Original Article

Investigation and analysis of the characteristics and drug sensitivity of bacteria in skin ulcer infections

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Abstract

Purpose: Skin ulcer is a common type of disease affecting patients’ health and quality of life, and bacterial infection increases the difficulty of its management.

Methods: The present study collected the results of bacterial culture sampled from the surface of 110 cases of skin ulcers at our hospital from January 2011 to December 2012. We analyzed the constituent ratios of ulcer surface bacteria, the change in the main infectious bacteria and the results of drug-sensitivity testing for common bacteria. In addition, the characteristics of bacterial infection of skin ulcers were summarized.

Result: Of the 110 samples, 90 isolated bacteria were cultured. Sixty-one were Gram-negative bacteria, mainly comprising Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae and Escherichia coli. In addition, 23 isolates were Gram-positive bacteria, mainly comprising Staphylococcus aureus and Enterococcus faecalis. The probability of a negative bacterial culture in 2012 was significantly lower than that in 2011 (16.7% vs. 40.0%, p < 0.01). Moreover, the probability of P. aeruginosa infection in 2012 was significantly higher than that in 2011 (31.7% vs. 14.0%, p < 0.01). P. aeruginosa was resistant to seven commonly used antibiotics. Both K. pneumoniae and E. coli had higher resistance to ampicillin. E. cloacae were not sensitive to piperacillin/tazobactam. Acinetobacter baumannii was resistant to all the tested drugs. S. aureus, E. faecalis and Staphylococcus epidermidis had high resistance to clindamycin. There was other drug resistance to reflect the higher rate of skin bacterial resistance.

Conclusion: Skin bacterial resistance rate is high. Gram-negative bacteria gradually account for the majority, and P. aeruginosa becomes the most important skin infection pathogen. These characteristics of bacterial infections of skin ulcers provide a significant reference for guiding the selection of antibiotics, better controlling infections of skin ulcers and accelerating the healing of skin ulcers.

Introduction

Skin ulcer is tissue defects of the skin that extends to the dermis and hypodermis, which can be induced by multiple causes. It is a clinically common disease that often affects patients’ quality of life and results in high medical costs. In addition, severe cases can threaten patient’s life. Skin ulcers are frequently accompanied by bacterial infections, making the treatment procedure hard and outcome unsatisfactory. In particular, with the increasing use and misuse of antibiotics, the probability of infections by various opportunistic infectious bacteria and drug-resistant bacteria has significantly increased, which further increases the difficulty of treatment. To explore the constituent ratios and drug-resistance of the surface bacteria on skin ulcers, the present study analyzed 110 such cases hospitalized in the Dermatology Department of Daping Hospital, Third Military Medical University, China from Jan. 2011 to Dec. 2012 and presented the results below.
Materials and methods

General data

Altogether 110 patients with skin ulcers were enrolled in this study, including 74 male and 36 female, with the mean age of 52.7 years, minimally 5 years and maximally 92 years. The shortest course of disease was 3 days and longest 34 years, mean 17 months. The causes included various wounds (burns, electric shock, scald, traffic accident wounds, scratch wounds, etc), surgical operations, diabetes, varicose veins and others. The involved areas included the feet (49 cases, 44.5%), the shank (45 cases, 40.9%), the torso (10 cases, 9%), the upper limbs (3 cases, 2.7%) and the head and face (3 cases, 2.7%). All the patients had received some treatment like dressing, debridement, folk recipe, etc before admission to our department, and 72 (65.5%) of them experienced external application of Chinese medicinal herbs, headache powder or antibiotic powder.

Bacterial culture

On the day of admission to the hospital, for each patient, the secretion from the surface of the skin ulcer was collected using a sterile cotton swab, according to the established protocol, before any antibiotics have been administered. The samples were placed in sterile test tubes, sealed and immediately sent for testing. Pathogenic bacteria isolation and culturing were carried out based on the sterile test tubes, sealed and immediately sent for testing. Pathogenic bacteria isolation and culturing were carried out based on the sterile test tubes, sealed and immediately sent for testing. Pathogenic bacteria isolation and culturing were carried out based on the National Clinical Testing Protocols (China).

Bacterial identification and drug sensitivity test

Bacterial identification and drug sensitivity test employed the VITEK 60 automated microbe analyzer (bioMérieux, France) and its supporting identification cards and drug sensitivity cards. Mueller-Hinton (M-H) agar was purchased from Hangzhou Tianhe Microbiology Ltd, China.

Statistical processing

SPSS 13.0 statistical processing software was used for statistical analysis, and data were expressed as constituent ratios. Data analysis adopted the descriptive method, and the intergroup comparison of constituent ratio employed the \( \chi^2 \) test, with \( p < 0.05 \) indicating a statistically significant difference and \( p < 0.01 \) indicating a very significant difference.

Results

Pathogenic bacteria culture

Of the 110 samples, 30 (27.3%) bacterial culture results were Gram-positive and 80 (72.7%) were Gram-negative. Altogether, 90 isolated bacteria were cultured; and the cultures from 10 patients demonstrated superinfection with 2 types of bacteria.

Constituent ratios of pathogenic bacteria

Among the 90 identified bacterial isolates, 61 (67.8%) were Gram-negative bacteria, mainly comprising Pseudomonas aeruginosa (26 isolates, 28.9%), Klebsiella pneumoniae (8 isolates, 8.9%), Enterobacter cloacae (6, 6.7%) and Escherichia coli (E. coli) (6, 6.7%). In addition, 23 isolates were Gram-positive bacteria (25.6%), mainly comprising Staphylococcus aureus (14 isolates, 15.6%) and Enterococcus faecalis (5 isolates, 5.6%). The constituent ratios of the bacterial cultures are shown in Table 1.

Table 1

| Pathogenic bacteria (n = 90) | No. | Percentage (%) |
|-----------------------------|-----|----------------|
| Gram-negative bacteria      | 61  | 67.8           |
| Pseudomonas aeruginosa      | 26  | 28.9           |
| Klebsiella pneumoniae       | 8   | 8.9            |
| Enterobacter cloacae        | 6   | 6.7            |
| Escherichia coli            | 6   | 6.7            |
| Acinetobacter baumannii     | 3   | 3.3            |
| Serratia marcescens         | 3   | 3.3            |
| Proteus                     | 3   | 3.3            |
| Morganella morgani Morgan subsp. | 2 | 2.2 |
| Raj's Pseudomonas Salmonella | 2  | 2.2            |
| Klebsiella oxytoca          | 1   | 1.1            |
| Keshi Citrobacter           | 1   | 1.1            |
| Gram-positive bacteria      | 23  | 25.6           |
| Staphylococcus aureus       | 14  | 15.6           |
| Enterococcus faecalis       | 5   | 5.6            |
| Staphylococcus epidermidis  | 3   | 3.3            |
| Staphylococcus haemolyticus | 1   | 1.1            |
| Others (Contaminating bacteria) | 6 | 6.7 |

Notes: Others are contaminating bacteria, such as coagulase negative staphylococcus, etc.

Analysis of bacterial changes

This analysis was performed for the two main types of infectious bacteria in the ulcer surface, S. aureus and P. aeruginosa, according to the number of positive specimens obtained from Jan. 2011 to Dec. 2011 (year of 2011) and Jan. 2012 to Dec. 2012 (year of 2012). Results showed that the probability of a negative bacterial culture in 2012 was significantly lower than that in 2011 (16.7% vs. 40.0%, \( p < 0.01 \)). Moreover, the probability of P. aeruginosa infection in 2012 was significantly higher than that in 2011 (31.7% vs. 14.0%, \( p < 0.01 \)) (Table 2).

Table 2

| Year | Bacterial species Total | No bacteria | S. aureus | P. aeruginosa | Others |
|------|------------------------|-------------|-----------|--------------|--------|
| 2011 | 20                     | 6           | 7         | 24           | 50     |
| 2012 | 10                     | 8           | 19        | 26           | 60     |
| \( \chi^2 \) | 7.486          | 0.044       | 4.716     | 0.240        |       |
| \( p \) | 0.006*               | 0.835       | 0.030*    | 0.625        |       |

\( *p < 0.05 \), compared with the two years.

Analysis of the drug sensitivity of ulcer surface bacteria

Drug sensitivity of Gram-negative bacteria (Table 3)

For P. aeruginosa (26 isolates), all (100.0%) of the isolates were resistant to ampicillin, ampicillin/sulbactam, cephazolin, cefotetan, ceftriaxone, furantoin and sulfamethoxazole/trimethoprim (SMZ/TMP); the vast majority (>90.0%) of the isolates were sensitive to amikacin, imipenem and piperacillin/tazobactam. For K. pneumoniae (8 isolates), <50.0% of the isolates were sensitive to piperacillin, ampicillin and furantoin; whereas all (100.0%) were sensitive to amikacin, ciprofloxacin, imipenem, piperacillin/tazobactam, SMZ/TMP and levofloxacin. For E. cloacae (6 isolates), <50.0% were sensitive to piperacillin/tazobactam and ampicillin/sulbactam, whereas all (100.0%) were sensitive to amikacin, aztreonam, ceftazidime, ceftriaxone, imipenem and levofloxacin. For E. coli (6 isolates), <50.0% of the isolates were sensitive to gentamicin, tobramycin and ampicillin, whereas all (100.0%) were sensitive to amikacin, imipenem, ertapenem, cefotetan and others.
The present study analyzed the bacterial culture results from 110 skin ulcer patients treated in our department from Jan. 2011 to Dec. 2012. The results and bacterial characteristics are summarized as follows:

Drug sensitivity of Gram-negative bacteria (Table 4)

For *S. aureus* (14 isolates), <50.0% of the isolates were sensitive to azithromycin, clarithromycin, clindamycin, erythromycin and penicillin G, whereas all (100%) of the isolates were sensitive to linezolid, moxifloxacin, furantoin, quinupristin/dalfopristin, tigemycin and vancomycin. For *Enterococcus faecalis* (5 isolates), <50.0% were sensitive to quinupristin/dalfopristin, SMZ/TMP, tetracycline, clindamycin and erythromycin, whereas all (100%) of the isolates were sensitive to tigemycin, vancomycin and penicillin G. For *Staphylococcus epidermidis* (3 isolates), <50.0% were sensitive to levofloxacin, oxacillin, cefaclor, cefotaxime, cefuroxime, ciprofloxacin, gentamicin, SMZ/TMP, azithromycin, clarithromycin, clindamycin, erythromycin and penicillin G, whereas all (100%) of the isolates were sensitive to linezolid, furantoin, quinupristin/dalfopristin, tigemycin, rifampin and tetracycline.

Discussion

Skin ulcers compromise the skin's natural defense and lead to scar formation as well as poor blood perfusion, which further result in decreased local immunity. Moreover, the ulcer surface becomes an ideal colony for bacterial reproduction and invasion; as a result, mild cases may encounter difficulty in skin ulcer healing, and severe cases may need amputations or develop systemic infection and septicemia. These bad consequences significantly affect patients' physical health and their quality of life. Therefore, it is essential to culture ulcer surface bacteria, test drug sensitivity and analyze the bacterial infection pattern, through which we can monitor the distribution/variation of clinically common infectious bacteria and make appropriate selection of antibiotics.

(1) According to the constituent ratio for pathogenic bacteria, Gram-negative bacteria were predominantly isolated; of the 110 patients evaluated, 30 were infected with Gram-negative bacteria (27.3%). From these patients, a total of 90 bacterial isolates were cultured and 10 patients demonstrated simultaneous infection with 2 types of bacteria. There were 61 Gram-negative isolates (67.8%), of which the main cultured organism was *P. aeruginosa* (26 isolates, 28.9%), and 23 Gram-positive isolates (25.6%), among which the main cultured organism was *S. aureus* (14 isolates, 15.6%). The most common bacteria cultured from these ulcer infections were *P. aeruginosa* and *S. aureus*, which is consistent with the findings reported by Renner et al. However, it is worth noting that, unlike previous studies showing that Gram-positive bacteria, especially *S. aureus*, were the most...
common bacterial isolates in skin ulcer infections, Gram-negative bacteria, especially *P. aeruginosa*, were more commonly isolated from the cases analyzed in the present study. We further performed statistical analysis of the annual changes in the types of infectious bacteria, and found that the probability of *P. aeruginosa* infection in 2012 was significantly higher than that in 2011 (31.7% vs. 14.0%, *p* < 0.01). There was no significant difference in ratios of other bacteria between the two years. The finding highlights the emergence of *P. aeruginosa* as one of the most important pathogenic bacteria in nosocomial infections in recent years. Decreases in immunity resulting from various causes, including certain types of operations and invasive treatment procedures, make patients even more susceptible to bacteria infections. In the present study, all of the patients evaluated had undergone treatment with various medicines (Chinese medicinal herbs, headache powder, folk recipe, etc) and debridement outside hospital; some patients also had external application of groundened penicillin or cephalosporin drugs. All these treatments likely contribute to the increased proportion of nosocomial bacterial infections. Moreover, the results of the present study suggest that changes in the type of bacterial infection may be correlated with improper diagnosis and treatment, as well as nosocomial infections, but this hypothesis requires further investigation.

(2) Infections with conditional pathogenic bacteria were detected. Conditional pathogenic bacteria are harmless in healthy human bodies but can become pathogenic under specific conditions. For example, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *E. cloacae*, *E. coli* and Proteus spp. were the most commonly isolated conditional pathogenic bacteria in the present study. Infection by conditional pathogenic bacteria is related to factors such as patient immunity, wound surface conditions and bacterial pathogenicity. The results of the present study suggest that, during the treatment, attention should be given to the patient’s systemic immune status, nosocomial infections and bacterial group imbalances, in an effort to reduce infections with conditional pathogenic bacteria.

(3) Analysis of the drug sensitivity results indicated that the drug resistance situation is serious. The present study found high rate of bacterial drug resistance. Among the Gram-negative bacteria, the drug-resistance rate to commonly applied antibiotics, including ampicillin, ceftriaxone, cephalzin and piperacillin, was atypically high. All isolates of *P. aeruginosa* demonstrated the widest range of antibiotic resistance to drugs including ampicillin, ampicillin/sulbac-tam, cephalzin, cefotetan, ceftriaxone, furantoin and SMZ/TMP. Moreover, all the three isolates of *A. baumannii* were resistant to all tested drugs, which made it a super drug-resistant bacterium. The antibiotics to which most Gram-negative bacteria were sensitive included amikacin, imipenem and piperacillin/tazobactam. Among the Gram-positive bacteria, the majority of isolates were resistant to most antibiotics, such as azithromycin, clarithromycin, clindamycin and erythromycin; however, the drug resistance of *S. epidermidis* was even greater. These results indicate that the infection bacteria at the ulcer surface have a strong resistance to the commonly used antibiotics. This situation may be related to the abuse of antibiotics in clinics, as well as the lag in research and development of antibiotics relative to bacterial evolution.

The severe and multiple drug resistance of *P. aeruginosa* and *A. baumannii* were especially prominent; moreover, these organisms can easily form bacterial biofilm on ulcer surfaces, which significantly compromises the treatment effect. Infections by *P. aeruginosa* and *A. baumannii* have become a difficult problem in clinical settings. Therefore, in clinical practice, we should standardize the procedures to avoid the abuse of antibiotics and cross infection. We also need to improve bacterial culture and drug sensitivity testing to guide clinical medication and improve the therapeutic effect. At the same time, it can reduce the waste of medical resources and patient time and money.

In summary, the present study analyzed the characteristics of infectious bacteria on the surfaces of skin ulcers in an attempt to provide reference for clinicians, laboratory technicians, microbiologists and pharmacologists. A timely understanding of the changes occurring in infectious bacterial groups can help to better control infections of skin ulcers, accelerate skin ulcer healing, reduce the probability of amputation/crippling and the spread of systemic infections, reduce drug abuse and guide the correct use of antibiotics.

References

1. Mansilha A, Brandão D. Guidelines for treatment of patients with diabetes and infected ulcers. *J Cardiovasc Surg (Torino)*. 2013;54(1 Suppl 1):193–200.

2. Renner R, Sticherling M, Rüger R, et al. Persistence of bacteria like Pseudomonas aeruginosa in non-healing venous ulcers. *Eur J Dermatol*. 2012;22(6):751–757. http://dx.doi.org/10.1684/ejd.2012.1865.

3. Gjødsbøl K, Christensen JJ, Karlsmark T, et al. Multiple bacterial species reside in chronic wounds: a longitudinal study. *Int Wound J*. 2006;3(3):225–231.

4. Bessa LJ, Fazii P, Di Giulio M, et al. Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. *Int Wound J*. 2012;9:47–52. http://dx.doi.org/10.1111/j.1742-481X.2012.120493.

5. Coetzee E, Rode H, Kahn D. Pseudomonas aeruginosa burn wound infection in a dedicated paediatric burns unit. *S Afr J Surg*. 2013;51:50–53. http://dx.doi.org/10.7196/sajs.1134.

6. Bowling FL, Dissanayake SU, Jude EB. Opportunistic pathogens in diabetic foot lesions. *Curr Diabetes Rev*. 2012;8(3):195–199.

7. Hurlow J, Bowler PG. Potential implications of biofilm in chronic wounds: a case series. *J Wound Care*. 2012;21:100–110.

8. Bowler PG, Welsby S, Towers V, et al. Multidrug-resistant organisms, wounds and topical antimicrobial protection. *Int Wound J*. 2012;9:387–396. http://dx.doi.org/10.1111/j.1742-481X.2012.00991.x.