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Distribution and Significance of Pathogenic Bacteria on the Traumatic Surface of Diabetic Foot Patients

Jiang Hou-Sen

Department of Orthopedics, Shandong Provincial Weifang People's Hospital Affiliated to Weifang Medical University, Shandong, China

Correspondence should be addressed to Jiang Hou-Sen, Department of Orthopedics, Shandong Provincial Weifang People's Hospital Affiliated to Weifang Medical University, 151 Guangwen Road, Weifang, 261000, Shandong, China

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ABSTRACT

OBJECTIVE

To understand the distribution of traumatic pathogens and changes in drug resistance in diabetic foot patients in Weifang City, to provide theoretical support and clinical basis for the rational use of antibiotics and antibacterial materials in clinical diabetic foot.

METHODS

Retrospective analysis of the distribution and characteristics of traumatic pathogenic bacteria in patients with diabetic foot ulcers seen from January 2020 to December 2021.

RESULTS

A variety of different conditions such as G+ bacteria, G- bacteria, fungal and mixed bacterial infections were found in diabetic foot ulcer trauma.

CONCLUSION

In recent years, the number of pathogenic bacteria in diabetic foot patients has increased significantly and mixed infections are predominant, therefore early empirical use of drugs followed by selection of antimicrobial therapy based on drug sensitivity is the key to the treatment of diabetic foot infections.

KEYWORDS

Diabetic foot; Bacterial infection; G+ bacteria; G- bacteria; Fungi

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INTRODUCTION

Diabetic foot (DF) is one of the most common chronic complications in diabetic patients, and the formation and role of pathogenic microorganisms in complex infections has become a hotspot and focus of research once the wound becomes infected, bringing a huge medical burden to patients and society [1-3]. Diabetic foot and infection lead to 80% of non-traumatic lower limb amputations and a mortality rate of 43%-55% at 5 years after surgery, higher than the incidence of cancers such as breast cancer and Hodgkin's disease [4-6].

Chronic wounds are largely infected and colonised by polymicrobial communities that contribute to ongoing inflammation and delayed healing processes, significantly reducing the quality of life of patients [7]. A variety of different microbial communities are present on the skin surface [8-10] and these contribute to the health of the body. However, following a break in the skin or tissue damage, microorganisms enter the wound, colonisation occurs and changes in microbial metabolism occur, providing an opportunity for the onset of toxicity of commensal microorganisms. Interventions such as early glucose lowering, anti-infective treatment with antimicrobials and wound debridement are effective treatments. Early anti-infection not only prevents local and systemic spread of infection and promotes wound healing, but also preserves the patient's limb function and saves medical costs. With the widespread use of broad-spectrum antimicrobials since the 20th century, the distribution and drug sensitivity of DF pathogens have changed, and there are differences between patients in different regions and even different hospitals. Therefore, retrospective analysis of the distribution and drug resistance of pathogenic bacteria on the traumatic surfaces of diabetic foot patients in our hospital, to understand the microflora of the population with diabetic foot ulcers, to provide guidance for initial empirical antibiotic treatment in the local area, to strengthen the purpose of treatment, to reduce costs through gradual and more rational use of antimicrobials [11,12], and to provide theoretical support and clinical basis for the rational use of antibiotics in clinical diabetic foot.

INFORMATION AND METHODS

Study Population and Data

Bacterial distribution and drug resistance analysis of a total of 234 patients with DF (Wagner grading [13] grade 2-4) hospitalized in Weifang People's Hospital from January 2020 to December 2021 were collected, and all patients met the diagnostic criteria for diabetes mellitus given by the World Health Organization in 1999. The general data of the study subjects were retrospectively analysed including a number of indicators such as gender, age, duration of diabetes, whether insulin was used to control blood glucose, whether fever was present, diabetic foot grading (Wagner grading), duration of ulceration, and mode of surgery.

Study Methods

Traumatic secretion collection our patients' trauma specimens were collected

There are two types of specimen collection: open wound specimen collection: The surface of the lesion and the surrounding skin are cleaned with sterile saline, the basal purulent secretions are taken with a sterile cotton swab and placed in a sterile test tube, mostly at the time of dressing change; unopened or semi-open wound specimen collection: The wound is disinfected with a towel, the surface of the lesion and the skin around the wound are cleaned with sterile saline, then the surface of the wound is cut open with a scalpel to reveal the deep purulent secretions. This is taken with a sterile cotton swab and placed in a sterile test tube and sent immediately to the microbiology laboratory.

Pathogen culture and drug sensitivity analysis

All specimens were inoculated on blood agar plates and incubated in a CO_2: Incubator at 37°C for 24 hours.

Identification and drug sensitivity testing was performed using the French bioMérieux automated microbial identification/drug sensitivity system to determine bacterial resistance according to the 2014 American Society for Clinical Laboratory Standardization guidelines [14]. Glycated hemoglobin was determined by high performance liquid chromatography.

Statistical Methods

All data were statistically analysed using SPSS 19.0 software, and the mean ($\mu \pm s$) was used for measurement data, and the chi-square test was used for count data, with test water $\alpha = 0.05$.

RESULTS

General Characteristics of the Study Population

A total of 234 inpatients with diabetic foot were included in the study population. The general characteristics of the study population are shown in (Table 1 and Table 2), of which 142 were male and 92 were female, aged 35 years to 95 years. The subjects of this experimental study were selected from patients with diabetic foot ulcers who were hospitalized in our hospital and had the following major characteristics: Firstly, the duration of diabetes mellitus was long and the glycaemic control was unsatisfactory, with 132 about 56.41% of the patients having a history of diabetes mellitus of 10 years - 19 years; Secondly, the age span of the patients was large, with 12 below 40, 20 in the 40 segment - 49 segment, 50 in the 50 segment - 59 segment, 60- 69 80 people 56 people in the 70 segment - 79 segment, and 16 people above 80. The main age stage of their onset was concentrated in the 50 years - 80 years, with the 60 years - 69 years age group being the most frequent, accounting for about 34.19% of the onset population; see (Chart 1 and Chart 3) for a review of the specific age distribution. Thirdly, the disease is severe. According to the diabetes wager grading standard, patients were classified as II, III and IV, with 26.50% grade II, 68.38% grade III and 5.12% grade IV, especially grade II and III (Chart 2 and Chart 4), which was due to the fact that most of the patients were referred to our hospital after long periods of change of medication and antibiotic anti-infection treatment in lower hospitals or in the community before they were admitted, with poor results, or even aggravation This is due to the fact that most of the patients had been treated at lower hospitals or in the community for long periods of time before they were admitted to our hospital for anti-infective treatment. Fourthly, most patients with diabetic foot had complications of diabetes or other major diseases, with osteomyelitis, hypertension, peripheral vascular disease and coronary atherosclerotic heart disease being the most common. Of these, 192 were most commonly accompanied by osteomyelitis, about 73.50%; hypertensive disease was the second most common in 128 patients, 54.70%, which is largely consistent with the findings Malachias MVB [15]; in third place was coronary artery heart disease in 124 patients, about 52.99%, while also suffering from peripheral vascular disease in 118, 50.43%, which is significantly This is significantly higher than that reported by Hinchliffe R [16].

Demographic Details	No. of Patients	Percentage
Age(years)		
<39	12	5.12%
40-49	20	8.55%
50-59	50	21.37%
60-69	80	34.19%
70-79	56	23.93%
>80	16	6.84%
Sex		
Male	142	60.68%
Female	92	39.32%
Types of Diabetes		
Type1	0	0
Type2	234	100%
Duration of Diabetics (Years)		
<10	50	21.37%
10–19	132	56.41%
>20	52	22.22%
Duration of Ulcer (Months)		
3	136	58.12%
03-06	90	38.46%
>6	8	3.42%

Table 1: General information and profile of selected patients.

Variables (N = 234)	No. of Patients	Percentage
Classification of Wagner		
0	0	0
I	0	0
II	62	26.50%
III	118	58.97%
IV	22	9.40%
V	12	5.13%%
Insulin-Dependent	158	67.52%
Chronic Kidney Disease on Dialysis	6	2.56%
Coronary Heart Disease	124	52.99%
Cerebral Infarction	28	11.97%
Dyslipidemia	58	24.79%
Peripheralarterial Disease	118	50.43%
Hypertension	128	54.70%
Chronic Kidney Disease	28	11.97%
Osteomyelitison	192	73.50%
Lesion Location		
Right Limb	132	56.41%
Left Limb	82	35.04%
Surgical Procedure	124	52.99%
Major Amputation	20	8.55%
Minor Amputation	122	52.14%
Debridement	90	38.46%
Previous Antibiotic Use	180	76.92%
Previous Hospitalization	118	50.43%

Table 2: Clinical characteristics of the data of the selected patients.

There are of course some patients with major illnesses such as renal insufficiency, cerebral infarction and dialysis (Chart 2 and Chart 5), and some patients with multiple major illnesses and generally poor health. Diabetic foot ulcers are one of the most serious complications of diabetes mellitus. Once toe necrosis occurs, it proves that the lower limbs have been ischemic and hypoxic for a long time and that the peripheral vascular compensation cannot meet the requirements of wound healing. Such patients often have equivalent degrees of cardiac, renal and cerebrovascular pathology, and it is important to closely monitor the patient's condition while rehydrating to prevent the occurrence of heart failure and renal failure (Figure 1 - Figure 3).

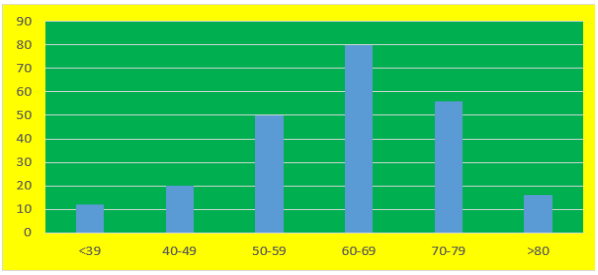

Figure 1: Age-specific distribution of patients.

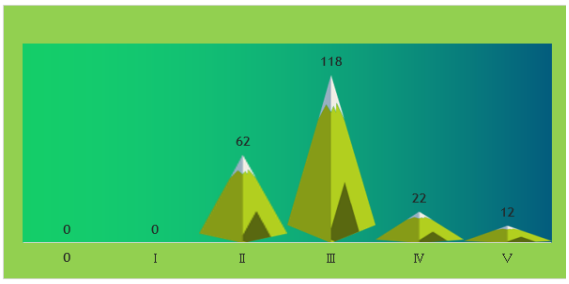

Figure 2: Diabetic foot wager grading.

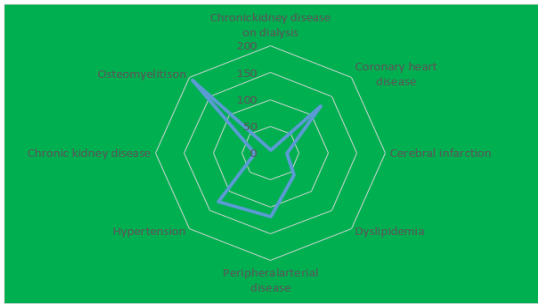

Figure 3: Complications or other conditions accompanying diabetes.

Bacterial Distribution Characteristics of Diabetic Foot Wounds

Pathogenic bacterial infections were present in 88.89% of diabetic foot patients, of which 27.35% were G+, 24.79% were G-, 35.90% were mixed, and 3.42% were fungal infections (Chart 6 and Chart 7); G+ bacteria included Staphylococcus (*Staphylococcus aureus, coagulase-negative Staphylococcus epidermidis*, etc.), *Streptococcus lactis, Enterococcus faecalis, Enterococcus avium,* and *Streptococcus* (Chart 9 and Chart 10), G- *Pseudomonas aeruginosa* (*Pseudomonas aeruginosa*), *Enterobacter cloacae, Klebsiella pneumoniae, Pneumocystis aeruginosa, Escherichia coli, Citrobacter vulgaris, Citrobacter minor, Morgan's bacillus, S. prodens* (Chart 11, Chart 12); fungi were Actinomyces, *Candida albicans, Pseudomycetes* (Chart 13 and Chart 14); mixed infections accounted for 35.90% of the infections, with 84 cases of both multiple G+ mixed infections and multiple G- mixed infections, as well as G+ and fungal, and G-fungal and fungal complex infections (Chart 8). The presence of at least two bacterial infections on the trabecular surface and three or more bacterial infections were also common. The complexity of trabecular infections explains why anti-

infective therapy is so ineffective in patients with diabetic foot ulcers. Fever symptoms were present in only 10 of 234 patients, accounting for only 4.27% of all patients. The reason considered was the chronic immune tolerance state of diabetic patients, who are in a chronic state of atypical inflammation.

The result is that the organism is not sensitive to the inflammatory response. In addition, patients often apply antibiotic anti-infection treatment for a long time. Bacterial cultures were negative in 26 cases. Considering that the rate of positive trauma cultures is influenced by a number of factors, the severe necrosis of the traumatized surface of the patients included in this experiment, with all the necrotic tissue in the centre of the traumatized surface being necrotic cells, may be one of the reasons. In addition, the long-term application of antibiotics can also interfere with the results of cultures.

Depending on the severity of the bacterial load, severe bacterial colonisation and infection can lead to delayed wound healing and even life-threatening sepsis or multi-organ failure [17]. Notably, Gram-positive bacteria, including Staphylococcus aureus (SA) and Streptococcus pyogenes (SP) were present in the early stages of infection, while Gram-negative bacteria, including Escherichia coli (EC) and Pseudomonas aeruginosa (PA), were present in the already developed wounds [18]. The results of this study show that Wager grade II has superficial ulcers as the main clinical manifestation and the wound infection is dominated by a single bacterium, allowing the selection of antibiotics with a narrower antibacterial spectrum; deep ulcers and gangrene as the main clinical manifestation are mainly mixed bacterial infections, often combined with anaerobic bacteria or even fungal infections, probably due to high glucose and high ester metabolism leading to a decrease in the body's immunity, combined with ischaemia of the local tissues, leading to ulcers or The deeper the ulcer, the lower the oxygen content, the more suitable it is for the growth of anaerobic bacteria and the proliferation of conditionally pathogenic bacteria (Figure 4 - Figure 9).

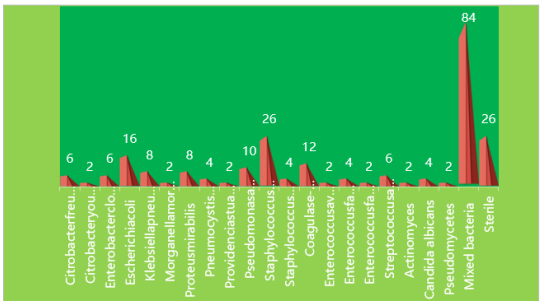

Figure 4: Distribution of bacteria specific to traumatic infections.

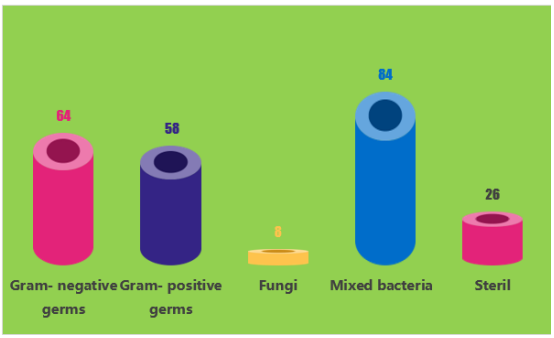

Figure 5: Broad classification of traumatic bacteria.

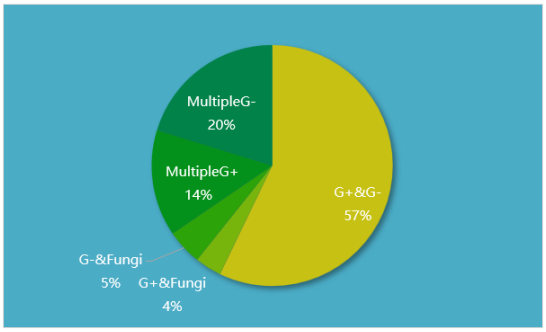

Figure 6: Classification of bacteria specific.

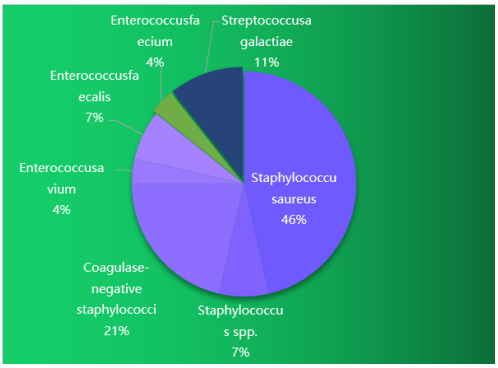

Figure 7: Specific distribution of Gram-positive bacteria.

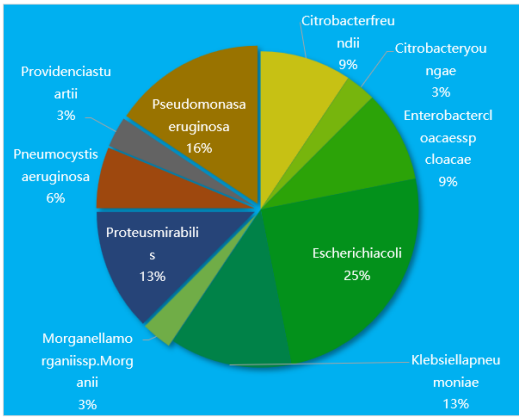

Figure 8: Specific distribution of Gram-negative bacteria.

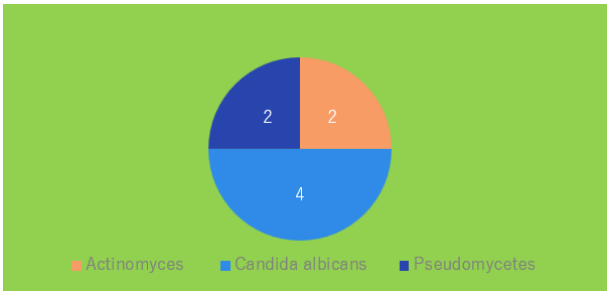

Figure 9: Specific distribution of fungal infections.

Analysis of the Drug Resistance of Bacteria

Due to the complexity of the bacterial species of the mixed infection, the mixed infection of G+ bacteria, from the drug sensitivity should be selected from the β-lactam broad-spectrum antibiotics at the same time combined with anti-anaerobic antibiotics; if the patient also combined with fungal infection, can be added with antifungal drugs. In patients with severe diabetic foot ulcers, necrosis and infected erosions, a combination of antibiotics should be considered in the early stages of the procedure, as one antibiotic alone may not be effective.

Analysis of Drug Resistance in G+ Bacteria

G+ bacteria mainly include *Staphylococcus spp.*, *Streptococcus spp.* and *Enterococcus spp*. The resistance of each genus to common antibiotics varies (Table 4). Staphylococcus spp. had higher resistance rates to penicillin G (95.24%), erythromycin (85.71%) and benzathine (85.71%), and were all sensitive to vancomycin, linezolid, daptomycin and teicoplanin; *Streptococcus spp.* had the highest resistance rate to erythromycin at 66.67%, followed by tetracycline (66.67%), all sensitive to vancomycin and all sensitive to teicoplanin. One strain of linezolid-resistant Streptococcus was also found; *Enterococcus spp.* showed higher resistance to ceftriaxone, clindamycin and erythromycin with 75.00%, 87.50% and 75.00% respectively, while one strain of linezolid-resistant *Enterococcus faecium* and three strains of vancomycin-resistant Enterococcus were found (Table 3).

Gram-Positive Germs	***Enterococcus***		***Streptococcus***		***Staphylococcus***	
Antibiotics	R/N	%	R/N	%	R/N	%
Ampicillin	02/08	25	-	-	40/42	95.24
Oxacillin	01/08	12.5	-	-	36/42	85.71
Ceftriaxone	-	-	02/06	33.33	24/42	57.14
Ceftazidime	01/08	12.5	02/06	33.33	19/42	45.24
Cefepime	0/8	0	01/06	16.67	15/42	35.71
Ciprofloxacin	03/08	37.5	-	11.76	22/42	52.38
Clindamycin	07/08	87.5	04/06	66.67	25/42	59.52
Levofloxacin	03/08	37.5	02/06	33.33	18/42	42.86
Erythromycin	06/08	75	04/06	66.67	36/42	85.71
Gentamycin	04/08	50	-	-	20/42	47.62
Linezolid	01/08	12.5	0/6	0	0/42	0
Rifampicin	04/08	50	-	-	5/42	11.9
Teicoplanin	0/8	0	0/6	0	0/42	0
Daptomycin	0/8	0	0/6	0	0/42	0
Vancomycin	01/08	12.5	0/6	0	0/42	0

Table 3: Resistance analysis of Gram-positive bacteria.
R: Number of Resistant Germs; N: Total Number of Isolated Germs; %: Percentage

Drug Resistance Analysis of G- Bacteria

G- bacteria were predominantly *Escherichia coli*, *Aspergillus chimaera* and *Pseudomonas aeruginosa*. The resistance rate of *E. coli.* to ampicillin sulbactam was the highest (87.50%), followed by ciprofloxacin, cefothiazole and ceftazidime (62.50%), while one strain of ertapenem-resistant and two strains of meropenem-resistant *E. coli.* were found; *P. aeruginosa* had the highest resistance rate to ampicillin sulbactam (100%), cefothiazole (70%), and was completely sensitive to tigecycline. Meropenem was resistant in only 1 case each; *Aspergillus chimaera* was significantly resistant to tetracycline (75.00%), ertapenem, tigecycline was completely sensitive and meropenem had only 1 resistant organism. *Klebsiella pneumoniae* had the highest resistance to *ampicillin sulbactam* and gentamicin, both at 62.50%, and were fully susceptible to tigecycline, ertapenem and *piperacillin tazobactam* (Table 4).

Gram-negative bacteria from the Enterobacteriaceae family were predominant.

Table 4: Analysis of drug resistance in Gram-positive bacteria.

Gram-Negative Germs	***Escherichia Coli***		***Proteus Mirabilis***		***Enterobactercloacae Ssp Cloacae***		***Pseudomonas Aeruginosa***		***Klebsiella Pneumoniae***	
Antibiotics	R/N	%	R/N	%	R/N	%	R/N	%	R/N	%
Amikacin	1/16	6.25	1/8	12.50	0/6	0	3/10	30.00	1/8	12.50
Ampicillin/Sulbactam	14/16	87.50	4/8	50.00	5/6	83.3	10/10	100.00	5/8	62.50
Ceftriaxone	10/16	62.50	3/8	37.50	2/6	33.33	7/10	70.00	4/8	50.00
Cefepime	5/16	31.25	1/8	12.50	2/6	33.33	2/10	20.00	2/8	25.00
Ceftazidime	9/16	56.25	3/8	37.50	3/6	50.00	10/10	100.00	3/8	37.50
Ciprofloxacin	10/16	62.50	3/8	37.50	1/6	16.67	3/10	30.00	3/8	37.50
Ertapenem	1/16	6.25	0/8	0	0/6	0.00	1/10	10.00	0/8	0.00
Gentamycin	7/16	43.75	3/8	37.50	1/6	16.67	2/10	20.00	5/8	62.50
Imipenem	1/16	6.25	2/8	25.00	0/6	0.	1/10	10.00	2/8	25.00
Levofloxacin	9/16	56.25	3/8	37.50	2/6	33.33	2/10	20.00	2/8	25.00
Meropenem	2/16	12.50	1/8	12.50	0/6	0	3/10	30.00	1/8	12.50
Minocycline	1/16	6.25	6/8	75.00	1/6	16.67	2/10	20.00	2/8	25.00
Tigecycline	0/16	0.00	3/8	37.50	0/6	0	0/10	0	0/8	0
Piperacillin/Tazobactam	2/16	12.50	1/8	12.50	1/6	16.6	2/10	20.00	0/8	0

R: Number of Resistant Germs; N: Total Number of Isolated Germs; %: Percentage

We observed that *Staphylococcus*, *Enterococcus* and *Streptococcus* were the three most common Gram-positive cocci isolated. In terms of bacterial resistance, Gram-positive bacteria, *Staphylococcus aureus*, were also significantly resistant to penicillin, erythromycin and clindamycin. Gram-negative bacteria showed a higher rate of resistance to ciprofloxacin and ampicillin. Given this extrapolated result, we recommend vancomycin, linezolid, carbapenems (Ertapenem, imipenem, etc.) or piperacillin-tazobactam as the initial empirical antibiotic therapy.

Based on our data, we further emphasise the importance of tissue bacteria and culture of diabetic foot ulcers, with initial use of advanced antibiotics or empirical control of infection as described above, and later treatment with sensitive antibiotics selected based on wound culture results to control infection early and reduce the amputation rate or plane of amputation.

The limitation of our study is that we selected the results of bacterial cultures from inpatients at our hospital in our region, some patients were admitted multiple times, and most patients were treated with antibiotics, whether given

orally or intravenously, which would have biased the culture results.

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