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Original Article

Pathogen distribution and drug resistance in a burn ward: a three-year retrospective analysis of a single center in China

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Abstract: To investigate the spread of multiple-resistant strain in a burn ward to inform clinical administration of antibiotic drugs, burn wound treatment and decision-making for infection control. A 3-year retrospective analysis was conducted. Specimens from wounds, blood, catheter, sputum, urine and stool collected from inpatients of the Second Affiliated Hospital of Zhejiang University of Medicine between January 1, 2011 and December 31, 2013 were cultured and strains were identified by automatic bacteria analysis. Sensitivity to 30 commonly used antibiotics was assessed by K-B disk diffusion. A total of 2212 strains of pathogenic bacteria or fungi were isolated (33.9% Gram-positive and 52.7% Gram-negative bacteria and 13.4% fungi), including 1466 from wound extracts, 128 from blood culture, 335 from urine culture, 5 from stool culture, 153 from sputum culture and 125 from catheters. The most frequently detected pathogens in wound secretions were *Staphylococcus aureus, Pseudomonas aeruginosa* and *Acinetobacter baumannii.* The Gram-positive bacteria *Staphylococcus epidermidis, Enterococcus faecalis* and *Enterococcus faecium*, and the Gram-negative bacteria *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Stenotrophomonas maltophilia, Proteus mirabilis* were also frequently detected. The most frequently detected strains of fungi were *Candida albicans; tropicalis*, *glabrata* and *parapsilosis,* and all were highly sensitive to itraconazole, fluconazole and voriconazole but resistant to ketoconazole. Attention should be paid to MRSA, multi-resistant *A. baumanni,* ESBL-producing enterobacteriaceae and Carbapenem-resistant *P. aeruginosa*. Understanding the distribution of bacterial infections in Chinese hospitals will be crucial to reduce hospital-acquired infection and drug resistance.

Keywords: Burns, wound infection, drug resistance, bacterial, drug resistance, fungal

Introduction

Although rapid debridement of burn wounds and the application of topical and systemic antimicrobial agents can improve the outcome of burn injury, infection of burn wounds can become systemic, causing sepsis, and organ failure [1-3]. It is estimated that infection accounts for 75% of mortalities in patients with burn injuries [1, 4-8].

Although the initial burn wound is sterile, within 48 hours of injury disruption of the skin's mechanical integrity can allow bacteria typically found on the surface of the skin, and in sweat glands and hair follicles to colonize the wound. The presence of devitalized, avascularized tissue provides a favorable niche for microbial growth, and later bacteria from the respiratory or digestive tract, hospital environment or healthcare workers can further contaminate the wound [4, 9, 10]. Immune suppression [11-15]; intestinal bacterial translocation; extended hospitalization and invasive diagnostic and therapeutic procedures including intubation and catheterization can all contribute to contamination of burn wounds and development systemic infection [16-21]. The emergence of multidrug resistant bacteria has also limited therapeutic options [22, 23] and increased mortality in burns patients [24].

Widespread use of antibiotics has been reported to hasten the spread of multidrug resistant nosocomial bacterial strains, predominantly *Staphylococcus aureus, Pseudomonas aerugi-*

nosa and Acinetobacter baumannii [3, 25-27]. Recent increases in the use of third-generation cephalosporins has also lead to emergence of new nosocomial infections in burn patients including the extended spectrum beta-lactamase (ESBL)-producing *Enter obacteriaceae* [17, 28, 29].

Excessive antibiotic drug use is a serious problem in China. In addition to widespread microbial resistance, hospital environments also contribute to hospital-acquired multiply drug resistant bacterial infections [30, 31]. Understanding the distribution of bacterial infections, and the extent of bacterial resistance to commonly used antibiotic drugs may inform development of procedures to reduce hospital-acquired infection and treatment guidelines designed to reduce the selection pressure for multiply-drug resistant pathogens.

In this study, we retrospectively characterized the pathogenic infections of 1942 inpatients of the burn ward of the second affiliated hospital of Zhejiang University of Medicine between January 1, 2011 and December 31, 2013. In total 2212 strains of pathogenic bacteria cultured from wound secretions, blood, catheter specimen, respiratory secretions, urine and stool were obtained for analysis and the distribution of bacterial species and antibiotic resistance was investigated.

Materials and methods

Patients

Patients treated at the burn ward of Zhejiang University of Medicine between January 2011 and December 2013 were included in this retrospective analysis. The inclusion criteria were as follows: patients with burns extending over more than 10% of their skin surface; with III degree burns extending over more than 1% of their skin surface area; or with burn injuries of the head or face, or burns accompanied with inhalation injury. A total of 1942 patients were enrolled, ranging from 1 to 97 years of age, including 1395 men and 547 women.

After admission, the burn patients strictly adhered to hospital infection control procedures. For superficial II degree and deep II degree wounds antibiotic cream or silver ions were applied after debridement, and wounds were dressed. Exposure therapy was applied to wounds of eschar III degree, which were then coated with PVP-I paste or silver sulfadiazine paste. For patients with superficial II degree wounds, dressing was changed the next day. Where possible patients with deep II degree received tangential excision treatment, and some patients received scab-dissolving treatment when dressings were changed. Where possible, patients with III degree wounds underwent excision of eschar, and skin grafting with protective xenoskin or allogenic skin.

Patients were discharged when wounds and skin donor sites had healed, and when underlying conditions such as hypertension and diabetes were controlled and improved.

After hospitalization, patients received routine prophylactic therapy with second to fourth generation cephalosporin antibiotics. Patients with burn area exceeding 30% received third to fourth generation cephalosporin antibiotics for triple treatment of Gram-positive and Gram-negative bacteria and fungi. Antibiotics were adjusted based on the susceptibility of pathogens.

Sample collection

Wound secretions were collected at patient admission and during hospitalization at least weekly. Respiratory secretions were collected from patients receiving preventive tracheotomy or ventilator support. During replacement of urinary catheters and deep vein catheter, samples were taken from this equipment. Bacterial cultures of these samples, and blood (two peripheral blood collected sites, or one peripheral blood intraductal blood, 2 sets of 4 bottles, with aerobic and anaerobic culture for each sample), urine and stool samples were made (2 sets of 4 bottles, with aerobic and anaerobic culture for each sample). For patients with suspected sepsis, temperature higher than 39°C or lower than 37°C, blood, urine, stool, phlegm and wound secretion culture was conducted for three consecutive days. Patients were treated according to the guidelines of the *D.iagnostic Criteria for Infection after Bum Injury* (Chinese Journal of Burns, 2007) [32] until 2012. Thereafter patients were treated according to the revised guide, *Diagnostic Criteria and Treatment Guideline for Infection*

Table 1. Annual distribution of Gram-positive and negative bacteria and fungi detected in clinical samples

Year	Patients Tested	Samples Tested	Strains identified (n)	Gram-positive bacteria [n (%)]	Gram-negative bacteria [n (%)]	Fungi [n (%)]
2011	644	700	723	235 (32.5)	417 (57.7)*	71 (9.8)*
2012	665	732	750	257 (34.3)	349 (46.5)	144 (19.2)
2013	633	725	739	258 (34.9)	400 (54.1)*	81 (11)*
Total	1942	2157	2212	750 (33.9)	1166 (52.7)	296 (13.4)

*$P < 0.005$ in comparison to 2012 data.

of Burns and *Guideline for Diagnosis, Prevention and Treatment of Invasive Fungal Infection after Burn Injury*.

Stool culture was only carried out when patients experienced diarrhea, other gastrointestinal symptoms or sepsis. Urine culture was regularly conducted in long-term catheterization or prior to catheter extraction. Conventional deep vein catheter indwelling was applied to critical patients, and swabs for culture were taken each time catheters were replaced, every 5-7 days.

Symptoms suggesting critical illness, or sepsis, include changes in color and smell of the wound secretions, granulation and increased bleeding in response to pressure, or inflammation of the wound and failure of the skin graft. Samples were taken more often from patients with more severe symptoms.

Species identification and antibiotic sensitivity

Species identification and drug sensitivity were assessed by laboratory staff of the Second affiliated hospital of Zhejiang University of Medicine (certificated by United States Association of pathologists, CAP) using the K-B disk diffusion method drug sensitive test disks, culture medium and quality-control strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *P. aeruginosa* ATCC 27853) (Oxoid Corporation). The American Clinical and Laboratory Standards Institute (CLSI) standard was used to evaluate outcomes.

All specimens were inoculated in the appropriate culture medium and incubated at 35°C in accordance with their respective requirements for 18 to 20 hours. An API identification strip or Vitek-2 Compact automatic Bacteria analyzer (BioMerieux, France) was employed was used to identify strains. A 30 g cefoxitin disk was used to detect the Methicillin-resistant staphylococci. Methicillin resistance was detected in coagulase positive Staphylococcus where the diameter of the inhibition zone was ≤ 21 mm. Methicillin resistance was excluded in coagulase negative Staphylococcus where the diameter of the inhibition zone was ≤ 24 mm.

Statistical analysis

WHONET 5.5 software and SPSS 19.0 (SPSS, Inc., Chicago, IL, USA) were used for statistical analysis. Categorical data are presented as frequencies and percentages. The categorical variables were analyzed using the Pearson's chi-square test. *P*-values ≤ 0.05 were considered statistically significant.

Results

Annual distribution of pathogenic bacteria in clinical samples

A total of 1942 patients treated at the burn ward of Zhejiang University of Medicine between January 2011 and December 2013 were included in this retrospective analysis, ranging from 1 to 97 years of age, including 1395 men and 547 women.

From cultures of burn patient wound secretions, respiratory secretions, catheter or main line samples, and blood, urine or stool samples, a total of 2212 strains of bacteria and fungi were identified. Of the 2212 strains identified, 750 (33.9%) were gram-positive bacteria, 1166 (52.7%) were gram-negative bacteria, and 296 (13.4%) were fungi (**Table 1**). Whilst there was no significant trend in the detection of gram-positive bacteria between 2011 and 2013, the fraction of gram-negative bacteria and fungi detected was significantly higher in 2012 than in 2011 and 2013 ($P < 0.05$, **Table 1**).

As illustrated in Supplementary Table 1, gram-negative bacteria were most prevalent in urine culture, sputum culture, wound secretions and deep vein catheter culture. In sputum culture,

Table 2. Annual distribution of pathogens detected in clinical samples

2011			2012			2013		
Pathogen	Strain (n)	Percentage (%)	Pathogen	Strain (n)	Percentage (%)	Pathogen	Strain (n)	Percentage (%)
Staphylococcus aureus	96	20.5	*Staphylococcus aureus*	86	16.6	*Staphylococcus aureus*	94	19.6
Pseudomonas aeruginosa	81	17.3	*Pseudomonas aeruginosa*	59	11.4	*Acinetobacter baumannii*	57	11.9
Acinetobacter baumannii	60	12.8	*Acinetobacter baumannii*	52	10.0	*Pseudomonas aeruginosa*	57	11.9
Staphylococcus epidermidis	31	6.6	*Escherichia coli*	28	5.4	*Escherichia coli*	30	6.3
Escherichia coli	21	4.4	*Klebsiella pneumoniae*	28	5.4	*Klebsiella pneumoniae*	27	5.6
Klebsiella pneumoniae	17	3.6	*Candida albicans*	26	5	*Staphylococcus epidermidis*	21	4.4
Candida albicans	16	3.4	*Staphylococcus epidermidis*	20	3.9	*Candida albicans*	20	4.2
Proteus mirabilis	15	3.2	*Enterococcus faecalis*	16	3.1	*Enterococcus faecalis*	17	3.5
Candida tropicalis	12	2.6	*Enterococcus faecium*	16	3.1	*Enterococcus faecium*	17	3.5
Stenotrophomonas maltophilia	12	2.6	*Staphylococcus haemolyticus*	16	3.1	*Enterobacter cloacae*	16	3.3
Enterococcus faecalis	11	2.3	*Candida albicans*	16	3.1	*Candida tropicalis*	11	2.3
enterococcus faecium	10	2.1	*Proteus mirabilis*	13	2.5	*Corynebacterium striatum*	11	2.3
Enterobacter cloacae	9	1.9	*Stenotrophomonas maltophilia*	13	2.5	*Proteus mirabilis*	11	2.3
Candida glabrata	6	1.3	*Candida glabrata*	12	2.3	*Stenotrophom Stenotrophomonas maltophilia*	11	2.3
Staphylococcus haemolyticus	5	1	C.parapsilosis	9	1.7	Staphylococcus haemolyticus	10	2.1

Table 3. Rate of resistance of Gram-positive bacteria to antibiotic drugs (%)

Antibiotic	*Staphylococcus epidermidis*			*Staphylococcus aureus*			*Enterococcus faecalis*			*Enterococcus faecium*		
	2011 (n=31)	2012 (n=20)	2013 (n=21)	2011 (n=96)	2012 (n=86)	2013 (n=94)	2011 (n=11)	2012 (n=17)	2013 (n=17)	2011 (n=10)	2012 (n=16)	2013 (n=17)
Methicillin	14.3	0	14.3	75.3	80.8	82.5	100	-	-	-	-	-
Oxacillin	80	94.7	80	76	81.2	86	100	-	-	-	-	-
Vancomycin	0	0	0	0	0	0	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0	5.9	18.8	0	6.2	0
Teicoplanin	0	0	0	0	0	0	0	0	0	0	0	0
Clindamycin	66.7	55	66.7	58.9	67.4	83	100	100	100	100	100	100
Erythromycin	71.4	83.3	71.4	64.3	88	83	20	75	70	75	100	91.7
Ciprofloxacin	57.1	33.3	57.1	50	76	83	14.3	29.4	33.3	100	81.2	93.8

the proportion of gram-negative bacteria was as high as 85.7%, while the proportion of gram-positive bacteria was only 12.5%. Only in blood culture were more strains of gram-positive bacteria identified than strains of gram-negative bacteria.

The most frequently detected pathogens in wound secretions were *S. aureus, P. aeruginosa* and *A. baumannii* in 2011, 2012 and 2013 (**Table 2**). The gram-positive bacteria *Staphylococcus epidermidis, enterococcus faecalis* and *enterococcus faecium* were also among the ten most frequently detected bacteria each year, and the gram-negative bacteria *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Stenotrophomonas maltophilia, Proteus mirabilis* were also frequently detected (**Table 2**). The most frequently detected strains of fungi were *Candida albicans* and *Candida tropicalis* (**Table 2**).

Antibiotic resistance

The susceptibility of isolated bacteria and fungi to commonly used antibiotic or antifungal drugs was investigated (**Tables 3**-**5**). As indicated in **Table 3**, a minority of *S. epidermidis* isolates (0 to 14.3%) was resistant to methicillin (MRSE), but a majority of *S. aureus* isolates (75.3 to 82.5%) were resistant to methicillin (MRSA). No *S. aureus, Enterococcus faecalis* or *Enterococcus faecium* isolates were resistant to the glycopeptide antibiotic vancomycin. No *S. aureus* isolates were resistant to the oxazolidinone linezolid or the glycopeptide teicoplanin, but most *S. aureus* isolates were resistant to the narrow-spectrum beta-lactam antibiotic oxacillin, the lincosamide Clindamycin, the macrolide erythromycin and the second-generation fluoroquinolone ciprofloxacin. In 2011 all *E. faecalis* isolates were resistant to the beta-lactam antibiotic oxacillin, but no other *E. faecalis* or *E. faecium* isolates were tested for oxacillin resistance. All *E. faecalis* and *E. faecium* isolates were resistant to clindamycin and most were resistant to erythromycin and ciprofloxacin (**Table 3**).

As illustrated in **Table 4**, of the five most commonly isolated strains of gram-negative bacteria, fewer *P. aeruginosa* isolates were resistant to amikacin, ciprofloxacin and ceftazidime. However more gram-negative isolates were resistant to carbon penicillin drugs such as imipenem and meropenem, and ceftriaxone and cefotaxime, while few were resistant to enzyme inhibitor complex antibiotics, such as cefoperazone/sulbactam or piperacillin/tazobactam. 8% of gram-negative isolates were resistant to polymyxin B in 2011, but no polymyxin B resistance was detected in 2012 or 2013. *A. baumannii* isolates were mostly resistant to carbapenem, aminoglycoside, cephalosporin, enzyme inhibitor complex antibiotics, fluoroquinolones and other antibiotics. No resistance to polymyxin B was detected in 2011 and 2012, but 2% of strains isolated in 2013 were resistant to this antibiotic *K. pneumoniae* isolated were often resistant to aminoglycoside, cephalosporin, enzyme inhibitor complex antibiotics, fluoroquinolones and other antibiotics, and between 21.7% and 63% of isolates were resistant to carbapenem antibiotics. Many *E. coli* isolates were resistant to cephalosporins and quinolones, while few were resistant to enzyme inhibitor complex antibiotics such as piperacillin/tazobactam and cefoperazone/sulbactam, carbon penicillins or amikacin. No resistance to polymyxin B was detected. Between 25% and 42.9% *Proteus mirabilis* iso-

Table 4. Rate of resistance of Gram-negative bacteria to antibiotic drugs (%)

Bacteria/year Antibiotic	*Pseudomonas aeruginosa*			*Acinetobacter baumannii*			*Klebsiella pneumoniae*			*Escherichia coli*			*Proteus mirabilis*		
	2011 (n=81)	2012 (n=59)	2013 (n=57)	2011 (n=60)	2012 (n=52)	2013 (n=57)	2011 (n=17)	2012 (n=28)	2013 (n=27)	2011 (n=21)	2012 (n=28)	2013 (n=30)	2011 (n=15)	2012 (n=13)	2013 (n=11)
Imipenem	70	17.2	41.8	89.7	84.6	82.5	41.2	32.1	63	0	7.1	3.3	0	60	88.9
Meropenem	64.5	18.2	36.5	83.8	62.5	80.4	33.3	21.7	54.2	0	0	0	0	8.3	0
Ertapenem	-	-	-	-	-	-	41.2	34.8	59.1	0	11.1	3.8	0	0	0
Amikacin	48.8	13.6	23.2	55.9	38.5	35.1	23.5	14.3	55.6	14.3	7.1	16.7	6.7	7.7	0
Gentamicin	55	20.3	46.4	86.4	80.8	75.4	35.3	60.7	63	66.7	71.4	63.3	46.7	23.1	18.2
Tobramycin	57.4	12.3	20	72.9	80.8	75.4	35.7	25	55.6	42.9	35.7	36.7	38.5	7.7	9.1
Ceftazidime	35.3	16.4	30.9	84.5	87	87.8	50	63	75	76.2	69.2	70.8	42.9	33.3	25
Ampicillin	100	100	100	100	100	100	100	100	100	94.4	92.9	93.3	66.7	46.2	36.4
Ampicillin/Sulbactam	97.2	96.4	100	89.1	84.8	93.3	58.8	59.3	80	78.9	69.2	100	30.8	50	-
Piperacillin/Tazobactam	64	22	46.3	89.7	84.3	82.5	41.2	35.7	63	15	3.6	13.3	6.7	0	0
Aztreonam	64.9	41.4	47.4	100	92.3	96.5	56.2	64.3	77.8	76.2	66.7	76.7	40	30.8	36.4
Levofloxacin	35.9	11.9	14	65	57.7	57.9	52.9	21.4	59.3	81	44.4	66.7	53.3	23.1	9.1
Ciprofloxacin	23.1	10.7	20	87.9	86.5	82.5	58.8	22.2	59.3	81	50	70	64.3	30.8	27.3
Polymyxin B	2.8	0	0	0	0	2	0	0	0	0	0	0	100	-	100
Tigecycline	-	-	-	-	-	-	-	0	-	-	0	-	-	-	-
Cefoperazone/Sulbactam	63.5	19.1	41.8	74.3	63	70.9	33.3	66.7	65.4	0	0	13.6	0	0	0
Cefuroxime	-	100	-	83.3	100	-	50	60	80	80	70	100	55.6	66.7	-
Cefepime	65	24.6	40.4	90	86.5	82.5	52.9	64.3	77.8	76.2	64.3	73.3	40	30.8	36.4
Cefotaxime	74.3	61.2	72.1	92.1	90.9	93.8	60	60	80	75	66.7	100	40	33.3	-
Cefatriaxone	73.1	60.3	68.4	95.6	98	84.2	58.3	65.2	77.8	75	66.7	69.8	40	30	36.4

(-) drug susceptibility was not performed.

Table 5. Rate of resistance of isolated fungi to antifungal drugs (%)

Antifungal Agents	2011 (n=41)	2012 (n=77)	2013 (n=43)
Amphotericin B	3.1	5.5	2.3
Itraconazole	4.5	3.6	0, 0
Ketoconazole	50	56.2	38.1
Fluconazole	2	6.7	4.9
Miconazole	3.6	5.6	3.2
Clotrimazole	0	11.1	18.6
Nystatin	0	3.9	0, 0

lates were resistant to cephalosporin drugs, but few isolates were resistant to enzyme inhibitor complex antibiotics such as piperacillin/tazobactam and cefoperazone/sulbactam, amikacin, carbon penicillin such as meropenem and ertapenem. Surprisingly, 60% and 88.9% of isolates were resistant to imipenem in 2012 and 2013, respectively. Resistance of *Proteus mirabilis to* quinolones declined during this period, however all isolates were resistant to polymyxin B in 2012 and 2013.

Candida albicans, tropicalis, glabrata and *parapsilosis* are highly sensitive to itraconazole, fluconazole and voriconazole, but were commonly resistant to ketoconazole (**Table 5**). Few fungal isolates were resistant to amphotericin B, nystatin or itraconazole, but many were resistant to ketoconazole.

Discussion

Understanding the distribution of bacterial infections in Chinese hospitals will be crucial for the development of treatment guidelines designed to reduce hospital-acquired infection and drug resistance. In this study, we characterized the pathogenic infections of a large sample of 1942 inpatients of the burn ward of the second affiliated hospital of Zhejiang University of Medicine between 2011 and 2013. In total 2212 strains of pathogenic bacteria or fungi were cultured from wound secretions, blood, catheter swabs, respiratory secretions, urine and stool. Of the 2212 strains identified, 33.9% were gram-positive bacteria, 52.7% were gram-negative bacteria, and 13.4% were fungi. Gram-negative bacteria were most prevalent in urine culture, sputum culture, wound secretions and deep vein catheter culture. Only in blood culture were more strains of gram-positive bacteria identified than strains of gram-negative bacteria.

The most frequently detected pathogens in wound secretions were *S. aureus, Ps aeruginosa* and *A. baumannii* each year. The gram-positive bacteria *S. epidermidis, Enterococcus faecalis* and *Enterococcus faecium*, and the gram-negative bacteria *Escherichia coli, K. pneumoniae, E. cloacae, S. maltophilia, Proteus mirabilis* were also frequently detected. The most frequently detected strains of fungi were *Candida albicans* and *tropicalis*.

Within the three-year period of study no significant trends in pathogen distribution were observed, and our findings were in line with similar studies at other hospitals in China and abroad. A previous, smaller study of 492 inpatients treated for burns at the Jishuitan Hospital between 2003 and 2005 found a higher proportion of gram-negative bacteria in cultures of wound secretions [33]. 54.5% of the pathogens identified in that study were gram-negative bacteria, and 42.8% were gram-positive bacteria. In their samples the most frequently detected bacteria were *S. aureus* (16.9%), *P. aeruginosa* (12.5%), and the most frequently detected gram-negative bacteria were *P. aeruginosa*, *E. coli*, *A. baumannii*, *E. cloacae* and *K. pneumoniae*. Similarly in culture of wound secretions from Swiss ICU patients between 1986 and 2005 *S. aureus* was also the most frequently identified pathogen, accounting for 20.8% of all isolates, followed by *E. coli* (13.9%), *P. aeruginosa* (11.8%), coagulase negative Staphylococcus (10.9%), enterococcus (9.7%), *E. cloacae* (5.6%), *K. pneumoniae* (5%), Acinetobacter (3.2%), *Proteus mirabilis* (2%), and *S. maltophilia* (1.4%) [3]. In a sample of children suffering from burns at a hospital in Tehran between 2005 to 2009, 66.8% of wound secretions were found to be *Staphylococcus* positive, and 12% of blood cultures contained *P. aeruginosa* [34]. Within the three-year period of study no significant trends in bacterial or fungal drug resistance were observed. Gram-positive isolates included both MRSA and *methicillin-sensitive S. aureus* (MSSA) and both MRSE and methicillin-sensitive *S. epidermidis* (MSSE). The rate of *S. aureus* methicillin resistance peaked at about 80%, lower than that detected at a burn center of Southwest Hospital [35], but higher than that detected at Shanghai Ruijin Hospital in 2003 [36].

Of the gram-positive bacteria detected, five and 11 strains of *Corynebacterium striatum* were detected in our department in 2012 and 2013, respectively, including samples collected from one patient in septic shock. Between 2004 and 2005, 36 strains of *C. striatum* were isolated in our hospital, indicating that this problem may be declining in our hospital [35]. *C. striatum* is a non-spore-forming gram-positive *corynebacterium* and considered to be a hyperparasite of the surface of skin or mucosa. Long-term bedridden and immunocompromised patients are susceptible to septicemia induced by *C. striatum* [36]. The CLSI lacks interpretive standards of susceptibility for non-spore forming gram-positive corynebacterium, but our screening revealed that isolated *C. striatum* was sensitive to Vancomycin, Teicoplanin and Imipenem, while less sensitive to quinolones, sulfonamides, macrolide and other antibiotics [35, 37].

Although it may seem strange that the wound secretions were dominated by gram-negative bacteria, while the blood culture was dominated by gram-positive bacteria, this trend has been previously reported by Karimi *et al.* who found that coagulase-negative staphylococci dominated in wounds, while *P. aeruginosa* dominated in the blood [34], and Chim *et al.,* reported that *A. baumannii*, MRSA and *P. aeruginosa*, dominated in the wound while coagulase-negative staphylococci dominated in the blood [38]. These observed differences may result from the different collection times of wound secretions and blood culture specimen. In wound secretions *S. aureus, P. aeruginosa* and *A. baumannii* were detected most frequently in all three years of our study, consistent with previous reports [38, 39]. Keen *et al.* also reported that *A. baumannii, P. aeruginosa* and *K. pneumoniae* were most frequently detected [40] An eight-year study published by Feng *et al.* found the most frequent hospital infections to be *P. aeruginosa*, *S. aureus* and *Candida* [41], and Bayram *et al.* found that in a 3-year survey the most frequently detected pathogens were *A. baumannii,* coagulase-negative staphylococci and *P. aeruginosa* [42].

Detected coagulase-negative staphylococci included *S. epidermidis and Staphylococcus saprophyticus,* both typically part of the normal flora of skin and mucous membranes [43]. *S. epidermidis* can cause prosthetic valve endocarditis, venous catheter infection, peritonitis, vessel-related infection and artificial joint infection, while *S. saprophyticus* is the main pathogen responsible for urinary tract infection in women [44]. The other types of coagulase-negative staphylococci also have become important opportunistic pathogens of patients with impaired immune function.

Proteus mirabilis and *vulgaris* can induce primary and secondary infection in human, and are also commonly responsible for urinary system infection. *Acinetobacter* is an opportunistic pathogen found in the skin follicle, respiratory tract, and the environment. In a warm environment, *Acinetobacter* often colonizes wounds producing nosocomial infections. In recent years, studies have shown that *A. baumannii* is a prevalent pathogenic bacterium, and is often resistant to multiple antibiotic drugs.

Wet burn wounds coupled with long hospital stays, long-term administration of broad-spectrum antibiotics, delayed wound processing and prolonged invasive procedures provide a favorable niche for development of multiply drug resistant strains of *P. aeruginosa. P. aeruginosa* has been demonstrated to evade antibiotic therapy via mutations in the efflux system and outer membrane proteins, production of inactivating enzymes such as lactamase, and bacterial biofilm formation [45-47].

We found low rates of meropenem and amikacin resistance in *P. aeruginosa*, however the rates of resistance were higher than in previous reports [39, 41, 48]. *A. baumannii* was highly resistant to third generation cephalosporins such as ceftazidime, carbapenem and other antibiotics, but highly sensitive to polymyxin B. Gram-negative bacteria were sensitive to tigecycline (a glycylcline antibiotic derivative of minocycline), whereas *P. aeruginosa* is naturally resistant to this class of antibiotics [49]. *Enterobacteriaceae*, *K. pneumoniae* and *P. mirabilis* resistant to Carbapenem appeared in the last 2 years, and only *E. coli* remained highly sensitive. In vitro studies of tigecycline have demonstrated efficacy against *A. baumannii*, Enterobacter and MRSA [50].

We found *K. pneumoniae* had a similar resistance profile with *A. baumannii* and *P. aeruginosa*. Resistance of *K. pneumoniae* is worthy of further attention. Our findings confirm previous

reports that multi-drug resistant *A. baumannii*, ESBL-producing Enterobacteriaceae and carbapenem-resistant *P. aeruginosa* pose major problems for the treatment of gram-negative bacterial infections [42, 51].

S. aureus was among the first pathogen detected in wound secretions, and *S. epidermidis* was also rapidly prevalent in wounds. These bacteria are sensitive to glycopeptide antibiotics, and no resistant strains of *S. epidermidis* were found, however a majority of *S. aureus* isolates were methicillin-resistant, a far higher rate than reported elsewhere [20, 42].

We identified a higher rate of fungal pathogens in 2012 than 2011 and 2013. Specifically, *Candida albicans, tropicalis, glabrata* and *parapsilosis* were prevalent. These isolates were highly sensitive to itraconazole, fluconazole and voriconazole with a rate of resistance under 10%, while resistance to ketoconazole was more prevalent. Fungi were most prevalent in urine cultures. Further investigation will be required to determine the impact of patient age, antibiotic administration, sedentary lifestyle during hospitalization and long-term catheterization on for these high rates of infection.

In addition to local treatment of burn wounds and systemic antibiotic therapy, our center has adopted a bundle of hospital infection control measures. The cross-infection control measures currently applied in our department include nursing procedures, aseptic procedures, hand hygiene, disinfection and isolation, ward flow control, microbial monitoring and regular replacement of deep venous catheter location. Before 2012, patients were treated according to the guidelines of the *D.iagnostic Criteria for Infection after Bum Injury* (Chinese Journal of Burns, 2007) [33]. Our center conducts regular bacteriological monitoring of severe burn patients to ensure targeted medication, and ensures rapid application of tailored therapy. Antibiotic drugs were selected based on experience prior to identifying pathogens. The principle of "de-escalation" was adopted in antibiotic administration, stressing "early use, early stop" and "perioperative application". Antibiotics were applied to wounds, or administered systemically when they could not be applied to burn wound. Similar guidelines or strategies have been followed worldwide [20, 50, 52].

The Southwest Hospital Affiliated to Third Military Medical University reported that the detection rate of MRSA was gradually decreasing, and resistance to clindamycin, erythromycin and other macrolide drugs also declined [39, 41]. In this survey, the detection rate of MRSA and rates of resistance to clindamycin and erythromycin increased over the three-year study period, while resistance of *P. aeruginosa* to ciprofloxacin, levofloxacin and other fluoroquinolones remained relatively low. Westh *et al.* reported that resistance against macrolides was associated with usage of quinolones [53].

Limitations

This retrospective study only included data from patients treated at one site, and did not record patient clinical or demographic characteristics. The timespan of three years was too short to allow any trends in pathogen diversity or antibiotic or antifungal resistance to be defined. No samples were retained for more in-depth molecular identification and homology analysis, and as specimens were collected after hospitalization we could not differentiate between environmental community-acquired or hospital-acquired infection.

Conclusions

In this study, we characterized the pathogenic infections of 1942 inpatients of the burn ward of the second affiliated hospital of Zhejiang University of Medicine between 2011 and 2013. In total 2212 strains of pathogenic bacteria or fungi were cultured from wound secretions, blood, catheter specimens, respiratory secretions, urine and stool. Their sensitivity to commonly used antibiotic and antifungal drugs was retrospectively analyzed. Of the 2212 strains identified, 33.9% were gram-positive bacteria, 52.7% were gram-negative bacteria, and 13.4% were fungi. Gram-positive bacteria mainly included *S. aureus, S. epidermidis Enterococcus,* gram-negative bacteria mainly included *P. aeruginosa, A. baumannii, K. pneumoniae and E. coli*, while fungi mainly included *C. albicans* and *tropicalis*. Understanding the distribution of bacterial infections in Chinese hospitals will be crucial for the development of treatment guidelines designed to reduce hospital-acquired infection and reduce drug resistance. In-depth attention should be paid to proportion of MRSA, multi-resistant *A. baumanni,*

ESBL-producing enterobacteriaceae and Carbapenem-resistant *P. aeruginosa*.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Annual distribution of Gram-positive and negative bacteria and fungi detected in clinical samples

Year	Strains identified (n)	Gram-positive bacteria [n (%)]	Gram-negative bacteria [n (%)]	Fungi [n (%)]
Wound Secretion Culture				
2011	468	168 (35.9)	259 (55.3)	41 (8.8)
2012	518	188 (36.3)	253 (48.8)	77 (14.9)
2013	480	187 (39)	250 (52.1)	43 (8.9)
Sputum Culture				
2011	56	9 (16.1)	46 (82.1)	1 (1.8)
2012	41	10 (24.4)	30 (73.2)	1 (2.4)
2013	56	7 (12.5)	48 (85.7)	1 (1.8)
Urine Culture				
2011	100	13 (13)	62 (2)	25 (25)
2012	116	22 (19)	38 (32.8)	56 (48.2)
2013	119	27 (22.7)	59 (49.6)	33 (27.7)
Stool Culture				
2011	2	0 (0)	1 (50)	1 (50)
2012	3	0 (0)	0 (0)	3 (100)
2013	0	0 (0)	0 (0)	0 (0)
Blood Culture				
2011	49	26 (53)	22 (44.9)	1 (1.1)
2012	35	20 (57.1)	12 (34.3)	3 (8.6)
2013	44	23 (52.3)	20 (45.5)	1 (2.2)
Deep vein catheter culture				
2011	48	19 (39.6)	27 (56.3)	2 (4.1)
2012	37	17 (46)	16 (43)	4 (11)
2013	40	14 (35)	23 (57.5)	3 (7.5)