

Original Article

Distribution and drug sensitivity of bacteria causing nosocomial infection in patients with hand and foot trauma

Meihong Wang^{1*}, Hongyong Zhu^{2*}, Yanling Li³, Lexin Wang⁴, Zengwu Han⁵, Jigang Zhou⁶

¹Department of Laboratory, Chunan Hospital of Traditional Chinese Medicine, Hangzhou, Zhejiang Province, China; ²Department of Orthopedics, Penglai Traditional Chinese Medicine Hospital, Yantai, Shandong Province, China; Departments of ³Integration of Neck, Shoulder, Waist and Leg Pain, ⁴Orthopedics, Rizhao Hospital of Traditional Chinese Medicine, Rizhao, Shandong Province, China; ⁵The Second Department of Orthopedics, Gucheng County Hospital of Hebei Province, Hengshui, Hebei Province, China; ⁶Department of Orthopedics, Jinxiang People's Hospital, Jining, Shandong Province, China. *Equal contributors and co-first authors.

Received December 12, 2019; Accepted January 8, 2020; Epub April 15, 2020; Published April 30, 2020

Abstract: Objective: To provide a reference for the treatment of bacteria causing nosocomial infection in patients with hand and foot trauma. Methods: This retrospective study was conducted in 160 patients with bacteria causing nosocomial infection in hand and foot trauma. Pathogenic bacteria were isolated and identified by an automatic bacterial identification system. Distribution characteristics and drug resistance of these bacteria were then analyzed. Results: In total, 48 gram-positive bacteria (28.9%) and 118 gram-negative bacteria (71.1%) were identified. Most gram-positive bacteria had strong drug resistance. Their resistance to penicillin and cefotaxime were strong (both about 10.0%). However, their resistance to vancomycin was weak (over 90.0%). Gram-negative bacteria commonly present in hospitals are resistant to most antibacterial drugs at different levels. However, they were sufficiently sensitive to imipenem and Shupushen, reaching a sensitivity of over 85.0%. Conclusion: Gram-negative bacteria are the main bacteria causing nosocomial infection in patients with hand and foot trauma. These bacteria are resistant to antibacterial drugs commonly used in clinical practice. The distribution characteristics and drug resistance of pathogenic bacteria play an important role in rational clinical medication.

Keywords: Hand and foot trauma, gram-positive bacteria, gram-negative bacteria, distribution of pathogenic bacteria, drug sensitivity analysis

Introduction

In recent years, the incidence of hand and foot trauma has significantly increased. Patients with moderate and severe hand and foot trauma are treated with surgery. Some open surgical wounds contaminated to different degrees are prone to wound infection. A variety of complications are observed and risks of surgery are raised. Patients can be in danger of losing their life. Therefore, the prevention of anti-infection is always performed during surgery [1, 2].

With the advent and widespread application of antibacterial drugs, infection has been efficiently controlled for a long time. However, there are more and more bacteria with drug resistance. The development of antibacterial

drugs falls behind the drug resistance of pathogenic bacteria, making infection a global medical problem [3, 4]. Targeted application of antibacterial drugs and decreased range and frequency of broad-spectrum drugs contribute to the reduction of both generation and spread of drug-resistant bacteria. There is an urgent need to solve this major medical problem as soon as possible [5].

Here, an epidemiological study of bacteria causing nosocomial infection in patients with hand and foot trauma was conducted. The types of pathogenic bacteria were identified, and their drug resistance was analyzed. With a more standardized anti-infection treatment and controlled generation and spread of drug-resis-

Distribution and drug sensitivity of bacteria

tant bacteria, the application of antibacterial drugs is more rational and effective.

Materials and methods

General information

A retrospective study was performed in 160 patients diagnosed with bacteria causing nosocomial infection in hand and foot trauma in Jinxiang People's Hospital between September 2017 and September 2019. To be specific, there were 88 males and 72 females. These patients were between 17 and 54 years old, and the average age was 32.5 ± 12.4 years old. They were diagnosed under the criteria for bacteria causing nosocomial infection described in document [2001] No. 2, which was enacted by the Department of Pharmaceutical Affairs of the Ministry of Health [6]. Wound exudates, sputum, and urine were the three major sample types.

This study was approved by the Ethics Committee of Jinxiang People's Hospital. An informed consent was signed by patients.

The isolation, cultivation and identification of pathogenic bacteria were conducted according to the methods described in the National Guidelines for Clinical Inspection Operation. Drug sensitivity test was also performed using the standardized methods.

Instruments, reagents and bacteria strains

Instruments: Full-automatic microbial identification instrument (Biolog, USA); CO₂ incubator (Shanghai Yiheng Scientific Instrument Co., Ltd., China); biological safety cabinet (Jinan Xinbeixi Biotechnology Co., Ltd., China); optical microscope (Olympus, Japan).

Reagents: Antimicrobial susceptibility test strips (Beijing Tiantan Biological Products Co., Ltd., China); culture bottles (Shandong Xinke Biotechnology Co., Ltd., China); bacteria identification and drug sensitivity test plates (BioMérieux, France); blood, China blue, chocolate, and MacConkey agar medium (Beijing Baiaolaibo Technology Co., Ltd., China).

Bacteria strains: *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27853, and *Escherichia coli* ATCC25922 (Jiangsu

provincial Center for Disease Prevention and Control).

Methods

Isolation, cultivation and identification of pathogenic bacteria: Isolation and cultivation: The infected area of the patients was cleaned using 0.9% physiological saline. After cleaning, the exudate existing in patients' infected part was collected by a sterile cotton swab. The swab was then preserved in a sterile bag and sealed before delivering to the cultivation room. Sputum and urine were directly packaged in a sterile bag. Samples were handled in a biological safety cabinet. With the help of inoculating loops, these samples were seeded in plates prepared with China blue, blood, and McCain agar medium, respectively. Thereafter, they were cultured at 37°C for 48 h [7].

Identification: The whole procedure was performed using VITEK 2 Compact System software. Specifically, bacteria were made into suspensions and injected into the test card (gram-positive or gram-negative) of the full-automatic microbial identification instrument. After sealing, the card was placed into the CO₂ incubator. The test result, which is based on the growth of bacteria cultured in the biochemical reaction wells, was recorded by the instrument [8].

Drug sensitivity test: The pathogenic bacteria successfully isolated from the three kinds of samples were purified. Drug sensitivity assay, which was performed using K-B paper diffusion method, was conducted on bacteria with a high prevalence. The procedure is described as below: inoculate bacteria on the surface of drug sensitivity test plates, and then put filter papers containing special antibacterial drugs on the surface of the plates. With the permeation of drugs, a transparent antibacterial circle was formed around the paper sheet. The stabilized size of the antibacterial circled was applied to evaluate the sensitivity of bacteria to drugs. Bacteria were divided into drug-resistant, moderately sensitive, and sensitive. Among them, moderately sensitive and sensitive bacteria were not drug-resistant [9].

Statistical analysis

The enumeration data were analyzed using SPSS statistical software version 14.0.

Distribution and drug sensitivity of bacteria

Table 1. Bacteria causing nosocomial infection in patients with hand and foot trauma

Bacteria	Number (n)	Ratio (%)
Gram-positive bacteria	48	28.9%
Staphylococcus aureus	18	10.8%
Enterococcus	16	9.6%
Staphylococcus aureus	5	3.0%
Staphylococcus epidermidis	4	2.4%
Other gram-positive bacteria	5	3.0%
Gram-negative bacteria	118	71.1%
Pseudomonas aeruginosa	27	16.3%
Acinetobacter baumannii	21	12.7%
Escherichia coli	20	12.0%
Serratia fading	11	6.6%
Enterobacter cloacae	11	6.6%
Klebsiella pneumoniae	8	4.8%
Aeromonas hydrophila	8	4.8%
Proteus vulgaris	5	3.0%
Other gram-negative bacteria	7	4.2%
In total	166	100%

Results

Distribution of pathogenic bacteria

Bacteria strains: In total, 48 gram-positive bacteria (28.9%) and 118 gram-negative bacteria (71.1%) were successfully identified. Among them, *Staphylococcus aureus* (10.8%) and *Enterococcus* (9.6%) were the top two gram-positive bacteria, while *Pseudomonas aeruginosa* (16.3%), *Acinetobacter baumannii* (12.7%), and *Escherichia coli* (12.0%) were the three main gram-negative bacteria (**Table 1**). As displayed in **Figure 1**, the number of pathogenic bacteria was different. Moreover, the total number of gram-negative bacteria (118) was higher than that of gram-positive bacteria (48).

Morphological identification

Bacteria with high prevalence were observed under oil immersion microscope (100×, 1.25 oil). Enterococci were round or oval, arranged in single, paired or short chain, and without flagella (**Figure 2A**); *Staphylococcus aureus* was golden yellow, arranged in short and irregular grape-shapes (**Figure 2B**); *Pseudomonas aeruginosa* was arranged irregularly, and the edges of colonies were extended in the shape of an umbrella (**Figure 2C**); *Escherichia coli* was pink-

colored, rod-shaped, and with a few sticky colonies (**Figure 2D**). The results observed under the microscope were consistent with that of instrument examination.

Drug sensitivity of major gram-positive bacteria

As displayed in **Table 2**, resistances of *Staphylococcus aureus* and *Enterococcus* to different antibacterial drugs were about 60%. Specifically, their resistances to penicillin and cefotaxime were strong (about 90.0% resistant), while their resistance to vancomycin was weak (about 8%). Similarly, **Figure 3** showed that resistances of major gram-positive bacteria to penicillin and cefotaxime were strong (91.17%), while resistance to vancomycin was weak (8.8%). These results indicated that gram-positive bacteria present in our hospital are resistant to most antibacterial drugs, and vancomycin is the preferred antibacterial drug for the treatment of these bacteria.

Drug sensitivity of major gram-negative bacteria

As shown in **Table 3**, gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Escherichia coli*, were widely present in our hospital and resisted most antibacterial drugs with a sensitivity ranging from 50% to 70%. However, their resistances to imipenem and Shupushen were only about 15%, suggesting that these bacteria were sufficiently sensitive to the two drugs. Similarly, **Figure 4** displayed that resistance of major gram-negative bacteria to imipenem (17.65%) and Shupushen (16.17%) were strong. These results suggested that imipenem and Shupushen are the two preferred antibacterial drugs for the treatment of gram-negative bacteria present in our hospital.

Discussion

Recently, the incidence of hand and foot trauma has significantly increased. Patients with large wounds are prone to contamination. They can suffer from delayed prognosis recovery and even disability, which severely influences the life quality of patients [10, 11]. Therefore, it is necessary to take effective measures to prevent nosocomial infection. The primary approach applied in the prevention and treat-

Distribution and drug sensitivity of bacteria

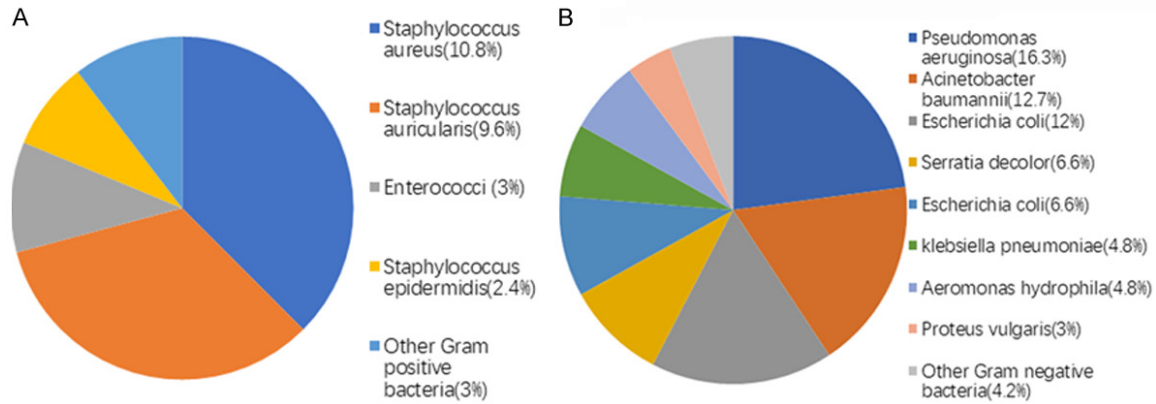


Figure 1. Distribution characteristics of pathogenic bacteria. A. Distribution characteristics of gram-positive bacteria. B. Distribution characteristics of gram-negative bacteria.

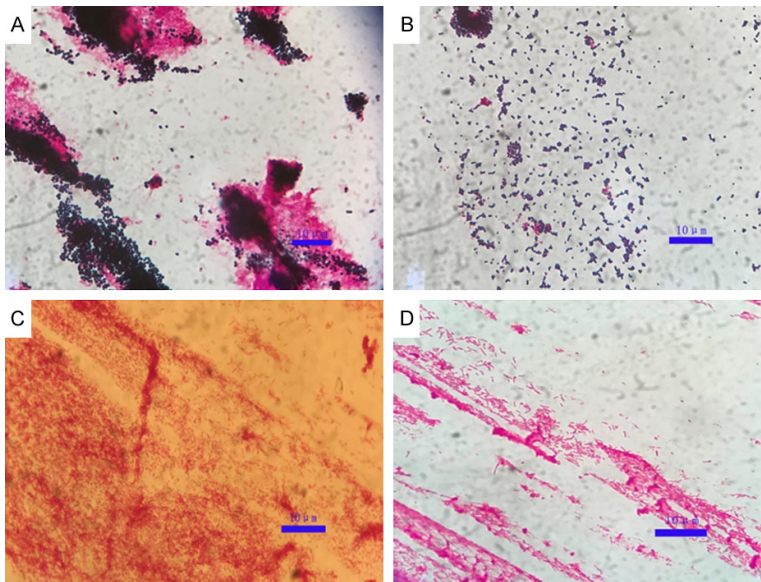


Figure 2. Morphology of several pathogenic bacteria (100 \times , 1.25 oil). A. Enterococcus (gram-positive bacteria). B. Staphylococcus aureus (gram-positive bacteria). C. Pseudomonas aeruginosa (gram-negative bacteria). D. Escherichia coli (gram-negative bacteria).

Table 2. Resistance of major gram-positive bacteria to antibacterial drugs (n, %)

Antibacterial drugs	Staphylococcus aureus (n=18)	Enterococcus (n=16)
Penicillin	16 (88.9%)	15 (93.8%)
Erythromycin	11 (61.1%)	10 (62.5%)
Gentamicin	12 (66.7%)	9 (56.2%)
Vancomycin	2 (11.1%)	1 (6.25%)
Piperacillin	13 (72.2%)	10 (62.5%)
Levofloxacin	13 (72.2%)	11 (68.8%)
Cefazolin	11 (61.1%)	9 (56.2%)
Cefotaxime	17 (94.4%)	14 (87.5%)
Clindamycin	12 (66.7%)	10 (62.5%)
Tigecycline	12 (66.7%)	10 (62.5%)

ment of nosocomial infection is antibacterial therapy. Novel antibacterial drugs have been generated and upgraded year by year. However, resistance of pathogenic bacteria to commonly used antibacterial drugs is gradually increasing, and rises even faster than before [12, 13]. The two main challenges we are facing are the rational selection and abuse control of antibacterial drugs. In order to improve the therapeutic effect of antibacterial drugs and avoid the generation of drug-resistant bacteria, doctors should choose antibacterial drugs rationally [14-16]. Here, 160 patients diagnosed with bacteria causing nosocomial infection in hand and foot trauma in our hospital were enrolled in this study. The distribution characteristics and drug resistance of these bacteria were analyzed.

As shown in this study, the bacteria were mainly gram-negative. In addition, Pseudomonas aeruginosa, Acinetobacter baumannii, and Escherichia coli were the top three (41%). There was no fungal infection, indicating that the use of broad-spectrum antibacterial drugs is relative-

Distribution and drug sensitivity of bacteria

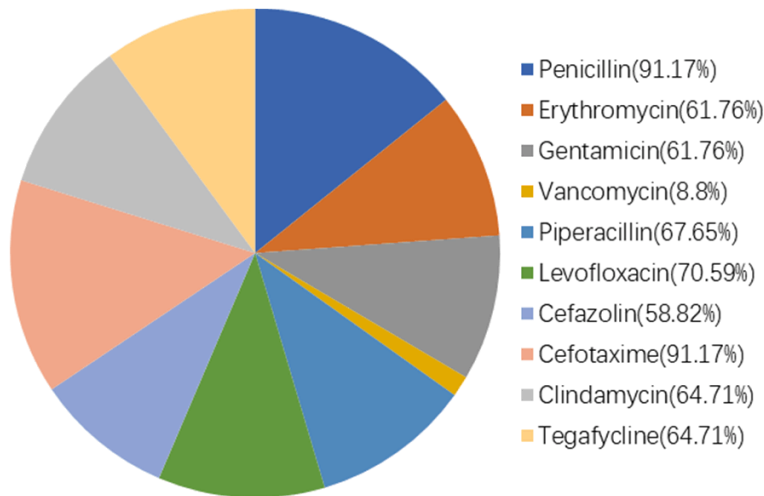


Figure 3. Resistance of major gram-positive bacteria to antibacterial drugs.

Table 3. Resistance of major gram-negative bacteria to antibacterial drugs (n, %)

Antibacterial drugs	<i>Pseudomonas aeruginosa</i> (n=27)	<i>Acinetobacter baumannii</i> (n=21)	<i>Escherichia coli</i> (n=20)
Tobramycin	16 (59.3%)	14 (66.7%)	11 (55.0%)
Amikacin	17 (63.0%)	13 (61.9%)	14 (70.0%)
Shupu Shen	4 (14.8%)	3 (14.3%)	4 (20.0%)
Aztreonam	16 (59.3%)	11 (52.4%)	11 (55.0%)
Ceftazidime	14 (51.9%)	12 (57.1%)	12 (60.0%)
Cefazolin	14 (51.9%)	11 (52.4%)	10 (50.0%)
Imipenem	5 (18.5%)	4 (19.0%)	3 (15.0%)
Ceftriaxone	16 (59.3%)	14 (66.7%)	11 (55.0%)
Levofloxacin	17 (63.0%)	13 (61.9%)	12 (60.0%)
Cefoperazone	15 (55.6%)	11 (52.4%)	14 (70.0%)

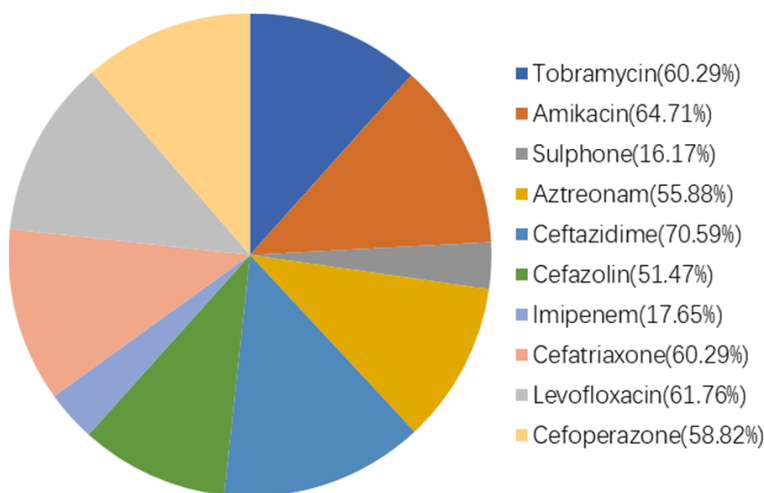


Figure 4. Resistance of major gram-negative bacteria to antibacterial drugs.

ly standardized. Wieland et al. reported that *Pseudomonas aeruginosa* is the main bacteria causing nosocomial infection, which is consistent to our result [17-19]. *Staphylococcus aureus* and *Enterococcus* were the main gram-positive bacteria. When compared with other regions, the prevalence of *Staphylococcus aureus* in our hospital was not high. The difference might be related to the types of sample [20]. The incidence of complex nosocomial infection was rare, suggesting that the control of multiple nosocomial infections of hand and foot wound trauma in our hospital was comprehensive and effective. Medical staff in our hospital pay great attention to the process of hand washing. It was reported that nosocomial infection caused by medical staff account for about 1/3 of infection. Qualified hand washing is the most economical and effective method to prevent the spread of diseases [21].

At present, the increased resistance of pathogenic bacteria to antibacterial drugs was caused by the abuse of these drugs. Nosocomial infection is becoming more and more serious, making the effective treatment of this disease more and more difficult. In this study, we found that resistance of major gram-positive bacteria to antibacterial drugs was strong. The sensitivity of these bacteria to penicillin and cefotaxime were only about 10.0%, while 90.0% resistant to vancomycin. Resistance of major gram-negative bacteria to most antibacterial drugs was different. The sensitivity of these bacteria to imipenem and Shupushen

were both over 85.0%, displaying sufficient anti-infection effect. These results indicated that rational selection of antibacterial drugs in clinical practice is of great importance for the prevention and treatment of nosocomial infection. As for the application of antibacterial drugs, China is facing a grim situation. Phodha et al. reported that useless treatment of about 30% hospitalized patients is caused by the resistance of pathogenic bacteria [22].

In order to improve the therapeutic effect, medical staff should classify and grade susceptibility status of pathogenic bacteria. Here, we only studied the distribution characteristics and drug resistance of pathogenic bacteria at the present stage, and performed no longer-term follow-up analysis. In addition, we merely discussed the distribution characteristics of bacteria causing nosocomial infection in patients with hand and foot trauma. Patients with other diseases were not included. Moreover, the sample size was insufficient. Last but not the least, there were no comprehensive comparisons concerning the occurrence of nosocomial infection and many other factors, such as the quality of medical care, nursing professionalism, medical environment, etc. Therefore, follow-up study should be regularly conducted to monitor, analyze, summarize, and optimize the present research. Subsequent studies will concentrate on expanding the breadth and depth of this study to provide a more rational prevention and treatment measure for nosocomial infection.

In summary, patients with hand and foot trauma are mainly infected with gram-negative bacteria. These bacteria are resistant to antibacterial drugs commonly used in clinical practice. We should be aware of the distribution characteristics and drug resistance of pathogenic bacteria, which are of great importance for the selection of rational clinical medication.

Disclosure of conflict of interest

None.

Address correspondence to: Jigang Zhou, Department of Orthopedics, Jinxiang People's Hospital, No. 117 Jinfeng East Road, Jining 272200, Shandong Province, China. Tel: +86-0537-6560385; Fax: +86-0537-6560385; E-mail: zhoujigang86sy@163.com

References

- [1] Li HK, Rombach I, Zambellas R, Walker AS, McNally MA, Atkins BL, Lipsky BA, Hughes HC, Bose D, Kumin M, Scarborough C, Matthews PC, Brent AJ, Lomas J, Gundle R, Rogers M, Taylor A, Angus B, Byren I, Berendt AR, Warren S, Fitzgerald FE, Mack DJF, Hopkins S, Folb J, Reynolds HE, Moore E, Marshall J, Jenkins N, Moran CE, Woodhouse AF, Stafford S, Seaton RA, Vallance C, Hemsley CJ, Bisnauthsing K, Sandoe JAT, Aggarwal I, Ellis SC, Bunn DJ, Sutherland RK, Barlow G, Cooper C, Geue C, McMeekin N, Briggs AH, Sendi P, Khatamzas E, Wangrangsimakul T, Wong THN, Barrett LK, Alvand A, Old CF, Bostock J, Paul J, Cooke G, Thwaites GE, Bejon P and Scarborough M. Oral versus intravenous antibiotics for bone and joint infection. *N Engl J Med* 2019; 380: 425-436.
- [2] Aldape MJ, Bayer CR, Rice SN, Bryant AE and Stevens DL. Comparative efficacy of antibiotics in treating experimental clostridium septicum infection. *Int J Antimicrob Agents* 2018; 52: 469-473.
- [3] Qiao M, Ying GG, Singer AC and Zhu YG. Review of antibiotic resistance in China and its environment. *Environ Int* 2018; 110: 160-172.
- [4] Wistrand-Yuen E, Knopp M, Hjort K, Koskinie-mi S, Berg OG and Andersson DI. Evolution of high-level resistance during low-level antibiotic exposure. *Nat Commun* 2018; 9: 1599.
- [5] Grenni P, Ancona V and Barra Caracciolo A. Ecological effects of antibiotics on natural ecosystems: a review. *Microchemical Journal* S0026265X17301108.
- [6] Wang YH, Chen JH and Xi-Sheng LI. Distribution and drug susceptibility of pathogens causing nosocomial infections in trauma patients of orthopedics department. *Chinese Journal of Nosocomiology* 2014; 24: 3312-3313.
- [7] Nicoloff H, Hjort K, Levin BR and Andersson DI. The high prevalence of antibiotic heteroresistance in pathogenic bacteria is mainly caused by gene amplification. *Nat Microbiol* 2019; 4: 504-514.
- [8] Kępa M, Mikłasińska-Majdanik M, Wojtyczka RD, Idzik D and Wąsik TJ. Antimicrobial potential of caffeic acid against staphylococcus aureus clinical strains. *Biomed Research International* 2018; 2018: 1-9.
- [9] Bogiel T, Deptula A and Gospodarek E. Evaluation of different methods for detection of metallo-beta-lactamases in pseudomonas aeruginosa clinical isolates. *Pol J Microbiol* 2010; 59: 45-8.
- [10] Del Piñal F, Urrutia E and Klich M. Severe crush injury to the forearm and hand: the role of microsurgery. *Clin Plast Surg* 2017; 44: 233-255.

Distribution and drug sensitivity of bacteria

- [11] Kim JT and Kim SW. Improvement of ischemic or congested wound conditions by reconstruction with microsurgical flaps. *Microsurgery* 2018; 38: 388-394.
- [12] Liu S, Wang M, Zheng L and Guan W. Antimicrobial resistance profiles of bacteria causing nosocomial infection in regional China: a brief report from two tertiary hospitals in China. *Med Sci Monit* 2018; 24: 8602-8607.
- [13] Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y, Ouellette M, Outerson K, Patel J, Cavaleri M, Cox EM, Houchens CR, Grayson ML, Hansen P, Singh N, Theuretzbacher U and Magrini N; WHO Pathogens Priority List Working Group. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018; 18: 318-327.
- [14] Plachouras D, Karki T, Hansen S, Hopkins S, Lyytikainen O, Moro ML, Reilly J, Zarb P, Zingg W, Kinross P, Weist K, Monnet DL and Suetens C; The Point Prevalence Survey Study Group. Antimicrobial use in European acute care hospitals: results from the second point prevalence survey (PPS) of healthcare-associated infections and antimicrobial use, 2016 to 2017. *Euro Surveill* 2018; 23.
- [15] Xie S, Guo Z, Zhou J, Chen G, Feng S and Li Z. Surveillance of antimicrobial resistance in dongguan tungwah hospital during 2016. *Chinese Journal of Infection & Chemotherapy* 2018; 18: 76-83.
- [16] Nimer NA, Abdel Dayem SA, Karim AbouNouar GA and Husni Dakkah AN. Evaluating antibiotic sensitivity patterns of pseudomonas in relation to specimen type in jordanian hospital. *J Pak Med Assoc* 2019; 69: 168-173.
- [17] Wieland K, Chhatwal P and Vonberg RP. Nosocomial outbreaks caused by acinetobacter baumannii and pseudomonas aeruginosa: results of a systematic review. *Am J Infect Control* 2018; 46: 643-648.
- [18] Pachori P, Goyalwal R and Gandhi P. Emergence of antibiotic resistance pseudomonas aeruginosa in intensive care unit; a critical review. *Genes Dis* 2019; 6: 109-119.
- [19] Akbari R, Bafghi MF and Fazeli H. Nosocomial infections pathogens isolated from hospital personnel, hospital environment and devices. *J Med Bacteriol* 2018; 7: 22-30.
- [20] Lynch D, O'Connor PM, Cotter PD, Hill C, Field D and Begley M. Identification and characterisation of capidermicin, a novel bacteriocin produced by staphylococcus capitis. *PLoS One* 2019; 14: e0223541.
- [21] Damani N. Manual of infection prevention and control. *Occupational Medicine* 2012; 62: 311-312.
- [22] Phodha T, Riewpaiboon A, Malathum K and Coyte PC. Annual relative increased in inpatient mortality from antimicrobial resistant nosocomial infections in Thailand. *Epidemiol Infect* 2019; 147: e133.