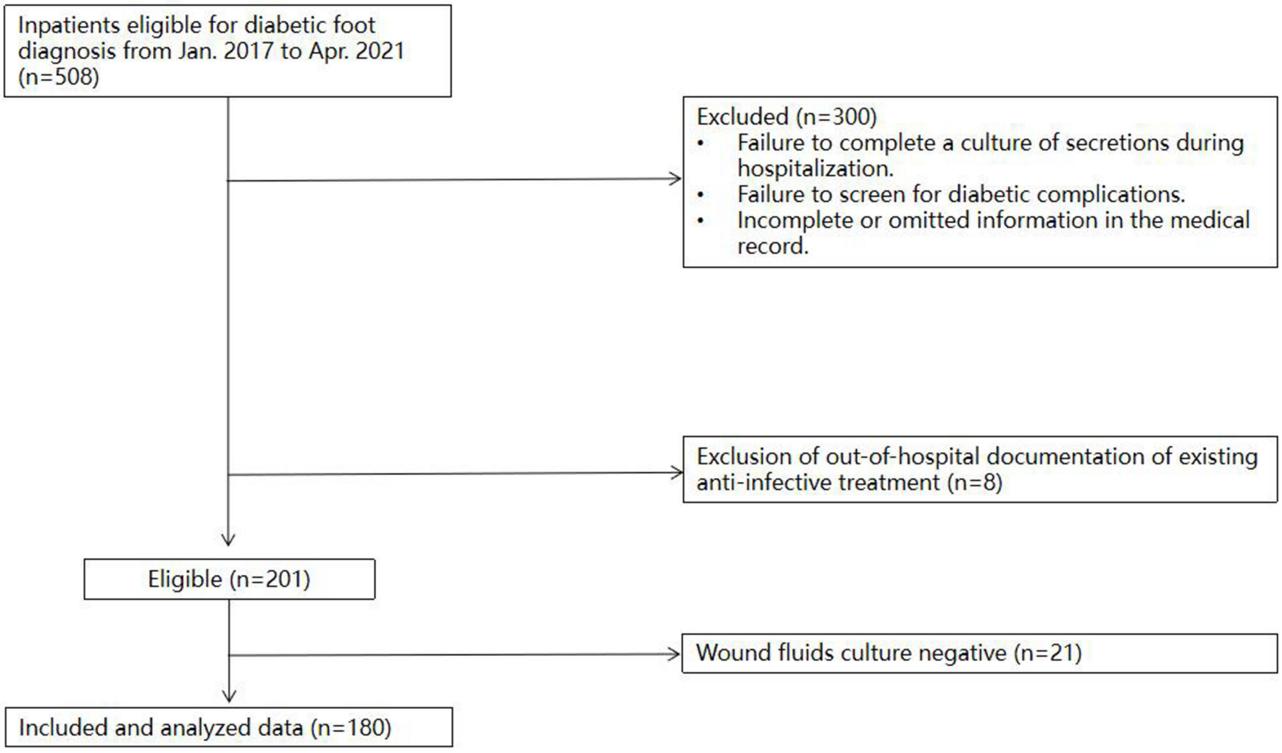

Figure 1 Inclusion of the study population.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS software version 28.0 (IBM Corporation, Armonk, NY, USA) for Windows. Classification variables are expressed in frequency and percentage. Continuous variables are expressed as the mean ± standard deviation (SD) or median and interquartile range (IQR). Chi-square test and Fisher's exact test were used to analyze classified variables. The continuity variables were tested by rank sum test. Multivariate Logistic regression analysis was used to determine the independent risk factors of infection in patients with DFUs between ESKAPE group and non-ESKAPE group, multidrug-resistant (MDR)-ESKAPE group and non-MDR-ESKAPE group, mild DFU group (Wagner 0–2) and severe DFU group (Wagner 3–5). Significance was defined as single factor $P < 0.1$, multi-factor $P < 0.05$.

Results

Clinical Characteristics

A total of 7966 patients with diabetes were hospitalized from January 2017 to April 2021, of which 508 (6.4%) were diagnosed with DFUs. In this study, 180 patients with DFUs were included in the following analysis. The average age of the patients was 63.42 ± 10.98 years old, and 38.8% were female. Hypertension (57.7%) and anemia (38.3%) were the two most common underlying comorbidities. Peripheral neuropathy (85.6%) was most associated with DFUs among other diabetes complications, and neuropathy was the most common type of ulcers (51.2%). The Wagner grade of the patients with diabetic foot infection was generally between 2 and 4. The median course of diabetes in these patients was 10.21 ± 7.172 years, and oral hypoglycemic drugs (38.3%) were the most common treatment (Table 1).

Distribution of Bacterial Isolates

A total of 206 isolates were isolated from 180 patients with DFUs. In addition, 25 patients (11.9%) were positive to two or more isolates during hospitalization. Among the 46 species of microorganisms present, 98 isolates (47.6%) were gram-negative bacteria and 97 isolates (47.1%) were gram-positive bacteria, and 11 isolates (5.3%) were fungi. *S. aureus* (52.6%) was the most common isolate, followed by *Proteus, E. coli, P. aeruginosa* and *Enterobacter* spp., accounting for

Table 1 Clinical Characteristics of 180 Patients with Diabetic Foot Infection

Variable	Statistics
Age, mean (years)	63.42 ± (range = 33–92)
Sex, n (%)	
Male	123 (61.2%)
Female	78 (38.8%)
Diabetes duration (years), mean ± SD	10.21 ± 7.172
Antidiabetes medication, n (%)	
Insulin	60 (29.9%)
Oral antidiabetics	77 (38.3%)
Insulin + oral antidiabetic	45 (22.4%)
Untreated or irregular medication	19 (9.5%)
Underlying comorbidities, n (%)	
Hypertension	116 (57.7%)
Hyperlipidemia	63 (31.3%)
Cerebral infarction	32 (15.9)
Coronary heart disease	68 (33.8%)
Heart failure	27 (13.4%)
Abnormal liver function	13 (6.5%)
Chronic hepatitis B	14 (7.0%)
Atherosclerosis	53 (26.4%)
Arterial plaque	19 (9.5%)
Arteriosclerotic occlusive disease of the lower extremity	38 (18.9%)
Osteoporosis	56 (27.9%)
Tumor	19 (9.5%)
Other chronic kidney disease (CKD)	32 (15.9%)
Estimated glomerular filtration rate (eGFR)	79.27 ± 31.615
Fracture	11 (5.5%)
Infection in other parts	46 (22.9%)
Sleep disorder	2 (1.0%)
Mental disorder	10 (5.0%)
Anemia	77 (38.3%)
Other long-term diabetic complications, n (%)	
Diabetic peripheral neuropathy	172 (85.6%)
Diabetic autonomic neuropathy	108 (53.8%)
Diabetic peripheral vascular disease	90 (44.8%)
Diabetic kidney disease	129 (64.2%)
Diabetic eye disease (retinopathy)	86 (42.8%)
Poor blood sugar control	151 (75.1%)
Acute complications of diabetes	4 (2.0%)
Types of diabetic foot ulcers, n (%)	
Neurotype	103 (51.2%)
Ischemic	17 (8.5%)
Mixed type	73 (36.3%)
Failed to classify	8 (4.0%)
History of amputation and/or foot ulceration, n (%)	
Toe removal/Amputation	31 (15.4%)
Foot ulceration	30 (14.9%)
Wound classification based on Wagner grade, n (%)	
Grade 1	10 (5.0%)
Grade 2	60 (29.9%)
Grade 3	38 (18.9%)

(*Continued*)

Table 1 (Continued).

Variable	Statistics
Grade 4	85 (42.3%)
Grade 5	8 (4.0%)
Dorsalis pedis artery, n (%)	
Normal	80 (39.8%)
Pulsation weakened	55 (27.4%)
Untouched pulsation	64 (31.8%)
Unknown	2 (1%)
Cigarette smoking, n (%)	76 (37.8%)
Alcohol consumption, n (%)	54 (26.9%)
Out-of-hospital wound care, n (%)	68 (33.8%)
Treatment of wound on admission, n (%)	
Debridement, dressing change and drainage (only)	107 (53.2%)
Invasive treatment	
+Toe removal	75 (37.3%)
+Amputation	9 (4.5%)
+Percutaneous transluminal angioplasty/Vascular stent implantation	7 (3.5%)
+Refusal of surgery	3 (1.5%)
HbA1c (%)	10.16 ± 2.483
Length of hospital stay (day), mean ± SD	28.49 ± 23.833
The duration of systemic antibiotic treatment (24h), mean ± SD	23.66 ± 17.093
Other Chronic kidney disease (CKD)	33 (16.4%)
Number of culture-positive cases, n (%)	180 (89.6%)

22.5, 18.3, 17.4 and 11.2%, respectively (Figure 2). Of the 206 isolates, 87 (42.2%) were ESKAPE pathogens, including 4 isolates of *E. faecium*, 51 isolates of *S. aureus*, 3 isolates of *K. pneumoniae*, 1 isolate of *A. baumannii*, 17 isolates of *P. aeruginosa*, and 11 isolates of *Enterobacter* spp.

Antimicrobial Susceptibility Profiles

The antimicrobial susceptibility test was carried out on all ESKAPE pathogens (Tables 2 and 3). *S. aureus* was highly resistant to ampicillin and penicillin, accounting for 88.2 and 86.3%, respectively. It is worth noting that a total of 8 isolates of methicillin-resistant *S. aureus* (MRSA) were detected in this study. All *E. faecium* were resistant to ciprofloxacin, levofloxacin, and erythromycin. Both *K. pneumoniae* and *A. baumannii* were relatively susceptible, even though only 3 isolates and 1 isolate were collected, respectively. Meanwhile, all the resistance rates of the antimicrobial agents tested on *P. aeruginosa* and *Enterobacter* spp. were less than 36%.

Comparison of Diabetic Foot Infection Between the ESKAPE Group and Non-ESKAPE Group

Compared with patients with non-ESKAPE infection, patients infected with ESKAPE pathogens were more likely to cigarette smoking (45.6% vs 33.7%, $P = 0.100$) and had more peripheral vascular disease complications (57.0% vs 40.6%, $P = 0.029$). However, the length of hospital stay (27.54 ± 31.557 vs 29.78 ±17.907, $P = 0.040$) and the duration of systemic antibiotic treatment (21.76 ± 19.503 vs 25.52 ± 15.668, $P = 0.021$) were shorter (Table 4). Patients with non-ESKAPE infection had a higher proportion of diabetic kidney disease (68.3% vs 54.4%, $P = 0.057$) and mental disorders (9.9% vs 0%, $P = 0.004$) (Table 4). Logistic regression analysis showed that peripheral vascular disease (odds ratio [OR] = 2.096; 95% confidence interval [CI], 1.100–3.992) and cigarette smoking (OR = 1.958; 95% CI, 1.015–3.777) were independent risk factors for ESKAPE infection in patients with DFUs (Table 5).

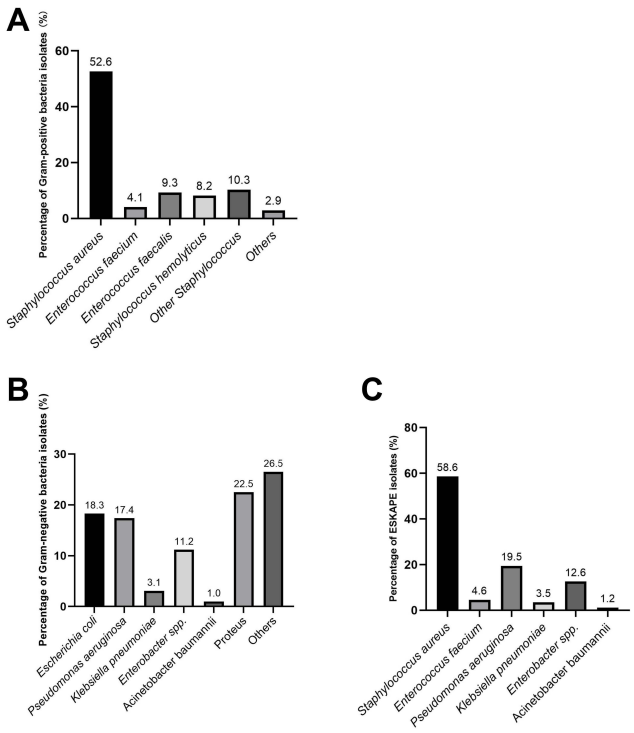

Figure 2 Distribution characteristics of pathogens isolated from DFUs. (**A**) Distribution of various types of Gram-positive bacteria; (**B**) Distribution of various types of Gram-negative bacteria; (**C**) Distribution of various types of ESKAPE pathogens.

Comparison of Diabetic Foot Infection Between the MDR-ESKAPE Group and Non-MDR-ESKAPE Group

Patients with non-MDR-ESKAPE infection were more prone to develop lower extremity arteriosclerotic occlusive disease (8.8% vs 24.4%, $P = 0.083$), and weakened or non-palpable dorsalis pedis artery (50.0% vs 73.3%, $P = 0.330$). In contrast, the MDR-ESKAPE infection was more likely to be associated with patients who had infections in other sites. (29.4% vs 13.3% $P = 0.078$). However, no independent risk factors for MDR-ESKAPE infection in patients with DFUs were found.

Table 2 Antimicrobial Susceptibility of ESKAPE: Gram-Positive Pathogens

Antimicrobial Agents	*S. aureus*, n (%) (n=51)	*E. faecium*, n (%) (n=4)
Penicillin	44 (86.3)	2 (50.0)
Ampicillin	45 (88.2)	2 (50.0)
Oxacillin	14 (27.5)	0 (0)
Ciprofloxacin	12 (23.5)	4 (100)
Levofloxacin	12 (23.5)	4 (100)
Daptomycin	1 (2.0)	0 (0)
Gentamicin	13 (25.5)	*
Erythromycin	30 (58.8)	4 (100)
Tetracycline	15 (29.4)	2 (50.0)
Nitrofurantoin	0 (0)	0 (0)
Vancomycin	0 (0)	0 (0)
Rifampin	2 (3.9)	2 (50.0)
Quinupristin–dalfopristin	1 (2.0)	*
Linezolid	1 (2.0)	0 (0)

Note: *Represents that the bacterial species are naturally resistant to the tested antibiotic.

Table 3 Antimicrobial Susceptibility of ESKAPE: Gram-Negative Pathogens

Antimicrobial Agents	*A. baumannii*, n (%) (n=1)	*P. aeruginosa*, n (%) (n=17)	*K. pneumoniae*, n (%) (n=3)	*Enterobacter* spp. n (%) (n=11)
Imipenem	0 (0)	0 (0)	0 (0)	0
Meropenem	0 (0)	0 (0)	0 (0)	0
Gentamicin	0 (0)	1 (5.9)	0 (0)	1 (8.3)
Tobramycin	0 (0)	1 (5.9)	0 (0)	0
Amikacin	0 (0)	0 (0)	0 (0)	0
Piperacillin-tazobactam	0 (0)	4 (23.5)	0 (0)	2 (16.7)
Ampicillin-Sulbactam	1 (100)	*	0 (0)	*
Cefuroxime	*	*	0 (0)	*
Cefotaxime	0 (0)	*	0 (0)	3 (25.0)
Ceftriaxone	1 (100)	*	0 (0)	3 (25.0)
Ceftazidime	0 (0)	6 (35.3)	0 (0)	2 (16.7)
Cefepime	0 (0)	5 (29.4)	0 (0)	2 (16.7)
Aztreonam	*	6 (35.3)	0 (0)	2 (16.7)
Ciprofloxacin	0 (0)	3 (17.6)	0 (0)	1 (8.3)
Levofloxacin	1 (100)	2 (11.8)	1 (33.3)	0 (0)
Cotrimoxazole	*	0 (0)	0	0 (0)

Note: *Represents that the bacterial species are naturally resistant to the tested antibiotic.

Table 4 Clinical Characteristics and Laboratory Records of Patients with ESKAPE Pathogen Infection

Variable	Non-ESKAPE (n=101)	ESKAPE (n=79)	*P*-value
Age (years)	64.12 ± 10.365	62.52 ± 11.740	
Sex			
Male	58 (57.4%)	54 (68.4%)	
Female	43 (42.6%)	25 (31.6%)	
Diabetes duration	10.96 ± 6.967	9.30 ± 6.688	
Antidiabetes medication			
Insulin	43 (42.6%)	29 (36.7%)	
Oral antidiabetics	26 (25.7%)	26 (32.9%)	
Insulin + oral antidiabetic	11 (10.9%)	7 (8.9%)	
Untreated or irregular medication	21 (20.8%)	17 (21.5%)	
Underlying comorbidities			
Hypertension	63 (62.4%)	41 (51.9%)	0.158
Hyperlipidemia	37 (36.6%)	21 (26.6%)	0.169
Cerebral infarction	20 (19.8%)	11 (13.9%)	0.300
Coronary heart disease	36 (35.6%)	26 (32.9%)	0.702
Heart failure	13 (12.9%)	11 (13.9%)	0.837
Abnormal liver function	5 (5.0%)	5 (6.3%)	0.689
Chronic hepatitis B	6 (5.9%)	7 (8.9%)	0.453
Atherosclerosis	9 (8.9%)	9 (11.4%)	0.582
Arterial plaque	25 (24.8%)	23 (29.1%)	0.511
Arteriosclerotic occlusive disease of the lower extremity	23 (22.8%)	13 (16.5%)	0.293
Tumor	9 (8.9%)	9 (11.4%)	0.582
Other Chronic kidney disease (CKD)	21 (20.8%)	11 (13.9)	0.232
Estimated glomerular filtration rate (eGFR)	76.47 ± 31.947	81.76 ± 29.862	0.246
Fracture	8 (7.9%)	3 (3.8%)	0.252

(*Continued*)

Table 4 (Continued).

Variable	Non-ESKAPE (n=101)	ESKAPE (n=79)	*P*-value
Infection in other parts	25 (24.8%)	16 (20.3%)	0.475
Sleep disorder	1 (1.0%)	1 (1.3%)	0.861
Mental disorder	10 (9.9%)	0 (0%)	**0.004**
Osteoporosis	33 (32.7%)	19 (24.1%)	0.205
Anemia	47 (46.5%)	27 (34.2%)	0.094
Other long-term diabetic complications			
Diabetic peripheral neuropathy	89 (88.1%)	65 (82.3%)	0.269
Diabetic autonomic neuropathy	55 (54.5%)	42 (53.2%)	0.863
Diabetic peripheral vascular disease	41 (40.6%)	45 (57.0%)	0.029
Diabetic kidney disease	69 (68.3%)	43 (54.4%)	**0.057**
Diabetic eye disease (retinopathy)	26 (25.7%)	13 (16.5%)	0.133
Retinopathy	21 (20.8%)	19 (24.1%)	0.602
Poor blood sugar control	35 (34.7)	30 (38.0%)	0.645
Acute complications of diabetes	2 (20%)	1 (1.3%)	0.710
Types of diabetic foot ulcers			
Neurotype	55 (54.5%)	31 (39.2%)	
Ischemic	6 (5.9%)	11 (13.9%)	
Mixed type	36 (35.6%)	34 (43.0%)	
Failed to classify	4 (4.0%)	3 (3.8%)	
History of amputation and/or foot ulceration			
Toe removal/Amputation	11 (11.0%)	16 (20.3%)	
Foot ulceration	15 (15.0%)	12 (15.2%)	
Wound classification based on Wagner grade			
Grade 1	6 (47.3%)	2 (2.5%)	
Grade 2	20 (19.8%)	29 (36.7%)	
Grade 3	18 (17.8%)	18 (22.8%)	
Grade 4	52 (51.5%)	28 (35.4%)	
Grade 5	5 (5.0%)	2 (2.5%)	
Dorsalis pedis artery, n (%)			
Normal	39 (38.6%)	27 (34.2%)	
Pulsation weakened	23 (22.8%)	27 (34.2%)	
Untouched pulsation	39 (38.6%)	25 (31.6%)	
Cigarette smoking	34 (33.7%)	36 (45.6%)	**0.100**
Alcohol consumption	25 (24.8%)	24 (30.4%)	0.400
Out-of-hospital wound care	35 (34.7%)	27 (34.2%)	0.947
Treatment of wound on admission			
Debridement, dressing change and drainage (only)	43 (42.6%)	48 (60.8%)	
Invasive treatment			
+Toe removal	47 (46.5%)	25 (31.6%)	
+Amputation	7 (6.9%)	2 (2.5%)	
+Percutaneous Transluminal Angioplasty/Vascular stent implantation	4 (4.0%)	4 (5.1%)	
HbA1c (%)	10.37 ± 2.385	9.97 ± 2.674	0.240
Length of hospital stay (day)	29.78 ± 17.907	27.54 ± 31.557	**0.040**
The duration of systemic antibiotic treatment (24h)	25.52 ± 15.668	21.76 ± 19.503	**0.021**

Note: Bold indicates $P < 0.10$.

Comparison of Diabetic Foot Infection Between the MDR-Pathogen Group and Non-MDR-Pathogen Group

Patients infected with MDR-pathogens were more likely to suffer from coronary heart disease (41.6% vs 29.1%, $P = 0.082$) and autonomic neuropathy (61.0% vs 48.5%, $P = 0.096$). Also, higher rate of alcohol consumption appeared in the

Table 5 Clinical Risk Factors for ESKAPE Pathogen Infection in Patients with DFUs

Variable	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	***P*-value**	**OR (95% CI)**	***P*-value**
Diabetic peripheral vascular disease	**1.937 (1.066–3.518)**	**0.029**	**2.096 (1.100–3.992)**	**0.024**
Mental disorder		0.004	< 0.001	0.999
Anemia	0.597 (0.325–1.095)	0.094	0.698 (0.355–1.371)	0.297
Diabetic kidney disease	0.554 (0.301–1.019)	0.057	0.708 (0.360–1.389)	0.315
Cigarette smoking	**1.650 (0.901–3.022)**	**0.104**	**1.958 (1.015–3.777)**	**0.045**
Length of hospital stay	0.996 (0.983–1.009)	0.040	1.014 (0.986–1.043)	0.322
The duration of systemic antibiotic treatment	0.987 (0.969–1.005)	0.021	0.978 (0.940–1.019)	0.288

Note: Bold indicates $P < 0.05$.

Table 6 Clinical Risk Factors for MDR-Pathogen Infection in Patients with DFUs

Variable	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	***P*-value**	**OR (95% CI)**	***P*-value**
Coronary heart disease	1.730 (0.930–3.221)	0.082	1.685 (0.893–3.179)	0.107
Alcohol consumption	**2.231 (1.145–4.349)**	**0.017**	**2.172 (1.104–4.272)**	**0.025**
Autonomic neuropathy	1.661 (0.912–3.024)	0.096	1.573 (0.852–2.905)	0.147

Note: Bold indicates $P < 0.05$.

Table 7 Clinical Risk Factors for Patients with Severe DFUs

Variable	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	***P*-value**	**OR (95% CI)**	***P*-value**
Anemia	2.149 (1.174–3.934)	0.013	1.648 (0.456–5.956)	0.446
Decreased pulse of dorsal foot artery	1.771 (0.957–3.278)	0.068	1.069 (0.360–1.389)	0.925
Invasive treatment	201.486 (61.49–660.209)	< 0.001	**326.642 (76.644–1392.08)**	**< 0.001**
Length of hospital stay	1.027 (1.007–1.047)	0.001	**1.145 (1.043–1.256)**	**0.004**
The duration of systemic antibiotic treatment	1.011 (0.996–1.026)	0.001	**0.918 (0.849–0.992)**	**0.030**

Note: Bold indicates $P < 0.05$.

MDR-pathogen group, and it was the only independent risk factor for MDR-pathogen infection in patients with DFUs (OR = 2.172; 95% CI, 1.104–4.272) (Table 6).

Comparison of Diabetic Foot Infection Between the Mild DFU Group and Severe DFU Group

As shown in Table 7, severe patients (Wagner 3–5) had a higher rate of anemia than those in the mild group (Wagner 0–2) (50.6% vs 32.3%, $P = 0.013$), and their dorsalis pedis arteries were weakened, or non-palpable and showed more abnormalities (70.1% vs 57.0% $P = 0.068$). In addition, a trend of higher incidence of invasive treatment (94.1% vs 7.5%, $P < 0.001$) was observed in patients with severe DFUs, and significantly higher rates of the duration of systemic antibiotic treatment (27.59±17.299 vs 20.4±17.063, $P = 0.001$) and length of hospital stay (31.66±18.513 vs 26.13 ±29.325, $P = 0.001$) were also observed in patients with severe DFUs. Importantly, invasive treatment (OR = 326.642; 95% CI, 76.644–1392.08), the duration of systemic antibiotic treatment (OR = 0.918; 95% CI, 0.849–0.992), and length of hospital stay (OR = 1.145; 95% CI, 1.043–1.256) were independent risk factors for patients with severe DFUs.

Discussion

As a metabolic syndrome, the clinical feature of diabetes is a hyperglycemic state resulting from decreased insulin secretion, insulin malfunction, or both.[3] Persistent hyperglycemia will lead to systemic diabetic chronic inflammation due to increased inflammatory cytokine levels and secretion of cytotoxic mediators.[19,20] As a result, both the hyperglycemic state and the chronic systemic inflammation put patients with diabetes at risk of developing several disabling and life-threatening complications, leading to an increased need for medical care, reduced quality of life, and premature death.[1] During the lifetime of a patient with diabetes, the chances of developing DFUs are as high as 25%.[9] DFUs are always colonized by microorganisms and more than 60% of ulcers end up with infections.[21] Amputation is required for approximately 20% of patients with moderate to severe levels of DFUs. After undergoing a diabetes-related amputation, death still occurs in over 70% of patients within 5 years.[2]

ESKAPE pathogens are involved in nearly one-fifth of nosocomial infections and are associated with the highest mortality rates, resulting in increased healthcare burdens.[22,23] With the increasing prevalence of antimicrobial resistance,[24] the number of antimicrobial agents effective against ESKAPE pathogens are decreasing, and there is even no drug for some pan-drug resistant pathogens. Novel antibiotics are being developed at a rate that is far slower than the rate of resistance development, suggesting that early identification and diagnosis of ESKAPE infection are extremely important.

In this study, bacteria were the dominant microorganisms isolated from wound fluids of DFUs, with roughly similar number of gram-positive and gram-negative pathogens. Remarkably, *S. aureus* was the most common isolate in DFUs, accounting for approximately one third of all isolates and more than half of ESKAPE pathogens, which is consistent with other studies.[25–27] As a commensal organism and an opportunistic pathogen, besides being the most common pathogen responsible for skin and soft tissue infections worldwide,[28] *S. aureus* is the most frequently isolated pathogens from the bone tissue specimens from patients with diabetic foot osteomyelitis.[14] *P. aeruginosa* represents the second most common organism isolated from chronic wound infections, accounting for 17.3% of ESKAPE pathogens in our study. Here, *S. aureus* showed high antimicrobial resistance, and although the resistance rate of *P. aeruginosa* is not as high as that of *S. aureus*, the wound that was infected with *P. aeruginosa* was usually associated with a significantly greater area and a longer healing process and resulted in a serious treatment-challenging ESKAPE infections.[29]

This study revealed that cigarette smoking and peripheral vascular disease were independent risk factors for ESKAPE infection. The associations between cigarette smoking and risks of all types of diabetes have been reported in multiple studies, and cigarette smoking was confirmed as a risk factor for amputation in DFUs.[30,31] A possible reason is that cigarette smoking generates high levels of reactive oxygen species, which can induce cellular oxidative stress in blood vessels and nervous system, leading to inflammation, cellular damage, and apoptosis.[30] Choi et al determined that cigarette smoking can increase the concentration of hydroquinone, which can significantly inhibit interferon-gamma (IFN-γ) secretion in a dose-dependent manner. In addition, Tollerud et al and Hogan et al reported that cigarette smoking is associated with a decrease in the number and proportion of circulating natural killer T (NKT) cells and invariant NKT cells, respectively.[32,33] These findings seem to be consistent with the results of a previous study indicating that serum NKT% and IFN-γ were significantly decreased in patients with diabetic foot infection compared to patients with non-infected DFUs and diabetes only.[34] Furthermore, cigarette smoking can systemically decrease immune responses, leading to ESKAPE infection.[35] It has been shown that hyperglycemia affects the vasoconstriction response and platelet aggregation by inhibiting endothelial nitric oxide synthase activation and increasing reactive oxygen species, leading to atherothrombosis in the vessels and ultimately to peripheral vascular disease.[36] Diabetic vascular disease is diagnosed in about 30% of patients with DFUs, and both macrovascular and microvascular diseases contributed to ischemia in the foot tissue and delayed wound healing, resulting in increased chance of acquiring ESKAPE infection in persistently exposed wounds. Importantly, cigarette smoking weakens vasodilation in skin microvasculature and further decreases the tissue oxygen tension and blood flow, placing patients at high risk for ESKAPE infection.[37,38] Cigarette smoking has always been a major factor in the development of peripheral vascular disease caused by increased formation of atherosclerotic plaques. Therefore, the correlation between cigarette smoking and peripheral vascular disease increases the risk of infection and complicates treatment.

It has been shown that alcohol consumption is an important factor in the development of DFUs due to the impairment of the wound-healing process by the ethyl-toxic effect of alcohol and thus promotes wound infection.[39] Moreover, although

granulocytes are the front line of the innate immune defense against bacterial infection, chronic or acute alcohol consumption can impair granulocyte function and then hinder the immune response, leading to a significantly higher risk of MDR-pathogen infection.[40] The Wagner system is the most widely used classification system for DFUs, which depends on the depth of ulceration and extent of gangrene.[41] In our study, the results showed that the longer length of hospital stay, longer duration of systemic antibiotic treatment, and more invasive treatments were significantly associated with patients with severe DFUs (Wagner 3–5), which were consistent with the actual clinical situation. Although no independent risk factors for MDR-ESKAPE infection were identified in this study, it is still very important to monitor and control the spread of ESKAPE resistance.

This study has some limitations. First, the retrospective data may deviate from the accuracy of the diagnosis of diabetic foot and several complications studied. Second, we only included the complete baseline characteristics in the medical records, some of which were excluded due to incomplete records, such as body mass index and procalcitonin. These variables may also be risk factors for ESKAPE infection in DFUs. Finally, due to the small sample size, there may be a selection bias in the results.

Conclusion

DFUs and ESKAPE pathogens share a significant concern about the limited treatment strategy and increasing healthcare costs. In this study, the incidence of ESKAPE infection in patients with DFUs was nearly 50%. Given that cigarette smoking and alcohol consumption were associated with ESKAPE infection and MDR-pathogen infection, respectively, we encourage smoking cessation and abstention from alcohol as part of the therapeutic strategy for patients with DFUs. Moreover, clinicians should be vigilant in patients with DFUs accompanied with peripheral vascular disease, planning appropriate antimicrobial agents and rigorous wound care to avoid ESKAPE infection.

Ethics Statement

This study was approved by the Institutional Review Board of the affiliated Hospital of Southwest Medical University (KY2020043). Written informed consent was obtained from all participants, and was conducted according to the guidelines of the Declaration of Helsinki.

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Disclosure

The authors report no conflicts of interest in this work.

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