Bacterial distribution, changes of drug susceptibility and clinical characteristics in patients with diabetic foot infection

LING LIU[1], ZHIHUI LI[2], XINXIN LIU[1], SHAN GUO[1], LIMIN GUO[1] and XUELIAN LIU[3]

[1]Clinical Laboratory; [2]Department of Hematology and [3]Department III of Special Needs, Xinxiang Central Hospital, Xinxiang, Henan 453000, P.R. China

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Abstract. The present study aimed to investigate the bacterial distribution, changes in drug susceptibility and clinical characteristics in patients with diabetic foot infection (DFI). A retrospective analysis of 216 patients with DFI treated at Xinxiang Central Hospital between 2013 and 2016 was carried out to analyze the bacterial distribution, changes of susceptibility and clinical characteristics. A total of 262 pathogenic strains were isolated from 216 patients with DFI. Among them, 43.13% exhibited Gram-positive (G^+) bacteria, 45.04% exhibited Gram-negative (G^-) bacteria and 11.83% was other. Between 2013 and 2016, the susceptibility of pathogenic bacteria to common antibacterial drugs showed a declining trend year by year. G^+ bacteria had high susceptibility to vancomycin and acetazolamide; while G^- bacteria showed high susceptibility to dibekacin, panipenem and biapenem. The main clinical symptoms of the 216 patients included edema (98.61%), purulent secretions (62.96%) and lower extremity sepsis (58.80%). The top three complications of the 216 cases were lower extremity vascular disease (58.80%), peripheral neuropathy (39.81%) and kidney disease (26.39%). Logistic regression analysis showed that age [odds ratio (OR), 2.708; P=0.005], previous use of antibacterial drugs (OR, 3.816; P=0.007) and application of the third generation cephalosporins (OR, 3.014; P=0.008) were the independent risk factors of drug resistance in patients with DFI (P<0.05). There were numerous types of pathogens in patients with DFI, and all of them had certain drug resistance. The drug susceptibility was decreasing year by year. The pathogens and drug resistance in patients with DFI should be monitored to reduce the incidence of related complications and improve the prognosis of patients.

Correspondence to: Dr Ling Liu, Clinical Laboratory, Xinxiang Central Hospital, 56 Jinsui Avenue, Xinxiang, Henan 453000, P.R. China
E-mail: liuling0788@163.com

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Introduction

Diabetes mellitus (DM) is a common disorder of glucose metabolism and a non-communicable chronic disease. DM is a serious threat to human health after cardiovascular disease (1). DM is currently one of the global public health problems and shows an increasing trend year by year. It is estimated that the number of diabetic patients in the world will reach 500 million by 2030 (2). DM has a lot of complications, and diabetic foot (DF) is one of the most serious ones. DF patients suffer from inflammation and foot tissue damages caused by the invasion of pathogenic microorganisms. Approximately 70% of DF patients will have diabetic foot infection (DFI) (3). Many kinds of bacterial can infect DFI patients, and Gram staining differentiate them into Gram-positive (G^+) and Gram-negative (G^-) bacteria (4). The clinical manifestations of DFI are complex and the treatment cycle is long. In addition, the unreasonable use of antimicrobial drugs, and the changes of pathogens and drug resistance in recent years have complicated the treatments and finally led to gangrene and increased amputation rate (5). The distribution of bacteria in DFI patients, the changes of their susceptibility to antimicrobial drugs, and the clinical characteristics of DFI should be studied to achieve better use of antimicrobial drugs, and to provide a scientific basis for the prevention and rapid control of DFI.

Materials and methods

General information. A retrospective analysis of 216 cases of DFI treated at Xinxiang Central Hospital (Xinxiang, China) from 2013 to 2016 was conducted. The inclusion criteria included: i) Meeting the diagnostic criteria of DF (6) and ii) confirmation of bacterial infection by tissue culture of specimen from the foot wound. The exclusion criteria included: i) Liver and renal failure and ii) malignant tumor. All patients had a history of drug use of the drugs covered by this study. The general information of the patients was listed in Table I. The study was approved by the Ethics Committee of Xinxiang Central Hospital and informed consents were signed by the patients.

Methods

Sample collection and strain identification. Patients' foot wound was cleaned with 0.9% sodium chloride solution. A sterile cotton swab was used to collect specimen from the base

of the wound. Specimens from patients with deep abscesses were collected by using a sterile syringe to get the pus. The specimens were cultured in growth media (Oxoid Corporation, Basingstoke, UK) and the bacterial strains were identified using a VITEK 32 automated microbial analyzer (BioMérieux, Craponne, France).

Susceptibility analysis. The cultured bacteria were classified and tested for susceptibility by MH agar KB according to the National Committee for Clinical Laboratory Standards (NCCLS). Bacteria were immediately inoculated on blood agar plates and placed in a 37˚C incubator for 24 h. Bacterial identification was carried out using a VITEK2 automatic bacterial analyzer (BioMérieux). Kirby-Bauer disc diffusion method was used for drug susceptibility testing. *Escherichia coli* ATCC35218A, TCC25922, *Enterobacter cloacae* ATCC700323 and *Klebsiella pneumoniae* ATCC700603 were used as the quality controls for G^- bacteria, while *Staphylococcus aureus* ATCC29213 was the quality control for G^+ bacteria. All control strains were provided by Nanjing Clinical Biotechnology Co., Ltd. Results between sensitivity and drug resistance that emerged in this study were not subjected to statistical analysis.

Observation indicators. Local clinical features of the patient, including edema, defined as swelling of the lateral limbs and skin thickening starting at the foot and ankle involving the entire lower extremity, purulent secretions, defined as a thin pus overflow of the affected foot, lower extremity pus and blood, defined as pus-like material and blood from lower extremities, bone exposure is defined as the presence of varying degrees of bone tissue exposure in the foot, necrosis, defined as necrosis of skin of the affected foot and the surrounding skin, malodorous smell, defined as bad smell of secretion of the affected foot.

Statistical analysis. Epidata3.1 was used to do the data entry, and SPSS 19.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Count data were expressed as number or composition ratio. Logistic regression analysis was conducted to study the impact of different factors. $P<0.05$ was considered statistically significant.

Results

Distribution of pathogens. 262 strains of pathogens were isolated from 216 patients with DFI, including 113 strains of G^+ bacteria (43.13%), 118 strains of G^- bacteria (45.04%), and 31 strains of fungi (11.83%). The results were shown in Fig. 1.

Changes in drug susceptibility of pathogenic bacteria. During 2013 to 2016, there was a gradual declining trend about pathogenic bacteria susceptibility to conventional antibacterial drug, but there was no statistically significant difference among the different time points ($P>0.05$) (Table II).

Susceptibility of G^+ bacteria to antibacterial drugs. G^+ bacteria showed the highest susceptibility to vancomycin and acetazolamide, while they had low susceptibility to erythromycin, amoxicillin, norfloxacin and penicillin (Table III).

Susceptibility of G^- bacteria to antibacterial drugs. G^- bacteria showed high susceptibility to dibekacin, panipenem and biapenem, and low susceptibility to cefaclor, norfloxacin and erythromycin (Table IV).

Table I. General patient data.

Characteristics	Patients (n=216)
Sex, n (%)	
Male	101 (46.76)
Female	115 (53.24)
Age, years, range	30-78
Mean age, years	52.36±7.47
BMI, kg/m^2	23.54±3.73
Course of disease, years, range	1-25
Average course of disease, years	11.48±5.36
Fasting blood glucose (mmol/l) (8 h)	10.23±4.37
Ulcer duration, days	51.73±8.75
Diabetic Foot Wagner Grading, n (%)	
Level 0-2	31 (14.35)
Level 3 or higher	185 (85.65)

Data are presented as mean ± standard deviation, unless otherwise indicated.

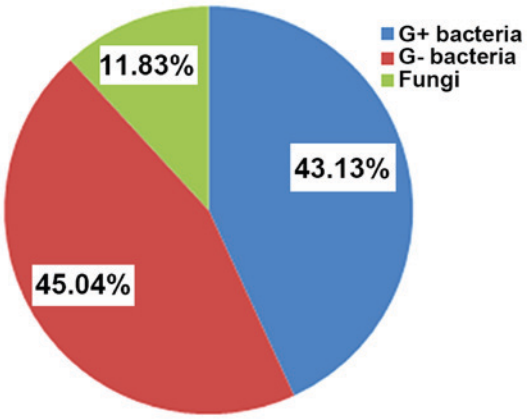

Figure 1. Distribution of pathogens in patients with diabetic foot infection.

Clinical symptoms of patients. The top three local clinical symptoms of the 216 patients were edema (98.61%), purulent secretions (62.96%), and lower extremity sepsis (58.80%) (Table V).

Complications of the patients. The top three complications were: Lower extremity vascular disease (58.80%), peripheral neuropathy (39.81%), and kidney disease (17.13%) (Table VI).

Analysis of factors affecting drug resistance in patients with DFI. For the analysis, the presence of drug resistance was used as the dependent variable, and patient age, hospitalization frequency, previous use of antibacterial drugs, combination with osteomyelitis, application of third-generation cephalosporins and the presence of more than three ulcers, were used as the independent variables. The results showed that age (OR=2.708, P=0.005), previous use of antibacterial drugs (OR=3.816,

Table II. Changes in drug susceptibilities of pathogenic bacteria during 2013-2016.

	G^+ bacteria, n (%)		G^- bacteria, n (%)		Fungi, n (%)	
Years	No. of identified strains	Susceptibility	No. of identified strains	Susceptibility	No. of identified strains	Susceptibility
2013	28	24 (85.71)	29	25 (86.21)	7	6 (85.71)
2014	26	21 (80.77)	27	21 (77.78)	8	6 (75.00)
2015	27	20 (74.07)	28	20 (71.43)	7	5 (71.43)
2016	32	22 (68.75)	34	23 (67.65)	9	4 (44.44)

Table III. Susceptibilities of G^+ bacteria to antibacterial drugs.

	Staphylococcus, n (%) (n=43)		*Streptococcus*, n (%) (n=39)		*Enterococcus*, n (%) (n=31)	
Antibacterial drugs	Resistance	Susceptibility	Resistance	Susceptibility	Resistance	Susceptibility
Erythromycin	41 (95.35)	2 (4.65)	36 (92.31)	1 (2.56)	29 (93.55)	1 (3.23)
Amoxicillin	40 (93.02)	3 (6.98)	35 (89.74)	2 (5.13)	27 (87.10)	2 (6.45)
Vancomycin	6 (13.95)	36 (83.72)	3 (7.69)	35 (89.74)	3 (9.68)	28 (90.32)
Acetazolamide	5 (11.63)	37 (86.05)	2 (5.13)	36 (92.31)	2 (6.45)	26 (83.87)
Norfloxacin	37 (86.05)	6 (13.95)	34 (87.18)	4 (10.26)	28 (90.32)	3 (9.68)
Penicillin	35 (81.40)	5 (11.63)	31 (79.49)	3 (7.69)	26 (83.87)	2 (6.45)

Table IV. Susceptibilities of G^- bacteria to antibacterial drugs.

	Proteus, n (%) (n=45)		*Escherichia coli*, n (%) (n=41)		*Klebsiella pneumoniae*, n (%) (n=32)	
Antibacterial drugs	Resistance	Susceptibility	Resistance	Susceptibility	Resistance	Susceptibility
Cefaclor	41 (91.11)	3 (6.67)	40 (97.56)	1 (2.44)	29 (90.63)	1 (3.13)
Dibekacin	7 (15.56)	35 (77.78)	5 (12.20)	32 (78.05)	2 (6.25)	29 (90.63)
Biapenem	6 (13.33)	37 (82.22)	3 (7.32)	37 (90.24)	3 (9.38)	28 (87.50)
Panipenem	5 (11.11)	38 (84.44)	4 (9.76)	36 (87.80)	2 (6.25)	26 (81.25)
Norfloxacin	40 (88.89)	5 (11.11)	37 (90.24)	2 (4.88)	28 (87.50)	2 (6.25)
Erythromycin	38 (84.44)	7 (15.56)	35 (85.37)	3 (7.32)	29 (90.63)	1 (3.13)

Table V. Clinical symptoms of patients.

Clinical symptoms	No. of patients	%
Edema	213	98.61
Purulent secretions	136	62.96
Lower extremity sepsis	127	58.80
Exposure of bones	76	35.19
Necrosis	61	28.24
Stinky smell	54	25.00

Table VI. Complications in patients.

Complications	No. of patients	%
Lower extremity vascular disease	127	58.80
Peripheral neuropathy	86	39.81
Kidney disease	37	17.13
Hyperlipidemia	36	16.67
Retinopathy	29	13.43

P=0.007), application of the third-generation cephalosporins (OR=3.014, P=0.008) were the independent risk factors for the resistance in patients with DFI (P<0.05) (Table VII).

Discussion

DM usually occurs in elderly patients. These patients have other diseases and low immunities. In addition, they suffer long-term

Table VII. Logistic regression analysis of the factors affecting the drug resistance in DFI patients.

Factors	β	SE	Wald	OR	95% CI	P-value
Age	0.618	0.673	6.424	2.708	1.106-3.854	0.005
Hospitalization frequency	0.362	0.435	4.126	0.619	0.493-0.874	0.316
Combination with osteomyelitis	0.615	0.314	3.427	0.716	0.496-0.862	0.218
Previous use of antibacterial drugs	0.563	0.606	7.703	3.816	1.075-4.712	0.007
Application of the third-generation cephalosporins	0.617	0.518	5.568	3.014	1.103-4.046	0.008
More than three ulcers	0.456	0.412	3.713	0.753	0.275-0.916	0.356

CI, confidence interval; OR, odds ratio; SE, standard error of the mean.

inadequate local blood supply because of blood glucose control and vascular diseases such as atherosclerosis. Thus, DFI has a high incidence in elderly DM patients (7). Under the influence of long-term hyperglycemia, patients have metabolic disorders and impaired immune systems. Weak immune system results in reduced chemotaxis, adhesion and phagocytosis of monocytes and neutrophils, which cannot resist the invasion of pathogens. In addition, the high levels of sugar and proteins in the exudates from foot wounds create a good environment for the survival and reproduction of bacteria, and thus can easily lead to the occurrence of infections (8). Wound repair in DFI patients is a complex physiological process. Patients usually have serious tissue damage and long courses of disease. Their wounds are difficult to heal and prone to drug resistance, and the prognosis is poor (9).

DFI is caused by a lot of pathogens. According to a relevant statistics (10), G^+ bacteria and G^- bacteria can be detected in DFI. The results of this study showed that G^+ bacteria accounted for 43.13%, G^- bacteria accounted for 45.04%, while others accounted for 11.83% of the pathogens. The majority was G^+ and G^- bacteria. Among the detected G^+ bacteria were *Staphylococcus*, *Streptococcus* and *Enterococcus*. *Staphylococci* is named after the fact that they look like clusters of grapes. *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Staphylococcus saprophyticus* can cause suppurative inflammation in DFI patients (11). *Streptococcus* are usually <2 μm in diameter, ovoid or spheroidal in appearance and has a chain-like appearance. They have a strong invasiveness and can produce a variety of exotoxins, which can aggravate the degree of infection in patients (12). *Enterococcus* are a group of intestine-dwelling bacteria in the shape of oval or spherical. They appear in short chains or pairs in liquid media and they do not produce spores. They are a group of important infectious pathogens (13). The G^- bacteria detected in this experiment mainly included *Proteus*, *Escherichia coli* and *Klebsiella pneumoniae*. *Proteus* are also called *Bacillus*. They are secondary infectious bacteria and are usually detected in the late phase of DFI. They cause corrosive tissue damage (14). *Escherichia coli* is a single-cell bacterium that lives in human intestine and is essential for human body (15). *Klebsiella pneumoniae* is one of the important infectious pathogens, especially in the immunocompromised population. It can lead to infections such as urinary tract infection, pneumonia and bacteremia with high mortality rate (16).

Clinically, DFI patients are often given broad-spectrum antibacterial drugs to kill bacteria and control infections (17). In clinical practice, antibacterial drugs need to be changed and patients usually need long-term use of a variety of drugs, especially those high-level antimicrobial drugs, resulting in increased drug resistance in patients. When bacteria invade into host cells, antibacterial drugs cannot effectively enter the cells. The compromised anti-infection effects result in delayed wound healing and cause great suffering in patients (18). The results of this study showed that the susceptibility of pathogens to conventional antibacterial drugs have declined year by year from 2013 to 2016, which was closely related to the unreasonable use of antibacterial drugs. In this study, we found that G^+ bacteria had high susceptibility to vancomycin and acetazolamide, while G^- bacteria were sensitive to dibekacin, panipenem and biapenem. These results indicated that drugs with low susceptibility should be avoided in the clinical treatment for G^+ bacteria-infected DFI patients, while vancomycin and acetazolamide should be chosen. Similarly, the third-generation cephalosporins should not be prescribed for G^- bacteria-infected DFI patients. The fourth-generation cephalosporins, dibekacin, panipenem and biapenem should be recommended. The use of drugs with high bacteria susceptibility can effectively control infections and avoid gangrene wounds.

DFI patients generally have low anti-infection abilities. Their clinical syndromes are obvious and mainly manifested as edema, purulent discharge, and lower extremity sepsis. Some patients also suffer bone exposure and necrosis (19). The long-term high blood sugar can easily lead to various complications, including lower extremity vascular disease (58.80%), peripheral neuropathy (39.81%) and kidney disease (26.39%). Lower extremity vascular disease is mainly because patients have low resistance to infections and persistent hyperglycemia can lead to metabolic disorders. Patients' limbs are vulnerable to bacterial invasion, resulting in damage to the endothelial cells on the arterial wall of the lower extremities, causing vascular endothelial dysfunction (20). In addition, the long-term dyslipidemia and other metabolic disorders impair the sympathetic and parasympathetic systems, triggering peripheral neuropathy (21). Continued inflammation in DFI patients also causes increased blood pressure, which in turn can impair renal function (22).

This study showed that age, previous use of antibacterial drugs and use of third-generation cephalosporins were independent risk factors for drug resistance in DFI patients ($P<0.05$). This is because autoimmune ability decreases as patients' age

increases. Their high glucose toxicity and oxidative stress can lead to changes in the expression of autophagy genes. The weakened autophagic function of the host cells makes it easier for bacteria but harder for antibacterial drugs to enter the cells, thereby increasing drug resistance (23). Because of lack of proper knowledge, the inappropriate use of third-generation cephalosporins and heavy use of antibacterial drugs in the past have led to a significant increase in drug-resistant pathogens.

In conclusion, DFI patients with bacterial infection mainly have G^+ and G^- bacteria. Their susceptibility to commonly used antibacterial drugs declines year by year. Proper clinical treatment of DFI needs to standardize the use of antibacterial drugs with drug susceptibility testing, so as to improve clinical symptoms and control of DFI.

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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LL wrote the manuscript. LL and ZL were responsible for patient susceptibility analysis. XiL and SG recorded and analyzed results. LG and XuL performed statistical analysis. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Xinxiang Central Hospital (Xinxiang, China) and informed consents were signed by the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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