CLINICAL ARTICLE

Epidemiologic Correlation and Drug Resistance Analysis of Pathogenic Bacteria in Different Open Limb Injury External Conditions

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Objective: To study the epidemiological correlation and drug resistance of external factors of infection caused by open injury of limbs to pathogens.

Methods: This experiment is a retrospective study. We took the geographical location and climate of Nanchang, Jiangxi Province, China as the background, analyzed 2017 strains of pathogens from 1589 patients with limb trauma infection in a University Affiliated Hospital from 2012 to 2017. Patients were divided into three groups according to the type of incision: I, In-hospital infection of clean limb incision, II, In-hospital infection with open injury, III, Community infection with open injury of the limb. Groups II and Groups III were divided into six subgroups according to the causes of trauma, including: accidents from non-motor vehicles, machinery, cutting/piercing, pedestrian injuries, struck by/against, pedal cycles, and other injuries. We found eight common pathogens of orthopedic infection, which were mainly divided into Gram-positive bacteria (G+, mainly including *Staphylococcus*) and Gram-negative bacteria (G-, mainly *Enterobacteriaceae*). The relationship between main pathogens and damage mechanism, apparent temperature and relative humidity was discussed in this study. SPSS v22.0 was used for statistical analysis of the data. Friedman's two-way ANOVA was used to analyze the difference between the injury mechanism and incidence of pathogenic bacteria. Linear regression was used to determine the trend between the incidence of major pathogens and seasonal temperature and humidity. The level of significance was set as $P < 0.05$.

Results: There was no significant difference in the distribution of pathogens between Groups II and Groups III ($P>0.05$). The drug resistance of Groups III was significantly higher than that of Groups II and Groups I. G+ bacteria were resistant to cephalosporin, ceftriaxone and other cephalosporins and erythromycin and other macrolides. They were sensitive to vancomycin and linezolid. G- were resistant to the first- and the second-generation cephalosporins, including cefotetan and cefazolin, and ampicillin and other penicillins, while they were sensitive to third-generation cephalosporins, such as ceftazidime, as well as to levofloxacin and other quinolones, meropenem, and other beta-lactamases. The correlation between the injury mechanism and infection of pathogenic bacteria was not significant. The monthly average apparent temperature and relative humidity were correlated with the infection rate of pathogenic bacteria.

Conclusion: In open injury of extremities, apparent temperature and relative humidity is an important risk factor for infection by pathogenic bacteria and the drug resistance of pathogenic bacteria in out-of-hospital infection was lower than that of hospital infection.

Key words: Apparent temperature; Drug resistance; Infection; Open injury of limbs; Pathogenic bacteria; Relative humidity

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Introduction

In recent decades, investigators in many institutions have been actively exploring the relationship between meteorological environment and human health to reduce the adverse impact of climate change on human health. People's bodies are highly susceptible to changes in the climate system, such as greenhouse effect CO_2, heat waves and cold waves, extreme high and low temperatures and air pollution. Studies have found environmental factors that induce diseases; for example, mumps, rubella, scarlet fever, hand, foot mouth disease, and other infectious diseases display regional factors, mainly concentrated in schools and kindergartens,[1] while a variety of foodborne diseases,[2] as well as tracheitis, encephalitis B,[3] and other diseases depend on various seasonal factors. Studies show that *Staphylococcus aureus* and coagulase negative *Staphylococcus* are seasonal according to meteorological and calendar data and are the main causes of infections after orthopedic surgery.[4,5] Apparent temperature, humidity, and precipitation are important factors for the survival of pathogens.[6] There may be many meaningful ecological relationships between the occurrence of diseases and development of natural environment and related climate factors.

With the development of society, there is a yearly increase in the incidence of high-energy injury, including traffic, sports, and mechanical injuries, such as knife injuries, smashing injuries, and fall injuries.[7,8] The level and mode of the energy that causes the injury leads to characteristic wound classification of soft tissue and bone, including: (i) sutured directly; (ii) exposure of epidermis/dermis; (iii) exposure of subcutaneous tissue; and (iv) exposure of musculoskeletal tissue.[9] In addition, in cases of traffic accident injury, contusion, and laceration, soil and sediment residues may be found that might contain an abundance of microorganisms. There can be billions of microorganisms in 1 g soil, glass debris left after glass cutting, fruit chips often left after fruit knife stabbing, etc., that may lead to wound infections and various types of colonizing bacteria. Skin as the natural barrier of isolation with the outside world, can resist external invasion and infection of various germs, maintain the relative balance of the internal environment. Once the skin is damaged and necrotic, it opens the door for microbial invasion, which can easily lead to infection and then lead to prolonged wound. If the infection is hard to control, it can require amputation and can even be life-threatening. Some studies have suggested that apparent temperature, relative humidity, rainfall, sunshine, and cloud cover have an impact on several foodborne diseases.[2] Studies have also shown that humidity and apparent temperature are potential important risk factors for increasing the risk of deep prosthetic joint infection in patients after Total knee arthroplasty.[10]

Nanchang is located in the north of central Jiangxi and has a subtropical humid monsoon climate, which is humid and mild with sufficient sunshine. The annual average temperature is 17°C–17.7°C; the highest and lowest temperatures ever recorded were 40.9°C and −15.2°C, respectively. Because of the varying monsoon intensities and advance and retreat time, the temperature fluctuates greatly, and the precipitation distribution is uneven. The annual rainfall is 1600–1700 mm, with 147–157 precipitation days, 5.6 annual average rainstorm days, and annual average relative humidity of 78.5%. To date, no study has been reported on external factors, including apparent temperature, humidity, and injury as risk factors of limb infection and their impact on the epidemiology and drug resistance analysis of pathogenic bacteria. Therefore, we conducted a retrospective cohort study in a tertiary hospital in Nanchang, Jiangxi Province, China, for the following research purposes: (i) to investigate the relationship between temperature, humidity and the epidemiology of pathogenic bacteria in open injury of limbs; (ii) to explore the relationship between the mechanism of trauma and the types and drug resistance of pathogens causing limb trauma infection; and (iii) the drug resistance of Gram-positive bacteria (G+) and Gram-negative bacteria (G-) in three groups were compared under different trauma mechanisms.

Methods

Inclusion Criteria

Inclusion criteria: (i) availability of complete clinical data; (ii) open injuries; and (iii) diagnosis of surgical site infection (i.e., purulent discharge from the wound, natural rupture of the wound, positive culture, or gram-positive staining) based on the criteria established by the Joint Committee of International Health Organization (2002)[12]; or finding of incision swelling or drainage, infection, or gaping of the incision wound by the surgeon; (iv) after aseptic surgery, it was diagnosed as nosocomial infection according to the diagnostic criteria for nosocomial infection[13]; and (v) bacterial culture in the secretion or incision drainage fluid was positive.

Exclusion Criteria

Exclusion criteria were: (i) repeated pathogens were cultured from the same site; (ii) contaminating bacteria; (iii) combination with other infectious diseases; and (iv) combination with autoimmune, infectious, or severe diseases and organ function damage.

Grouping Criteria

From January 2012 to July 2017, 1589 patients with limb infections in the Department of Orthopedics of the First Affiliated Hospital of Nanchang University were analyzed retrospectively, and they were divided into three groups: Group I (clean incision infection), infection of clean incision of limbs in our hospital[13]; Group II (in-hospital infection group with open injury), infection of open injury of limbs 48 h after hospitalization; and Group III (Community infection group with open injury of the limb), infection of open injury of limbs before 48 h hospitalization, including infection occurred before admission. These three groups showed 2017 strains of pathogenic bacteria. The causes of trauma included: accidents from non-motor vehicles, machinery, cutting/piercing, pedestrian injuries, struck by/against, pedal cycles, and other injuries.[11] Groups II and III were divided into six subgroups to explore the correlation between them and the incidence of eight common orthopedic pathogens[14]: gram-positive (mainly including *Staphylococcus*), gram-negative (mainly *Enterobacteriaceae*), and to compare the differences between them and the resistance of orthopedic ward colonized bacteria.

Collection of Meteorological Data

Meteorological data were collected from the China Meteorological data sharing network. The data were collected from the monthly report of the ground meteorological records submitted by the climate data processing departments of each province, city, and autonomous region. The data set is the monthly record of China's 613 basic and benchmark ground meteorological observation stations and automatic stations since 1951. These data includes the monthly average apparent temperature (°C), monthly average humidity, and monthly average temperature and humidity of Nanchang City from January 2012 to July 2017. The daily average weather data matching for climate factors in all locations in Nanchang area is uploaded by the base station.

Examination Method

The isolation media for the drug susceptibility tests were 90-mm Chinese blue agar medium (Shanghai Kemajia Biotechnology Co., Ltd. Shanghai, China) and Colombian blood agar medium (Shanghai Kemajia Biotechnology Co., Ltd. Shanghai, China). Pathogen identification and drug susceptibility test were performed using the VITEK-2 Compact automatic bacterial tester (BioMerieux, Hazelwood, MO, USA). Drug sensitivity was evaluated according to the Clinical and Laboratory Standards Institute (CLSI, 2017) standards.

The study was approved by the Ethics Committee of The First Affiliated Hospital of Nanchang University (approval #20170720). The comparison of general data includes the comparison of age, gender differences and the distribution of infection sites in each group.

Bacterial Distribution

Pathogens include Gram-positive bacteria and Gram-negative bacteria and distribution of pathogens in each group was compared. We used VITEK-2 Compact automatic bacterial tester for pathogen identification.

Drug Resistance of Pathogenic Bacteria

We cultured the bacteria in the isolation media for the drug susceptibility tests—90-mm Chinese blue agar medium and Colombian blood agar medium. Drug susceptibility tests were also performed using the VITEK-2 Compact automatic bacterial tester. Then we evaluated drug sensitivity according to the Clinical and Laboratory Standards Institute (CLSI, 2017) standards. After that we compared the drug resistance of pathogens in each group and the drug resistance of different pathogens to different antibiotics.

Statistical Analysis

SPSS v22.0 (Chicago, IL, USA) was used for statistical analysis of the data. The measurement data with non normal distribution were expressed by median (quartile) [M (p25-p75)]. The counting data were expressed as constituent ratio or percentage (%). To understand the differential relationship between pathogenic bacteria damage mechanism and incidence, Friedman's two-way method was used. The Pearson test was used to determine the relationship between the mean temperature, relative humidity and the mean monthly damage mechanism. Therefore, multiple linear regression analysis was used to analyze the relationship between the prevalence of main pathogens and seasonal temperature and humidity. The difference was statistically significant ($P < 0.05$). All data were stored in Microsoft Excel for statistical analysis.

Results

The Condition of the Site of Infection and the Comparison of General Data

There was no significant difference in gender and age among all groups ($P > 0.05$). The proportion of male patients (75%) was higher than female patients (25%). The average age (year) of Group I patients was 47.00 years, which was higher than that of Group II patients (43.50 years) and Group III patients (45.00 years) (Table 1). The statistical table of the affected sites is shown in Table 2.

Bacterial Distribution

Group II and Group III were mainly infected by G+ (Table 3). The eight groups of pathogenic bacteria that showed the highest incidence were *Enterobacter cloacae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Baumann/Acinetobacter haemolyticus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Enterococcus faecalis*.

TABLE 1 Comparison of general information [M (25%,75%)]

Group	Group I	Group II	Group III	*P*
Age	47.00 (37.25,54.75)	43.50 (35.00,53.50)	45.00 (39.00,55.00)	0.095
Gender (Male:Female)	405:128	557:198	148:42	0.422

TABLE 2 Distribution of infection sites in each group (n&%)

Group	Group I	Group II	Group III
Hand	50 (9.38%)	59 (7.81%)	144 (18.42%)
Foot	78 (14.63%)	155 (20.53%)	298 (34.21%)
Upper limb articular cavity	7 (1.31%)	16 (2.12%)	25 (1.05%)
Lower limb articular cavity	62 (11.63%)	88 (11.66%)	174 (12.63%)
The wrist is near the diaphysis	43 (8.07%)	79 (10.46%)	127 (2.63%)
The ankle joint is near the shaft	293 (54.97%)	358 (47.42%)	710 (31.05%)
Total	533 (100.00%)	755 (100.00%)	190 (100.00%)

TABLE 3 Damage mechanism and G + / G- Distribution

Damage mechanism	Non–motor vehicle transport		Machinery		Cutting/ piercing		Pedestrian		Struck by/ against		Pedal cyclist		Other injuries		Total		
	G+	G-	G+	G-	G+	G-	G+	G-	G+	G-	G+	G-	G+	G-	G+	G-	all
Group II	272	102	61	19	25	9	120	56	26	10	21	8	17	9	542	213	755
Group III	35	18	15	3	28	8	34	14	0	2	5	4	16	8	133	57	190
Total	307	120	76	22	53	17	154	70	26	12	26	12	33	17	675	270	945

TABLE 4 Distribution of pathogenic bacteria

Pathogenic bacteria	Group I number of cases (proportion)	Group II number of cases (proportion)	Group III number of cases (proportion)	*P*-value
Staphylococcus aureus	60 (11.3%)	87 (11.5%)	29 (15.3%)	0.307
Staphylococcus epidermidis	34 (6.3%)	51 (6.8%)	6 (3.2%)	0.176
Enterococcus faecalis	16 (3.1%)	24 (3.2%)	6 (3.2%)	0.983
klebsiella pneumoniae	25 (4.6%)	32 (4.2%)	5 (2.6%)	0.476
Enterobacter cloacae	71 (13.4%)	132 (17.5%)	27 (14.2%)	0.110
Escherichia coli	59 (11.1%)	63 (8.4%)	16 (8.4%)	0.228
Pseudomonas aeruginosa	51 (9.5%)	84 (11.1%)	26 (13.7%)	0.282
Baumann/Acinetobacter haemolyticus	47 (8.8%)	62 (8.3%)	11 (5.8%)	0.419
Others	170 (31.9%)	220 (29.0%)	64 (33.6%)	0.365

The pathogenic bacteria were the *E. cloacae*, *Staph. aureus*, *K. pneumoniae*, and *E. faecalis*. No significant differences were seen in the distribution of pathogenic bacteria (Table 4) ($P > 0.05$).

Drug Resistance of Pathogenic Bacteria

The drug resistance of group III was significantly lower than that of groups I and II. Drug resistance of pathogenic bacteria in out-of-hospital infection was lower than that in hospital infection. Among them, G- were resistant to the first- and second-generation cephalosporins, including cefotetan and cefazolin, and ampicillin and other penicillins, while they were sensitive to third-generation cephalosporins, such as ceftazidime, as well as to levofloxacin and other quinolones, meropenem, and other beta-lactamases. G+ were resistant to cephalosporin, ceftriaxone and other cephalosporins and erythromycin and other macrolides; penicillins such as oxacillin showed strong resistance. They were sensitive to vancomycin and linezolid (Figures 1 and 2).

Correlation Between Pathogenic Bacteria and External Conditions

Injury Mechanism and Distribution of Pathogenic Bacteria

Table 5 reflects the proportions of different pathogens under six trauma factors in groups II and III. The statistical distribution of six trauma factors and pathogens in groups II and III was calculated by the Friedman's two-way ANOVA of rank, $P > 0.05$. In other words, there was no significant difference in pathogen distribution under the six trauma factors (Table 5).

Pearson Analysis on Temperature, Humidity and Pathogenic Bacteria Occurrence Rate

Pearson analysis was performed on apparent monthly temperature, relative monthly humidity and infection rate of G+/G-. Temperature was an independent risk factor in part of the group III (non-motor vehicles, machinery, struck by/against injury and all group) and part of the group II

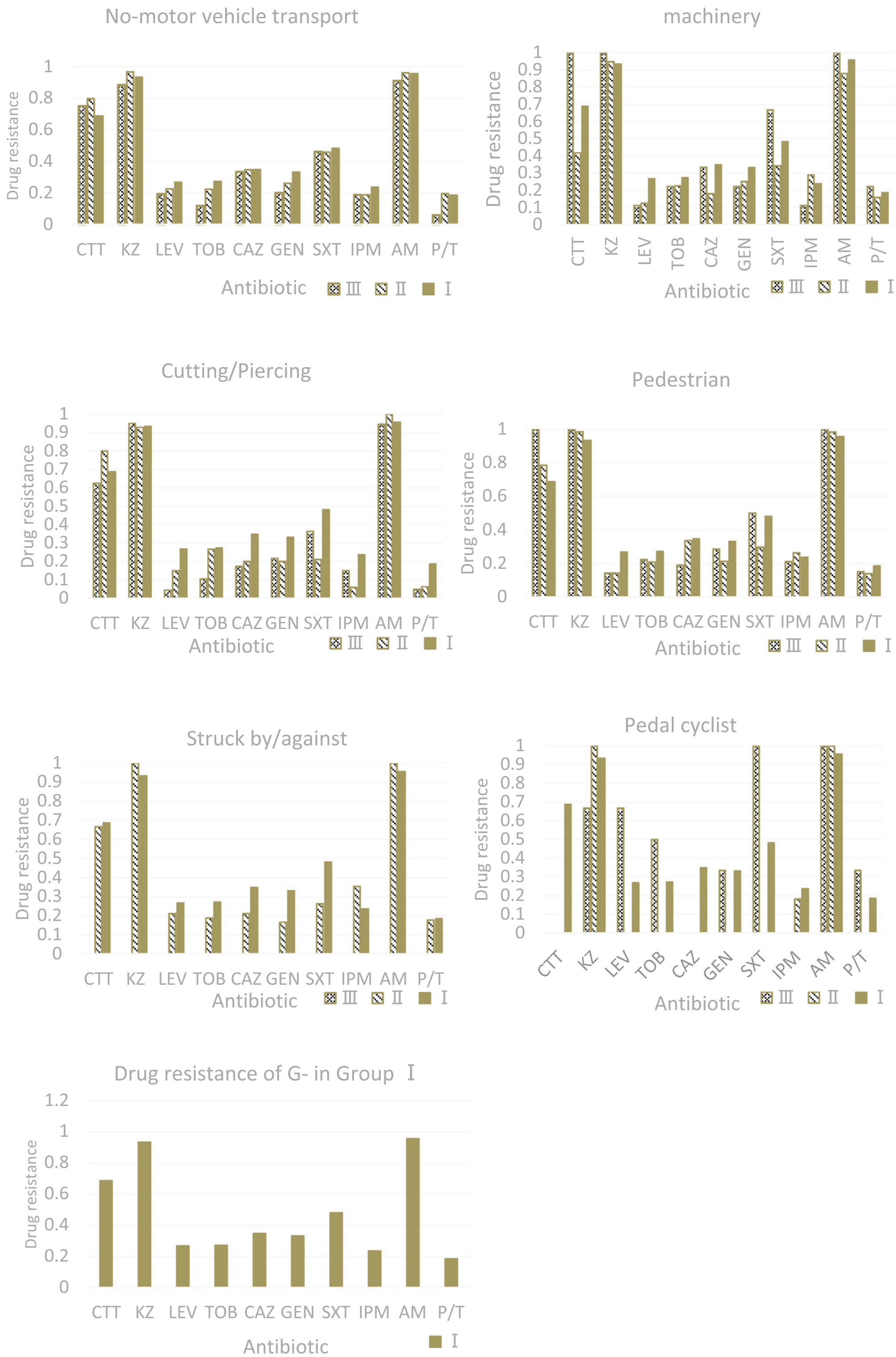

FIGURE 1 Comparison of G - drug resistance in group I, II and III infection caused by six trauma mechanisms. "CTT," cefotetan; "KZ," cefazolin; "Lev," levofloxacin; "TOB," tobramycin; "CAZ," ceftazidime; "GEN," gentamycin; "SXT," sulfamethoxazole; "IPM." imipenem; "Am." ampicillin; "P/T," piperacillin/tazobactam

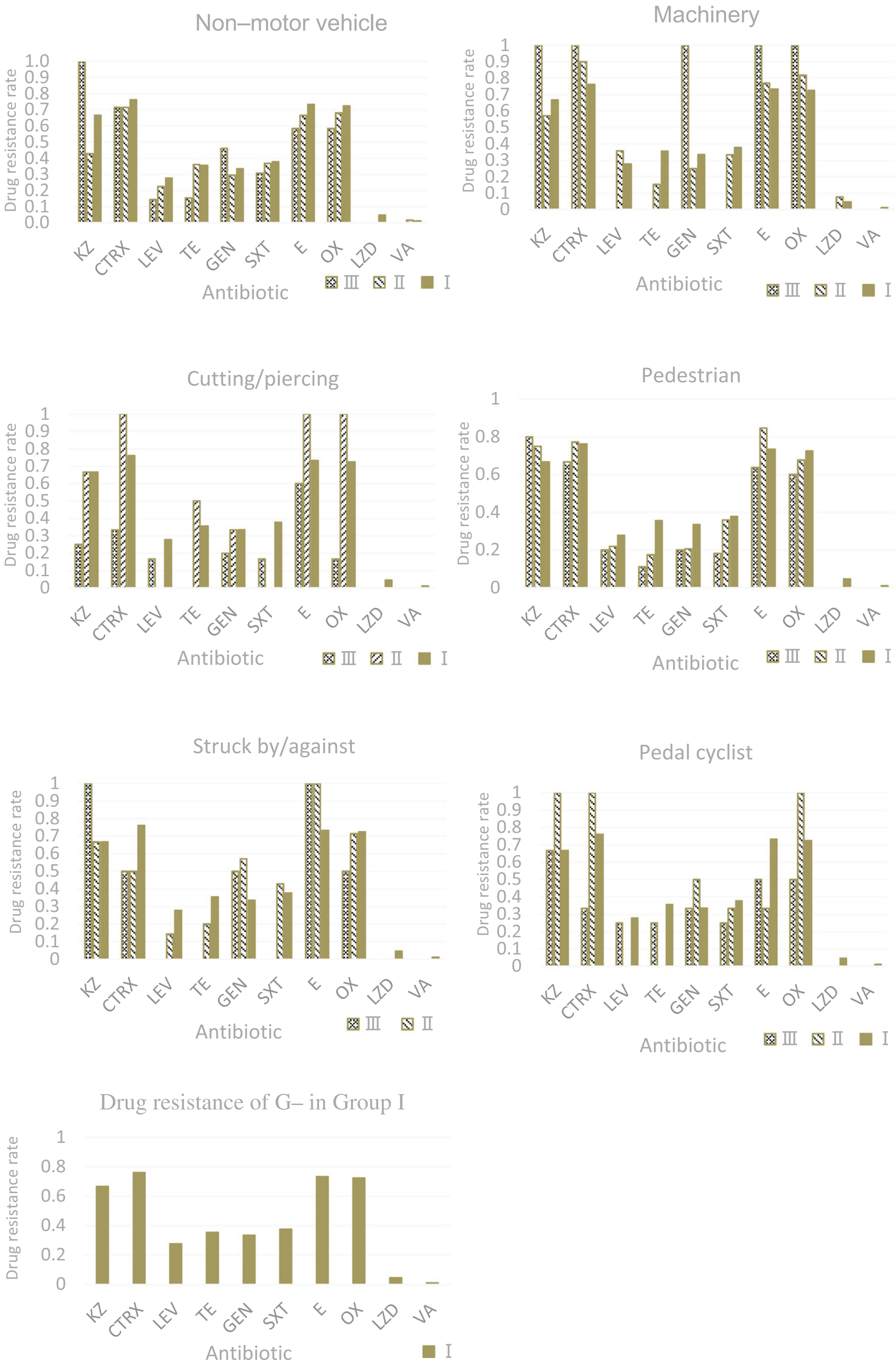

FIGURE 2 Comparison of G + drug resistance in group I, II and III infection caused by six trauma mechanisms. "KZ," cefazolin; "CTRX," ceftriaxone; "LEV"levofloxacin; "Te," tetracycline; "GEN," gentamicin; "SXT"sulfamethoxazole;"E," erythromycin; "OX," benzoxacillin; "LZD," linazolam; VA, vancomycin

(non-motor vehicles, struck by/against, pedal vehicle injuries) ($P < 0.05$). There was no significant correlation between the monthly mean relative humidity and the infection rate of G+/G-. On the analysis of six kinds of main pathogenic bacteria infection rate and the average monthly apparent correlation of temperature, relative humidity, temperature were independent risk factors in part of the group III (cutting/piercing, pedestrian injuries; *P. aeruginosa* infection) and subgroups in group II (machinery infection with *K. pneumoniae*, pedestrian injury infections with *Enterobacter cloacae*) ($P < 0.05$). The monthly average relative humidity was an independent risk factor in part of group II (for non - motor vehicles with *A. baumannii* infection, for cutting/piercing; *E. cloacae*) and part of group III (for machinery infection with *A. baumannii*, for pedestrian injury infections with *S. aureus*) ($P < 0.05$) (Tables 6 and 7).

Multiple Linear Regression Analysis on Temperature, Humidity and Pathogenic Bacteria Occurrence Rate

Multiple linear regression analysis of G+/G- infection rate and monthly mean apparent temperature, relative humidity revealed that average monthly temperature and part of the group III (cutting/piercing, all G +/G -), part of group II (pedestrian injuries infection of G-, pedal cycles infected G +, all G +/G -) positively correlated with the infection rates ($P < 0.05$); however, the highest R2 fitting degree was 0.211. There was no significant linear relationship between monthly average relative humidity and G+/G-. In the analysis of the relationships between the six main pathogenic bacteria and the monthly apparent temperature and relative humidity, no obvious linear relationships between pathogenic bacteria were found (Tables 6 and 7).

Discussion

The Drug Resistance of G+ and G-

No significant differences were seen in the distribution of pathogens among the three groups. The drug resistance of group III was significantly lower than that of groups II and I. The correlation between injury mechanism and infection by pathogenic bacteria was statistically significant. Univariate and multivariate analyses showed statistically significant relationships for G+, G-, and pathogenic bacteria with temperature and partial injury mechanism infection.

Relationship between Pathogen Types, Drug Resistance and Trauma Mechanism

We found no significant differences in the distribution of pathogens among the three groups. This is consistent with the results of the distribution of pathogenic bacteria infection in limb wound in China and abroad.[15–18] The overall drug resistance of pathogenic bacteria in group III was lower than that of groups I and II. At present, no relevant research in orthopedics has been found. In the fields of respiratory tract, urinary tract, infections and burns, it has been reported that the drug resistance of pathogenic bacteria in community-acquired infection was lower than that in hospital-acquired infection.[19–21] This may be related to the routine and widespread use of antibiotics in inpatients, and resistance of the pathogenic bacteria that lead to the presence of drug-resistant strains under the effect of antibiotic selection and ultimately lead to nosocomial infection.[22]

Non-motor vehicle transport injury is a serious high-energy injury, and the injury mechanism is complex. Comminuted fractures are common, often combined with severe soft tissue and other important organ injuries.[23] Mechanical injury is caused by direct contact between the moving (static) parts, tools, or machined parts of mechanical equipment and human body, including pinch, collision, shearing, entanglement, stranding, grinding, cutting, and stabbing. The exposed transmission parts (such as gears, shafts, tracks and reciprocating parts of all kinds of rotating machinery) may cause mechanical injuries and may lead to skin peeling, subcutaneous bleeding, trauma, fracture, visceral rupture, and limb amputation.[24] Most cutting/piercing injuries are linear, with varying lengths and depths of wounds, smooth edges, intact and opposite edge on both sides, sharp angles, flat walls, absence of tissue bridges in the wound cavity, and the ability to cut off the periosteum of the wound bottom leaving linear scratches on the bone. The degree of bleeding is related to the length and depth of the injury. When the human body contacts the ground at certain angles and speeds, the damage is often distributed on one or multiple parts of the body (when rolling occurs), and bruises are often formed on the exposed parts of the body (such as hands and faces).[25] Struck by/against injuries cause open, extensive tissue contusion, bleeding, and swelling. Although nerves, blood vessels, and tendons are injured by contusion and pressure, their continuity is maintained, resulting in tissue ischemia, edema, further liquefaction, and necrosis. Extensive scar, adhesion, and contracture are formed in the late stages.[26] Pedal vehicle injury refers to those caused by throwing of the human body resulting in falling on the ground under the action of a motor vehicle. Because the body absorbs the kinetic energy of the moving vehicle, the impact of the front and rear of the vehicle on the human body cannot only cause direct impact injury, but also cause the human body to fall, resulting in indirect damage.[25] Many trauma mechanisms have various energy levels, which generate the varying degrees of damage and shapes to soft tissues and bones, different types of wounds, and also different types of pollutants. We found no significant difference in bacterial infection caused by different trauma mechanisms. When skin mucosal protection and immune function are damaged to different degrees, there is no disease-causing bacteria that are more likely to colonize and invade.

Relationship between Temperature, Humidity and Pathogenic Bacteria

We found a significant correlation between apparent temperature and pathogen infection. Ramos *et al.* showed an incremental change in the incidence of specific infections associated with seasonal changes in temperature, humidity, and precipitation caused by P. *aeruginosa*.[27] Townsley *et al.* showed that the formation of biofilm of the bacteria was related to different apparent temperatures.[28] Di Ciccio *et al.* showed that the surface hydrophobicity of the cells increased with increase in the apparent temperature.[29] At 37°C and

TABLE 5 Damage mechanism and distribution of pathogenic bacteria

	Group III damage mechanism						Group II damage mechanism					
Pathogenic bacteria	Non–motor vehicle transport	Machinery	Cutting/ piercing	Pedestrian	Struck by/against	Pedal cyclist	Non–motor vehicle transport	Machinery	Cutting/ piercing	Pedestrian	Struck by/ against	Pedal cyclist
Staphylococcus aureus	17.0%	11.1%	13.9%	16.7%	50.0%	0.0%	11.2%	6.3%	11.8%	14.2%	16.7%	6.7%
Staphylococcus epidermidis	3.8%	0.0%	0.0%	4.2%	0.0%	11.1%	6.4%	7.5%	5.9%	8.5%	2.8%	3.3%
Enterococcus faecalis	3.8%	0.0%	0.0%	2.1%	50.0%	11.1%	3.7%	2.5%	2.9%	3.4%	0.0%	3.3%
klebsiella pneumoniae	1.9%	0.0%	0.0%	4.2%	0.0%	0.0%	3.7%	5.0%	8.8%	2.8%	8.3%	0.0%
Enterobacter cloacae	3.8%	22.2%	5.6%	20.8%	0.0%	11.1%	18.9%	13.8%	8.8%	19.3%	13.9%	23.3%
Escherichia coli	11.3%	0.0%	8.3%	6.3%	0.0%	33.3%	6.6%	11.3%	0.0%	6.8%	5.6%	10.0%
Pseudomonas aeruginosa	17.0%	44.4%	19.4%	14.6%	0.0%	0.0%	13.0%	8.8%	11.8%	8.5%	8.3%	3.3%
Baumann /Acinetobacter haemolyticus	11.3%	5.6%	2.8%	4.2%	0.0%	0.0%	9.6%	6.3%	11.8%	6.3%	5.6%	13.3%
Other pathogens	30.2%	16.7%	50.0%	27.1%	0.0%	33.3%	26.9%	38.8%	38.2%	30.1%	38.9%	36.7%

TABLE 6 *P*-values of correlation between temperature, humidity and G + /G- occurrence rate

	Mechanism		Total		Non–motor vehicle transport		Machinery		Cutting/piercing		Pedestrian		Struck by/against		Pedal cyclist	
Group	Value	*P*	G+	G-	G+	G-	G+	G-	G+	G-	G+	G-	G+	G-	G+	G-
II	Pearson	Apparent temperature	0.000	0.017	0.017	0.565	0.565	0.246	0.246	0.301	0.054	0.054	0.033	0.033	0.045	0.045
		Humidity	0.920	0.911	0.539	0.539	0.469	0.469	0.985	0.985	0.911	0.985	0.549	0.985	0.552	0.552
	Multiple linear regression	Adjusted R^2	0.211	0.057	−0.023	−0.019	0.020	−0.003	−0.024	−0.014	0.177	0.027	0.047	0.046	0.006	0.035
		Apparent temperature	0.000	0.017	0.518	0.549	0.103	0.261	0.631	0.304	0.000	0.056	0.031	0.032	0.163	0.048
		Humidity	0.599	0.837	0.750	0.525	0.389	0.498	0.619	0.951	0.403	0.974	0.595	0.479	0.552	0.600
III	Pearson	Apparent temperature	0.259	0.259	0.000	0.000	0.048	0.048	0.991	0.991	0.058	0.058	0.031	0.991	0.052	0.052
		Humidity	0.350	0.915	0.763	0.533	0.427	0.483	0.981	0.981	0.915	0.915	0.569	0.569	0.551	0.551
	Multiple linear regression	Adjusted R^2	0.111	−0.011	−0.011	−0.003	0.052	0.028	0.075	0.004	0.021	0.054	0.044	−0.002	−0.019	0.005
		Apparent temperature	0.003	0.264	0.348	0.256	0.641	0.247	0.009	0.283	0.073	0.626	0.035	0.217	0.506	0.132
		Humidity	0.370	0.943	0.568	0.478	0.641	0.505	0.953	0.313	0.816	0.099	0.574	0.603	0.572	0.907

TABLE 7 ***P*-values of correlation between temperature, humidity and pathogenic bacteria occurrence rate**

Group		II					III				
Items		Pearson		Multiple linear regression			Pearson		Multiple linear regression		
Pathogens	*P*	Apparent temperature	humidity	Adjusted R^2	Apparent temperature	humidity	Apparent temperature	humidity	Adjusted R^2	Apparent temperature	humidity
Non-motor vehicle transport	Sau	0.96	0.27	−0.012	0.997	0.275	0.688	0.912	−0.028	0.687	0.9
	Sep	0.412	0.389	−0.01	0.433	0.409	0.151	0.533	0.009	0.145	0.49
	Efa	0.646	0.569	−0.023	0.664	0.584	0.357	0.395	−0.007	0.377	0.417
	Knp	0.459	0.743	−0.02	0.454	0.721	0.235	0.817	−0.008	0.235	0.778
	Ecl	0.361	0.053	0.038	0.395	0.058	0.251	0.654	−0.006	0.246	0.619
	Eco	0.781	0.813	−0.029	0.775	0.805	0.883	0.945	−0.031	0.886	0.95
	Pae	0.541	0.848	−0.025	0.549	0.869	0.186	0.754	−0.003	0.193	0.794
	Aba	0.099	0.043	0.079	0.075	0.033	0.414	0.348	−0.006	0.392	0.331
Machinery	Sau	0.377	0.191	0.007	0.405	0.205	0.304	0.192	0.011	0.328	0.206
	Sep	0.202	0.78	−0.005	0.209	0.82	-	-	-	-	-
	Efa	0.397	0.481	−0.013	0.416	0.504	-	-	-	-	-
	Knp	0.047	0.762	0.033	0.046	0.694	-	-	-	-	-
	Ecl	0.121	0.976	0.007	0.124	0.924	0.547	0.356	−0.013	0.573	0.371
	Eco	0.702	0.317	−0.012	0.671	0.312	-	-	-	-	-
	Pae	0.061	0.571	0.03	0.059	0.511	0.66	0.623	−0.025	0.677	0.638
	Aba	0.268	0.128	0.022	0.291	0.139	0.179	0.012	0.088	0.196	0.014
Cutting/piercing	Sau	0.293	0.856	−0.013	0.3	0.89	0.998	0.863	−0.031	0.996	0.864
	Sep	0.488	0.186	0.003	0.521	0.197	-	-	-	-	-
	Efa	0.321	0.381	−0.005	0.34	0.404	-	-	-	-	-
	Knp	0.732	0.699	−0.027	0.745	0.711	-	-	-	-	-
	Ecl	0.333	0.049	0.041	0.364	0.054	0.161	0.405	0.009	0.173	0.435
	Eco	-	-	-	-	-	0.392	0.51	−0.012	0.379	0.488
	Pae	0.762	0.294	−0.013	0.795	0.303	0.006	0.595	0.088	0.006	0.498
	Aba	0.864	0.307	−0.014	0.831	0.307	0.398	0.592	−0.015	0.387	0.568
Pedestrian	Sau	0.483	0.502	−0.016	0.467	0.484	0.763	0.03	0.044	0.687	0.03
	Sep	0.596	0.625	−0.023	0.612	0.642	0.189	0.81	−0.003	0.195	0.851
	Efa	0.081	0.318	0.03	0.088	0.346	0.664	0.541	−0.023	0.684	0.555
	Knp	0.326	0.592	−0.012	0.339	0.62	0.971	0.2	−0.005	0.928	0.202
	Ecl	0.006	0.495	0.091	0.006	0.404	0.613	0.483	−0.019	0.594	0.473
	Eco	0.146	0.784	0.004	0.146	0.736	0.557	0.527	−0.02	0.576	0.545
	Pae	0.263	0.341	0.004	0.246	0.316	0.038	0.136	0.066	0.042	0.15
	Aba	0.444	0.613	−0.018	0.459	0.637	0.86	0.644	−0.027	0.845	0.641
Struck by/against	Sau	0.248	0.624	−0.005	0.242	0.589	0.447	0.926	−0.022	0.448	0.901
	Sep	0.321	0.748	−0.014	0.331	0.78	-	-	-	-	-
	Efa	-	-	-	-	-	0.321	0.381	−0.005	0.34	0.404
	Knp	0.632	0.776	−0.026	0.643	0.792	-	-	-	-	-
	Ecl	0.086	0.989	0.015	0.088	0.954	-	-	-	-	-
	Eco	0.593	0.526	−0.021	0.612	0.543	-	-	-	-	-
	Pae	0.052	0.493	0.033	0.056	0.536	-	-	-	-	-
	Aba	0.717	0.245	−0.007	0.68	0.242	-	-	-	-	-
Pedal cyclist	Sau	0.446	0.594	−0.018	0.462	0.617	-	-	-	-	-
	Sep	0.546	0.55	−0.02	0.564	0.569	0.235	0.817	−0.008	0.235	0.778
	Efa	0.664	0.541	−0.023	0.684	0.555	0.334	0.626	−0.013	0.346	0.655
	Knp	-	-	-	-	-	-	-	-	-	-
	Ecl	0.527	0.935	−0.025	0.527	0.914	0.398	0.592	−0.015	0.387	0.568
	Eco	0.962	0.612	−0.027	0.979	0.616	0.656	0.975	−0.028	0.657	0.96

TABLE 7 Continued

Group		II					III				
Items		Pearson		Multiple linear regression			Pearson		Multiple linear regression		
Pathogens	*P*	Apparent temperature	humidity	Adjusted R^2	Apparent temperature	humidity	Apparent temperature	humidity	Adjusted R^2	Apparent temperature	humidity
	Pae	0.334	0.626	−0.013	0.346	0.655	-	-	-	-	-
	Aba	0.802	0.794	−0.029	0.811	0.803	-	-	-	-	-

Note: "Sau," Staphylococcus aureus, "Sep," Staphylococcus epidermidis, "Efa," Enterococcus faecalis , "Knp," klebsiella pneumoniae"Ecl," Enterobacter cloacae , "Eco," Escherichia coli , "Pae," Pseudomonas aeruginosa , "Aba," Baumann/Acinetobacter haemolyticus,"-," not applicable.

12°C, the difference in hydrophobicity was statistically significant. Ochiai *et al.* showed that biofilm formation of *V. cholerae* was induced by increase in c-di-GMP levels at low temperature.[30] All strains missing within the gene framework of *V. cholerae* were predicted to encode either diguanylcyclase (DGCs) or phosphodiesterase (PDEs) and were screened to participate in low-temperature induced biofilm formation and *Vibrio* polysaccharide (VPS) gene expression. The results showed that DGCs were used to regulate the apparent temperature of cyclic-di-GMP (cdGMP). They also demonstrated that in gram-negative pathogens, the apparent temperature regulates the level of cdGMP in a similar manner, but not in *Listeria*. This reveals the role of apparent temperature in biofilm formation and cdGMP environmental regulation signaling. Our results show that the apparent temperature was related to gram-negative and positive bacterial infection with *E. cloacae*, *Pseudo aeruginosa*, and *K. pneumoniae*.

We also found that the monthly average relative humidity of open limb injury was related to the infection of pathogens; however, there was no obvious linear relationship. Some studies showed that humidity itself has an impact on the invasiveness of pathogens. Doroshenko *et al.* first reported the ecological significance of the development and function of hyphal prokaryotes in soil microbial communities under extremely low humidity.[31] When the relative humidity of *Streptomyces* was 0.50, its spores could germinate, and the length of mycelium increased. At this humidity, the formation of lateral branches (hyphal branches) was only observed in single bedbug bacteria, and this ability was most substantial in *Streptomyces odorus*. Studies on the growth of *Streptomyces* on agar medium with varying osmotic pressures show that when the relative humidity is 0.67, all the spores of *Streptomyces* germinate and produce hyphae, without microflora. More recently, Lindsay *et al.* identified that a change in average humidity between symptom onset and admission in the winter was significantly associated with severity of illness among children with hematogenous osteomyelitis in the USA.[32] Li's research showed that the relative humidity is an important factor affecting the growth and reproduction of microorganisms on filter media.[33] Under the condition of 15% low relative humidity, the growth and propagation of bacteria on the filter media will be inhibited under the condition of low humidity. However, 50% medium humidity condition has little effect on the growth and reproduction of bacteria. Therefore, under medium humidity conditions, bacterial growth will have a long stable period in the early and later stages of culture. When the relative humidity is in the range of 40%–60%, the relative humidity has no effect on the growth rate of bacteria. High humidity condition is most suitable for bacterial growth and reproduction. Under this condition, the growth curve of the microbes is similar to the typical growth curve of microbes, and the growth of microbes on the filter material will be apparent with the growth of culture time. The relative humidity change in Nanchang area is relatively large. In the last five years, the maximum monthly relative humidity change can reach 36%. The reason why there is no linear relationship may be that in addition to the monthly relative humidity, there are also many factors such as human environment and immune system homeostasis that affect the colonization of pathogenic bacteria.

Limitations and Expectations

This study had several limitations: (i) we did not record the specific apparent temperature and relative humidity at the time of open injury; therefore we could only use the monthly mean apparent temperature and relative humidity for the study ; (ii) because the number of patients with trauma shows seasonal variations, the number of patients with trauma in summer and autumn is significantly more than that in spring and winter; hence, we can only use composition comparison correlation for statistical analysis ; (iii) without the support of relevant basic experiments, it will be valuable to carry out basic research on colonization of pathogenic bacteria in human open wounds and apparent temperature, humidity, and injury mechanism, and establish research without other interference factors; (iv) Group II may not exclude some out-of-hospital infections whose incubation period exceeds 48 h. A small number of nosocomial pathogens are planted 48 h after admission; (v) the damage mechanism was studied. The effect of apparent temperature and humidity on pathogens is more suitable for areas with similar conditions, and its practicability may be limited in other conditions; (vi) ward air conditioner and fresh air system is the temperature and humidity of the ward, which is different from the outside. The temperature and humidity in the ward and the occurrence and development of wound infection are not included in this paper, which will be for future research. For patients with nosocomial infection of wounds in wards, the influence of the air conditioning system and fresh air system on infection needs to be further studied, and the drug resistance of pathogenic bacteria under different temperature and humidity needs further exploration of basic experiments.

Conclusion

In our study, apparent temperature and relative humidity is an important risk factor for infection with pathogenic bacteria; however, there is no obvious correlation with the injury mechanism. At present, more research is needed to confirm this possibility. The drug resistance of pathogenic bacteria in out-of-hospital infection was lower than that in hospital infection.

DATA AVAILABILITY STATEMENT

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

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Zhang JX. Control of seasonal infectious diseases. Lin Chuang He Li Yong Yao Za Zhi. 2014;7:115.
2. Park MS, Park KH, Bahk GJ. Interrelationships between multiple climatic factors and incidence of foodborne diseases. Int J Environ Res Public Health. 2018;15:2482.
3. Lin CL, Chang HL, Lin CY, Chen KT. Seasonal patterns of Japanese encephalitis and associated meteorological factors in Taiwan. Int J Environ Res Public Health. 2017;14:1317.
4. Sahoo KC, Marrone G, Pathak A, Sahoo S, Lundborg CS, Tamhankar AJ. Climatic factors and community-associated methicillin-resistant staphylococcus aureus skin and soft-tissue infections – a time-series analysis study. Int J Environ Res Public Health. 2014;11:8996–9007.
5. Leekha S, Diekema D, Perencevich E. Seasonality of staphylococcal infections. Clin Microbiol Infect. 2012;18:927–33.
6. Naumova EN. Mystery of seasonality: getting the rhythm of nature. J Public Health Policy. 2006;27:2–12.
7. Ma YD, Lin WM. Analysis and discussion on the surgical treatment of long bone fracture caused by high energy trauma. Zhong Guo Jiao Xing Wai Ke Za Zhi. 2007;15:107–9.
8. Luo JS. Clinical classification and surgical treatment of long bone fracture caused by high energy trauma. Xian Dai Yi Xue. 2015;21:43–4.
9. Ning N, He LX, Lu J, Liao DB, Wang YQ, Chen JL. Risk factors of acute wound infection in orthopedics. Zhong Guo Xiu Fu Chong Jian Wai Ke Za Zhi. 2014;28: 714–7.
10. Armit D, Gunasena R, Parkinson B, et al. Humidity a potential risk factor for prosthetic joint infection in a tropical Australian hospital. ANZ J Surg. 2018;88: 1298–301.
11. DiMaggio CJ, Avraham JB, Frangos SG, Lee DC, Wall SP. The epidemiology of emergency department trauma discharges in the United States. Acad Emerg Med. 2017;24:1244–56.
12. Sun GW, Dai MD, Wang DJ. Preliminary analysis on the characteristics of the joint committee international department hospital evaluation standard (2nd edition). Wei Sheng Ruan Ke Xue. 2004;03:131–3.
13. Ministry of health of the People's Republic of China. Diagnostic criteria for nosocomial infection (trial). Zhong Hua Yi Xue Za Zhi. 2001;05:61–7.
14. Xie BL, Guo RS, Yang XW, Wan L, Yao WY, Lai Q, et al. Epidemiology and drug resistance analysis of mixed infection in orthopedic surgical sites. Surg Infect (Larchmt). 2020;21:465–71.

15. Lee SO, Choi SH, Chung JW, et al. Risk factors for acquisition of imipenem-resistant Acinetobacter baumannii: a case-control study. Antimicrob Agents Chemother. 2004;48:224–8.
16. Li FX, Cui GH, Huang XL, Shen XK, Wang WS, Shi CH. Characteristics of pathogens and risk factors of surgical site infection in orthopedic patients in five consecutive years. Zhong Guo Gan Ran Kong Zhi Za Zhi. 2016;15:466–70.
17. Ma YH, Ban JH, Qian JF, Xu A-B, Sun DS. Analysis of pathogen resistance and risk factors in orthopedic sterile surgical incision infection. Zhong Hua Yi Yuan Gan Ran Xue Za Zhi. 2015;25:2828–30.
18. Ye ZJ, Wang WQ, Zhang XJ. Distribution and drug resistance of pathogenic bacteria in orthopedic trauma. Zhong Hua Yi Yuan Gan Ran Xue Za Zhi. 2011;21:1256–8.
19. Tsay R, Fung CP, Siu LK, Chang FY. Characteristics of bacteremia between community-acquired and nosocomial Klebsiella pneumoniae infection. Arch Intern Med. 2002;162:1021–7.
20. Yinnon AM, Butnaru A, Jerassy Z, Raveh D, Rudensky B. Klebsiella bacteraemia: community versus nosocomial infection. QJM. 1996;89:933–41.
21. Wang Z, Guo HB, Ji W, Tao YZ, Ding YF. Comparative studies on the composition and antibiotic-resistance of pathogenic bacteria between children with communityacquired and hospital-acquired pneumonia. Zhong Hua Yu Fang Yi Xue Za Zhi. 2011;45:211–6.
22. Hashemi SH, Esna-Ashari F, Tavakoli S, Mamani M. The prevalence of antibiotic resistance of Enterobacteriaceae strains isolated in community- and hospital-acquired infections in teaching hospitals of Hamadan, west of Iran. J Res Health Sci. 2013;13:75–80.
23. Du DY, Gao JM, Lin X, Zeng JB, Ran HL. A comparative study on the epidemiology of road traffic accident injury and fall injury. Chuang Shang Wai Ke Za Zhi. 2000;02:75–7.
24. Li QF. Risk assessment analysis and countermeasures for mechanical injury of stamping machines in China. Hei Long Jiang Ke Ji Xin Xi. 2017;16:61.
25. Guan DW (ed), Dictionary of forensic medicine. China: Chemical Industry Press; 2009.p. 251–7.
26. Zhu JK, Huang JF, Chen JS, Dictionary of surgery. China: Beijing Science and Technology Press; 2003.p. 197–201.
27. Ramos GP, Rocha JL, Tuon FF. Seasonal humidity may influence Pseudomonas aeruginosa hospital-acquired infection rates. Int J Infect Dis. 2013; 17:757–61.
28. Townsley L, Yildiz FH. Temperature affects c-di-GMP signalling and biofilm formation in vibrio cholerae. Environ Microbiol. 2015;17:4290–305.
29. Di Ciccio P, Vergara A, Festino AR, Paludi D, Zanardi E, Ghidini S, et al. Biofilm formation by Staphylococcus aureus on food contact surfaces: relationship with temperature and cell surface hydrophobicity. Food Control. 2015;50:930–6.
30. Ochiai Y, Hondo R, Mochizuki M, Takano T, Yamada F, Ueda F. Biofilm formation under different temperature conditions by a single genotype of persistent listeria monocytogenes strains. J Food Prot. 2014;77:133–40.
31. Doroshenko EA, Zenova GM, Zviagintsev DG, Sudnitsyn II. Spore germination and mycelial growth of Streptomycetes at different humidity levels. Mikrobiologiia. 2005;74:795–9.
32. Lindsay EA, Jo CH, Tareen N, Copley LA. Seasonal variation and weather changes related to the occurrence and severity of acute hematogenous osteomyelitis in children. J Pediatric Infect Dis Soc. 2018;7:e16–23.
33. Li Q. Experimental study on the effect of air temperature and humidity on the growth and reproduction of microorganisms on fiber materials. Tian Jin Da Xue. 2014;5:1–62.