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Season, weather and predictors of healthcare-associated Gram-negative bloodstream infections: a case-only study

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SUMMARY

Background: Recent studies reported seasonality in healthcare-associated infections (HCAI). The association of this phenomenon with other risk factors for HCAI is not clear.
Aim: To analyse the interplay of season, weather and usual predictors of healthcare-associated bloodstream infections caused by Gram-negative bacilli (GNB-BSI).
Methods: A case-only study was conducted in a teaching hospital in Brazil. The study enrolled 446 subjects with GNB-BSI diagnosed from July 2012 to June 2016. Demographic data, comorbidities, invasive procedures and use of antimicrobials were reviewed in medical charts. The season in which GNB-BSI occurred, and weather parameters on the day of diagnosis were recorded. Factors associated with occurrence of GNB-BSI in different seasons (reference category: winter) and caused by different GNB (reference category: *Escherichia coli*) were analysed. Uni- and multi-variable models of multi-nomial logistic regression were used for analysis.
Findings: GNB-BSI diagnosed in summer was more likely to be caused by *Klebsiella* spp. [odds ratio (OR) 5.33; 95% confidence interval (CI) 2.04–13.96] or *Acinetobacter baumannii* (OR 2.69; 95% CI 1.04–6.96), and there was an association between *Klebsiella* spp. and spring (OR 2.86; 95% CI 1.14–7.18). Average temperature on the day of diagnosis was associated with *Klebsiella* spp. (OR 1.19; 95% CI 1.07–1.33) and *A. baumannii* (OR 1.20; 95% CI 1.07–1.34).
Conclusion: Warm seasons and daily temperature impact on the aetiology of GNB-BSI, even in models adjusted for usual risk factors. One possible explanation for these findings is that seasonality of healthcare-associated pathogens is intrinsic to micro-organisms, and not associated with comorbidities, procedures or use of antimicrobials.

Introduction

Seasonality of healthcare-associated infections (HCAI) caused by Gram-negative bacilli (GNB) has been reported recently [1]. It is usually characterized by 'summer peaks' in

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incidence [2–5] or association with environmental temperature [6,7]. Coincidentally, a multi-centre study reported increased incidence of GNB bloodstream infections (GNB-BSI) in hospitals located closer to the Equator [8].

Factors underlying this phenomenon have not been elucidated. Increased environmental reservoirs (inside or outside healthcare settings), understaffing associated with summer holidays, and expression of virulence factors in GNB have been suggested as contributing causes [1,9]. Ecological studies [3,6,7], although valuable for identifying seasonality, have not contributed to reinforcing either of these hypotheses.

Seasonality in the incidence of healthcare-associated pathogens could reflect seasonal variations in their epidemiological determinants. For some years, traditional risk factors for healthcare-associated GNB (especially those that are multidrug-resistant) have been described repeatedly [10]. They include severity of illness, comorbidities, time of exposure to health care ('time at risk'), invasive procedures and use of antimicrobials [11,12]. It is therefore worth investigating if those factors vary between seasons, thus contributing to seasonality.

This was the rationale of this study. Briefly, an observational, individual-based study was conducted to identify the interplay of season, weather and usual predictors of healthcare-associated GNB-BSI.

Methods

Setting

This study was conducted in a 450-bed teaching hospital in Botucatu, São Paulo State, Brazil. The hospital is a centre for tertiary care for several cities in an area comprising one million inhabitants [7].

Study design

A case-only study was conducted, enrolling subjects with healthcare-associated GNB-BSI diagnosed from July 2012 to June 2016.

Inclusion criteria

All adult patients (aged ≥18 years) admitted to the hospital who acquired healthcare-associated GNB-BSI caused by *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas aeruginosa* or *Acinetobacter baumannii* during the study period were included in this study. The diagnosis of healthcare-associated GNB-BSI required that positive blood cultures were collected after three days of admission ('three-midnight rule'), according to the recommendations of the Society for Healthcare Epidemiology of America [13]. All patients who presented with infection upon admission were excluded. Finally, for subjects who presented more than one GNB in successive blood cultures, only the first culture result was recorded.

Data collection

Data were collected in medical charts and laboratory files. Demographic data and comorbidities, including the Charlson Comorbidity Index, were recorded [14]. Other data were collected for 'time at risk', counted from admission until the day of diagnosis of GNB-BSI (i.e. the day of collection of the first blood culture positive for the GNB of interest). These data included admission to an intensive care unit (ICU); invasive procedures and devices; use of steroids, antineoplastic and other immunosuppressing drugs; and use of antimicrobials.

Data were complemented by: (a) season in which cases were diagnosed; and (b) average temperature (in degrees Celsius), average relative humidity and rainfall on the day of diagnosis of each GNB-BSI. These data were collected at a nearby meteorological station (Faculty of Agronomical Sciences, Botucatu, Brazil).

Table I
Meteorological data from the days of diagnosis of Gram-negative healthcare-associated bloodstream infections

Parameter/pathogen	Average	SD	*P*-value
Temperature (°C)			
Overall parameter	20.73	3.43	
Total GNB	**21.23**	**3.28**	**0.01**
Escherichia coli	20.24	3.43	0.22
Enterobacter spp.	20.77	3.08	0.94
***Klebsiella* spp.**	**21.50**	**3.37**	**0.04**
Pseudomonas aeruginosa	21.11	3.11	0.40
Acinetobacter baumannii	**21.92**	**3.12**	**<0.001**
Relative humidity (%)			
Overall parameter	73.87	14.00	
Total GNB	73.91	13.77	0.96
Escherichia coli	73.74	12.48	0.94
Enterobacter spp.	72.99	14.72	0.62
Klebsiella spp.	75.89	14.61	0.10
Pseudomonas aeruginosa	74.35	13.70	0.79
Acinetobacter baumannii	72.13	13.03	0.20
Rainfall (mm)			
Overall parameter	4.12	11.03	
Total GNB	4.49	11.44	0.54
Escherichia coli	3.79	10.75	0.80
Enterobacter spp.	3.30	7.81	0.55
Klebsiella spp.	5.44	14.27	0.21
Pseudomonas aeruginosa	6.26	13.01	0.13
Acinetobacter baumannii	4.54	10.63	0.69

GNB, Gram-negative bacilli; SD, standard deviation.
Note: groups were compared with the overall parameter (average values for all days in the whole study period) using Student's *t*-test. Significant results ($P < 0.05$) are presented in bold type.

Statistical analysis

Two analyses were performed. The first analysis compared GNB-BSI diagnosed in different seasons. Cases diagnosed in winter were used as the reference category. Models of polytomous (multi-nomial) logistic regression were used for both uni- and multi-variable analysis [15]. In the multi-variable step, a stepwise forward strategy was used, using criteria of $P < 0.05$ and $P > 0.1$ for insertion and removal of variables [16]. However, according to recommendations from the Infectious Diseases Society of America [17], 'time at risk' and the Charlson Comorbidity Index were included in all models.

The second analysis compared GNB-BSI caused by different GNB, using *E. coli* as the reference category. The analysis was

Table II
Descriptive results and univariate analysis (multi-nomial logistic regression) of factors associated with acquisition of healthcare-associated Gram-negative bloodstream infections in different seasons

Factors	Winter (87) *N (%)*	Spring (129) *N* (%)	Summer (122) *N* (%)	Autumn (108) *N* (%)
Micro-organisms				
Escherichia coli (reference)	*20 (23.0)*	20 (15.5)	14 (11.5)	21 (19.4)
Enterobacter spp.	*15 (23.1)*	20 (15.5)	16 (13.1)	14 (13.0)
Klebsiella spp.	*16 (18.4)*	34 (26.4)	**44 (36.1)**[b]	32 (29.6)
Pseudomonas aeruginosa	*15 (17.2)*	24 (18.6)	16 (13.1)	9 (8.3)
Acinetobacter baumannii	*21 (24.1)*	31 (24.0)	32 (26.2)	32 (29.6)
Demographic data				
Female sex	*38 (43.7)*	59 (45.7)	54 (44.3)	40 (37.0)
Age, years, median (quartiles)*	*60 (50–74)*	57 (50–71)	64 (51–74)	63 (53–73)
Comorbidities				
Heart disease	*20 (23.0)*	**46 (35.7)**[a]	33 (27.0)	30 (30.6)
Lung disease	*12 (13.8)*	18 (14.0)	18 (14.8)	13 (12.0)
Renal disease	*18 (20.7)*	32 (24.8)	26 (21.3)	32 (29.6)
Liver disease	*10 (11.5)*	15 (11.6)	13 (10.7)	8 (7.4)
Diabetes mellitus	*22 (25.3)*	37 (28.7)	33 (27.0)	32 (29.6)
Central nervous system disease	*22 (25.3)*	28 (21.7)	36 (29.5)	25 (23.1)
Solid malignancy	*18 (20.7)*	20 (15.5)	21 (17.2)	21 (19.4)
Lymphoma/leukaemia	*2 (2.3)*	10 (7.8)	11 (9.0)	5 (4.6)
AIDS	*0 (0.0)*	5 (3.8)	6 (4.9)	3 (2.8)
Trauma	*6 (6.9)*	10 (7.8)	11 (9.0)	5 (4.6)
Charlson Comorbidity Index, median (quartiles)*	*2 (1–4)*	3 (1–4)	2 (1–4)	3 (1–5)
Admission data				
Time at risk, days, median (quartiles)*	*13 (8–85)*	12 (7–22)	13 (8–23)	16 (9–23)
Admission to ICU	*42 (51.2)*	66 (51.6)	48 (40.7)	53 (51.5)
Surgery	*57 (65.5)*	**62 (48.1)**[a]	**62 (50.8)**[a]	56 (51.9)
Placement of drains	*33 (37.9)*	36 (27.9)	33 (27.0)	30 (27.8)
Haemodialysis	*12 (13.8)*	22 (17.1)	16 (13.1)	18 (16.7)
Urinary catheter	*69 (79.3)*	88 (68.2)	84 (68.9)	79 (73.1)
Mechanical ventilation	*42 (48.3)*	56 (43.4)	49 (40.2)	52 (48.1)
Central venous catheter	*64 (73.6)*	91 (70.5)	83 (68.0)	69 (63.9)
Parenteral nutrition	*8 (9.2)*	11 (8.5)	8 (6.6)	7 (6.5)
Nasoenteral tube	*59 (67.8)*	72 (55.8)	**64 (52.5)**[a]	67 (62.0)
Use of steroids	*21 (24.5)*	39 (30.2)	37 (30.3)	34 (31.5)
Use of antineoplastic drugs	*3 (3.4)*	9 (7.0)	**14 (11.4)**[a]	**16 (14.8)**[a]
Use of other immunosuppressing drugs	*8 (9.2)*	10 (7.8)	8 (6.6)	5 (4.6)
Neutropenia	*2 (2.3)*	12 (9.3)	12 (9.8)[a]	8 (7.4)
Pressure ulcer	*20 (23.0)*	24 (18.6)	31 (25.4)	**42 (38.9)**[a]
Use of antimicrobials				
Penicillins	*2 (2.3)*	7 (5.4)	3 (3.3)	4 (3.7)
Penicillins plus beta-lactamase inhibitors	*25 (28.7)*	40 (31.0)	37 (30.3)	35 (32.4)
Cephalosporins, first/second generation	*31 (35.6)*	**24 (18.6)**[b]	29 (23.8)	**14 (13.0)**[b]
Cephalosporins, third/fourth generation	*20 (23.0)*	28 (21.7)	31 (25.4)	35 (32.4)
Carbapenems	*25 (28.7)*	32 (24.8)	32 (26.2)	36 (33.3)
Polymyxins	*12 (13.8)*	8 (6.2)	8 (6.6)	11 (10.2)
Quinolones	*15 (17.2)*	22 (17.1)	27 (22.1)	26 (24.1)
Vancomycin	*24 (27.6)*	32 (24.8)	28 (23.0)	34 (31.5)
Linezolid	*6 (6.9)*	**1 (0.8)**[a]	**1 (0.8)**[a]	2 (1.9)
Macrolides	*11 (12.6)*	12 (9.3)	11 (9.0)	9 (8.3)
Anti-anaerobic drugs	*16 (18.4)*	23 (17.8)	22 (18.0)	23 (21.3)
Trimethoprim/sulfamethoxazole	*4 (4.6)*	8 (6.2)	8 (7.4)	10 (8.2)
Number of antimicrobials used, median (quartiles)*	*2 (1–4)*	1 (0–3)	2 (1–3)	2 (1–4)
Healthcare-associated infections				
Central-line-associated bloodstream infection	*64 (73.6)*	91 (70.5)	83 (68.0)	69 (63.9)
Catheter-associated urinary tract infection	*27 (31.0)*	33 (25.8)	37 (30.3)	32 (39.8)
Pneumonia (non-ventilator-associated)	*11 (12.6)*	21 (16.3)	16 (13.1)	12 (11.1)

Table II (*continued*)

Factors	Winter (87) *N (%)*	Spring (129) *N (%)*	Summer (122) *N (%)*	Autumn (108) *N (%)*
Pneumonia (ventilator-associated)	*19 (21.8)*	18 (14.0)	27 (22.1)	22 (21.3)
Surgical site infection	*9 (10.3)*	13 (10.1)	12 (9.8)	11 (10.2)
Infection of skin/soft tissues	*8 (9.2)*	5 (3.9)	7 (5.7)	6 (5.6)
Other healthcare-associated infections	*6 (6.9)*	9 (7.0)	11 (9.0)	6 (5.6)
Two or more healthcare-associated infections	*38 (43.7)*	50 (38.8)	44 (36.1)	44 (49.7)

Data from the reference category are presented in italics.
AIDS, acquired immunodeficiency syndrome (defined as a positive test for human immunodeficiency virus plus an opportunistic infection and/or a CD4+ lymphocyte count below 200); ICU, intensive care unit; anti-anaerobic drugs, clindamycin and metronidazole.
Note: results are presented as *N* (%) except for variables marked with * (presented as median [quartiles]). Winter is the reference category for comparisons. Significant differences are presented in bold type. [a]$P < 0.05$; [b]$P < 0.01$.

generally similar, except that weather parameters (temperature, humidity and rainfall) were included among the study variables in this phase. Both analyses were performed using SPSS Version 20 (IBM Corp., Armonk, NY, USA).

Ethical issues

This study was approved by the local committee for ethics in research.

Results

This study enrolled 446 subjects with GNB-BSI caused by the following micro-organisms: *E. coli*, 75; *Enterobacter* spp., 65; *Klebsiella* spp., 126; *P. aeruginosa*, 64; and *A. baumannii*, 116. Central-line-associated BSI (CLABSI) accounted for 68.8% of cases. Overall, cases were more frequent in summer (27.4%) and spring (28.9%) than in winter (19.5%) and autumn (24.2%) ($P = 0.01$). Table I presents a description of meteorological data from the days of collection of positive blood cultures, with reference to overall parameters.

Uni- and multi-variable analyses of factors associated with diagnosis of GNB-BSI in different seasons are presented in Tables II and III. In multi-variable analysis, *Klebsiella* spp. and *A. baumannii* were found to be more likely to be involved in the aetiology of GNB-BSI during summer. There was also an association between *Klebsiella* spp. and spring. Finally, minor associations of seasons with other variables were identified, such as neutropenia (less likely to occur in winter) and the use of linezolid or first-/second-generation cephalosporins (greater during winter).

When, in turn, uni- and multi-variable models were used to analyse predictors of different pathogens (Tables IV, V and VI), higher average temperature on the day of diagnosis was found to be associated with *Klebsiella* spp. and *A. baumannii*. Other relevant associations with statistical significance ($P < 0.05$) included: greater relative humidity and *Klebsiella* spp.; admission to ICU and all pathogens (in comparison with *E. coli*); and use of carbapenems and *A. baumannii*.

Discussion

Seasonality of infectious diseases is a complex phenomenon, the determinants of which are not fully understood [18]. The impact of season and climate on parasite–host systems is relatively easy to understand for vector-borne diseases, but not as clear for human-to-human transmission, or for acquisition of pathogens from endogenous or environmental sources (such as occurs in HCAI) [19]. Even so, understanding the mechanisms and pattern of seasonality may provide clues regarding how and when control measures should be applied. An example is the promotion of yearly influenza vaccine campaigns during autumn [20]. Seasonality of HCAI should not be interpreted as a mere curiosity, but should point to opportunities for prevention and control. This is particularly relevant considering that working processes (e.g. surgical activity, admission of new trainees in teaching hospitals) in healthcare settings may present seasonal variations.

Given previous evidence of seasonality of GNB-BSI, this study focused on identifying variations of risk factors and aetiologic agents between seasons. Risk factors presented a rather stable pattern of occurrence through the year. Minor differences, such as greater use of cephalosporins in winter, might reflect seasonal variation in the intensity of surgical activities in the hospital, as these antimicrobials are often used for surgical prophylaxis. Others (e.g. neutropenia is more common in warm months) are puzzling, although greater use of linezolid during winter might be an indirect marker of seasonality of infections caused by vancomycin-resistant enterococci. This finding requires further investigation.

The 'summer peaks' of *Klebsiella* spp. and *A. baumannii* were consistent. This is consistent with previous reports [4,7]. These micro-organisms were also associated with greater environmental temperature, a finding that has been reported previously [21]. Interestingly, these associations persisted even after adjustment for specific risk factors for these micro-organisms, such as admission to ICU and use of carbapenems. In this respect, it is worth noting that even when the analysis was restricted to patients admitted to ICUs (which were units with climate control), the association of the outside temperature with *Klebsiella* spp. [odds ratio (OR) 1.31; 95% confidence interval (CI) 1.09–1.57; $P = 0.004$] and *A. baumannii* (OR 1.36; 95% CI 1.13–1.63; $P = .001$) persisted. Similar results were found when CLABSI cases alone were included in the multivariable models.

This phenomenon also impacted on overall antimicrobial resistance in GNB-BSI. Using imipenem as a marker of overall resistance, the proportion of resistant isolates for different pathogens in this study was: *A. baumannii*, 81.9%; *P. aeruginosa*, 23.4%; *Klebsiella* spp., 13.6%; *Enterobacter* spp., 3.1%; and *E. coli*, 1.4%. Thus, for instance, the predominance of *A. baumannii* in summer and/or on warmer days was associated with an increase in imipenem resistance, with implications for therapy and outcome of affected patients [22]. It is worth noting

Table III
Final multi-variable model of multi-nomial logistic regression for acquisition of healthcare-associated Gram-negative bloodstream infections in different seasons

Factors	OR (95% CI)		
	Spring	Summer	Autumn
Micro-organisms			
Escherichia coli (reference)			
Enterobacter spp.	1.78 (0.68–4.71)	2.09 (0.73–5.89)	0.89 (0.31–2.54)
Klebsiella spp.	**2.86 (1.14–7.18)**[a]	**5.33 (2.04–13.96)**[b]	2.17 (0.85–5.52)
Pseudomonas aeruginosa	2.35 (0.88–6.26)	2.22 (0.77–6.43)	0.63 (0.20–1.94)
Acinetobacter baumannii	1.89 (0.77–4.62)	**2.69 (1.04–6.96)**[a]	1.32 (0.53–3.30)
Other factors			
Charlson Comorbidity Index	1.05 (0.92–1.19)	1.03 (0.91–1.18)	1.07 (0.94–1.22)
Time at risk	0.99 (0.97–1.01)	0.99 (0.97–1.01)	0.99 (0.97–1.01)
Neutropenia	**6.40 (1.12–36.55)**[a]	**7.93 (1.34–45.63)**[a]	4.39 (0.74–25.97)
Pressure ulcer	0.95 (0.43–2.13)	1.49 (0.68–3.28)	**2.81 (1.28–6.16)**[a]
Use of cephalosporins, first/second generation	**0.43 (0.22–0.83)**[a]	0.66 (0.34–1.25)	**0.29 (0.13–0.62)**[b]
Use of linezolid	**0.06 (0.01–0.66)**[a]	0.06 (0.01–0.61)[a]	**0.13 (0.02–0.85)**[a]

OR, odds ratio; CI, confidence interval.
Note: winter is the reference category. Significant results are presented in bold type. [a]$P < 0.05$; [b]$P < 0.01$.

that temperature on the day of diagnosis was significantly associated with overall imipenem resistance on logistic regression (Table VII).

Two aspects from this study must be emphasized. First, it was conducted in a hospital located in a tropical area. This is interesting as most studies on seasonality of HCAI and GNB have been conducted in countries with temperate climates [1]. In the tropics, variations in weather (temperature, humidity) between different seasons are tenuous compared with those observed in temperate areas. Summer is warm and rainy, while a moderate decrease in temperature and humidity occurs during winter. While climate has been shown to be a relevant factor in the incidence of GNB-BSI [8], the association of warmer seasons and higher temperatures with specific GNB seems to be valid for most latitudes.

A second aspect concerns the overall patterns of demography, comorbidities and procedures among study subjects. They were fairly old (median age 62 years), with several comorbidities (median Charlson Comorbidity Index 3). Almost half of the patients (48.6%) were admitted to an ICU, 39.5% acquired HCAI in two or more sites, and most had invasive devices placed. Finally, 49.8% of patients died during admission. These are common characteristics of GNB-BSI [23]. Traditional risk factors for GNB-BSI were present at similar rates during different seasons. One hypothesis to explain seasonality in the absence of variations in risk factors is that intrinsic properties of micro-organisms (either invasiveness or the ability to survive in environmental reservoirs), rather than changes in healthcare processes, underlie seasonality.

This study had a few limitations. First, it was undertaken at a single centre, and subjects were selected over a relatively small time period. The impact of 'summer understaffing' on the incidence of GNB-BSI was not assessed [1,9]. Seasonal changes in patient or staff behaviour were also not assessed. The study design did not allow the possibility of ecological competition between different micro-organisms in the hospital setting to be taken into account – a phenomenon that has been reported [24]. Finally, the temperature and humidity on the day of diagnosis were used, while the impact of weather on the acquisition of GNB-BSI might have occurred on previous days. While conducting this study, the authors faced the dilemma of using the temperature on the day of diagnosis, or a moving average of temperature over three or seven previous days. However, the incubation period for HCAI is not clear. Therefore, and based on the fact that temperature on the day of diagnosis is likely to be similar to that on previous days, the latter was chosen. Of note, a recent study found similar results

Table IV
Results of univariate analysis of meteorological factors associated with acquisition of healthcare-associated Gram-negative bloodstream infections caused by different micro-organisms

Meteorological factors	*Escherichia coli* (75)	*Enterobacter* spp. (65)	*Klebsiella* spp. (126)	*Pseudomonas aeruginosa* (64)	*Acinetobacter baumannii* (116)
Temperature, °C, median (quartiles)	*20.7 (17.6–22.4)*	20.7 (19.1–22.9)	**21.5 (19.3–23.8)**[a]	21.6 (19.1–23.3)	**22.0 (20.0–24.0)**[a]
Humidity, %, median (quartiles)	*75.2 (65.3–81.8)*	75.3 (63.7–82.7)	76.9 (68.3–87.4)	74.8 (65.9–82.5)	75.0 (66.5–80.9)
Rainfall, mm, median (quartiles)	*0.0 (0.0–0.0)*	0.0 (0.0–1.4)	0.0 (0.0–4.73)	0.0 (0.0–8.97)	0.0 (0.0–4.9)

Data from the reference category are presented in italics.
Note: significant results are presented in bold type. [a]$P < 0.01$.

Table V
Descriptive results and univariate analysis (multi-nomial logistic regression) of factors associated with acquisition of healthcare-associated Gram-negative bloodstream infections caused by different micro-organisms

Factors	*Escherichia coli* (75) *N (%)*	*Enterobacter* spp. (65) *N* (%)	*Klebsiella* spp. (126) *N* (%)	*Pseudomonas aeruginosa* (64) *N* (%)	*Acinetobacter baumannii* (116) *N* (%)
Season					
Winter (reference)	*20 (26.7)*	15 (23.1)	16 (12.7)	15 (23.4)	21 (18.1)
Spring	*20 (256.7)*	20 (30.8)	34 (27.0)	24 (37.5)	31 (26.7)
Summer	*14 (18.7)*	16 (24.6)	**44 (34.9)**[b]	16 (25.0)	32 (27.6)
Autumn	*21 (28.0)*	14 (21.5)	32 (25.4)	9 (14.1)	32 (27.6)
Demographic data					
Female sex	*32 (42.7)*	27 (41.5)	54 (42.9)	27 (42.2)	51 (44.0)
Age, years, median (quartiles)	*64 (54–74)*	62 (52–74)	63 (50–74)	59 (47–74)	62 (47–72)
Comorbidities					
Heart disease	*22 (29.3)*	21 (32.3)	36 (28.6)	19 (29.7)	34 (29.3)
Lung disease	*10 (13.3)*	8 (12.3)	21 (16.7)	9 (14.1)	13 (11.2)
Renal disease	*9 (12.0)*	**19 (29.2)**[a]	**37 (29.4)**[b]	11 (17.2)	**32 (27.6)**[a]
Liver disease	*14 (18.7)*	5 (7.7)	14 (11.1)	5 (7.8)	**8 (6.9)**[a]
Diabetes mellitus	*22 (29.3)*	15 (23.1)	34 (27.0)	14 (2.19)	39 (33.6)
Central nervous system disease	*16 (21.3)*	17 (26.2)	25 (19.8)	21 (23.8)	32 (27.6)
Solid malignancy	*22 (29.3)*	12 (18.5)	25 (19.8)	8 (12.5)	13 (11.2)
Lymphoma/leukaemia	*3 (4.0)*	2 (3.1)	7 (5.6)	4 (6.2)	12 (10.3)
AIDS	*4 (5.3)*	1 (1.5)	2 (1.6)	4 (6.2)	3 (2.6)
Trauma	*4 (5.3)*	5 (7.7)	4 (3.2)	4 (6.2)	16 (13.8)
Charlson Comorbidity Index, median (quartiles)	*3 (1–6)*	**2 (1–4)**[a]	3 (1–4)	2 (1–4)	3 (1–4)
Admission data					
Time at risk, days, median (quartiles)	*10 (6–16)*	**13 (8–27)**[b]	**15 (8–25)**[b]	**16 (8–29)**[c]	**14 (9–23)**[b]
Admission to ICU	*19 (26.4)*	26 (41.3)	**54 (44.6)**[a]	**42 (66.7)**[c]	**68 (60.7)**[c]
Surgery	*36 (48.0)*	41 (63.1)	59 (46.8)	40 (62.5)	61 (52.7)
Placement of drains	*20 (60.7)*	21 (32.3)	37 (29.4)	22 (34.4)	32 (26.7)
Haemodialysis	*4 (5.3)*	8 (12.3)	**28 (22.2)**[b]	7 (10.9)	**21 (18.5)**[a]
Urinary catheter	*47 (62.7)*	39 (60.0)	84 (66.7)	48 (75.0)	**102 (87.9)**[c]
Mechanical ventilation	*21 (28.0)*	23 (35.4)	45 (35.7)	**38 (59.4)**[c]	**72 (62.1)**[c]
Central venous cateter	*39 (52.0)*	50 (76.9)[b]	85 (67.5)	**48 (75.0)**[b]	**85 (73.3)**[b]
Parenteral nutrition	*5 (6.7)*	7 (10.8)	11 (8.7)	4 (6.3)	7 (6.0)
Nasoenteral tube	*31 (41.3)*	32 (49.2)	67 (53.2)	**48 (75.0)**[c]	**84 (72.4)**[c]
Use of steroids	*12 (16.0)*	14 (21.8)	**44 (34.9)**[b]	**25 (39.1)**[b]	**36 (31.0)**[a]
Use of antineoplastic drugs	*9 (12.0)*	5 (7.7)	15 (11.9)	4 (6.2)	9 (7.8)
Use of other immunosuppressing drugs	*1 (1.3)*	3 (4.6)	**17 (13.5)**[a]	6 (9.4)	4 (3.4)
Neutropenia	*8 (10.7)*	2 (3.1)	8 (6.3)	4 (6.2)	12 (10.3)
Pressure ulcer	*8 (10.7)*	15 (23.1)	27 (21.4)	**23 (35.9)**[b]	**44 (37.9)**[c]
Use of antimicrobials					
Penicillins	*2 (2.7)*	0 (0.0)	6 (4.8)	2 (3.1)	7 (6.0)
Penicillins plus beta-lactamase inhibitors	*12 (16.0)*	19 (29.2)	33 (26.2)	**26 (40.6)**[b]	**47 (40.5)**[b]
Cephalosporins, first/second generation	*16 (21.3)*	20 (30.8)	24 (19.0)	19 (29.7)	19 (16.4)
Cephalosporins, third/fourth generation	*12 (16.0)*	10 (8.8)	31 (24.6)	15 (23.4)	**46 (39.7)**[b]
Carbapenems	*4 (5.3)*	**12 (18.5)**[a]	**31 (24.6)**[b]	**21 (32.8)**[c]	**57 (49.1)**[c]
Polymyxins	*2 (2.7)*	7 (10.8)	7 (5.6)	**10 (15.6)**[a]	**13 (11.8)**[a]
Quinolones	*18 (24.0)*	**6 (9.2)**[a]	227 (21.4)	9 (14.1)	30 (25.9)
Vancomycin	*7 (9.3)*	11 (16.9)	**26 (20.6)**[a]	**21 (32.8)**[b]	**53 (45.7)**[c]
Linezolid	*0 (0.0)*	0 (0.0)	1 (0.8)	4 (6.2)	5 (4.3)
Macrolides	*5 (6.7)*	6 (9.2)	8 (6.3)	6 (9.4)	18 (15.5)
Anti-anaerobic drugs	*15 (20.0)*	8 (12.3)	17 (13.5)	9 (14.1)	35 (30.2)
Trimethoprim/sulfamethoxazole	*6 (8.0)*	1 (1.1)	9 (7.1)	4 (6.2)	10 (8.6)
Number of antimicrobials used, median (quartiles)*	*1 (0–2)*	1 (0–2)	2 (0–3)	**2 (1–4)**[b]	**3 (2–5)**[c]

(*continued on next page*)

Table V (*continued*)

Factors	*Escherichia coli* (75) *N (%)*	*Enterobacter* spp. (65) *N* (%)	*Klebsiella* spp. (126) *N* (%)	*Pseudomonas aeruginosa* (64) *N* (%)	*Acinetobacter baumannii* (116) *N* (%)
Healthcare-associated infections					
Central-line-associated bloodstream infection	*39 (52.0)*	**50 (76.9)**[b]	**85 (67.5)**[a]	**48 (75.0)**[b]	**85 (73.3)**[b]
Catheter-associated urinary tract infection	*23 (30.7)*	17 (26.2)	45 (35.7)	25 (39.1)	30 (25.9)
Pneumonia (non-ventilator-associated)	*9 (12.0)*	6 (9.2)	13 (10.3)	11 (17.2)	21 (18.1)
Pneumonia (ventilator-associated)	*5 (6.7)*	**13 (20.0)**[a]	**21 (16.7)**[a]	**16 (25.0)**[b]	**32 (27.6)**[b]
Surgical site infection	*5 (6.7)*	8 (12.3)	11 (8.7)	9 (14.1)	12 (10.3)
Infection of skin/soft tissues	*2 (2.7)*	6 (9.2)	5 (4.0)	3 (4.7)	10 (8.6)
Other healthcare-associated infections	*13 (17.3)*	**2 (3.1)**[a]	11 (8.7)	**0 (0.0)**[b]	**6 (5.2)**[b]
Two or more healthcare-associated infections	*16 (21.3)*	**26 (40.0)**[a]	42 (33.3)	**33 (51.6)**[b]	**59 (50.9)**[b]

Data from the reference category are presented in italics.
AIDS, acquired immunodeficiency syndrome (defined as a positive test for human immunodeficiency virus plus an opportunistic infection and/or a CD4+ lymphocyte count below 200); ICU, intensive care unit; anti-anaerobic drugs, clindamycin and metronidazole.
Note: results are presented as *N* (%) except for variables marked with * (presented as median [quartiles]). Winter is the reference category for comparisons. Significant differences are presented in bold type. [a]$P < 0.05$; [b]$P < 0.01$; [c] $P < 0.001$.

Table VI
Final multi-variable model of multi-nomial logistic regression for acquisition of healthcare-associated Gram-negative bloodstream infections caused by different micro-organisms

Factors	OR (95% CI)			
	Enterobacter spp.	*Klebsiella* spp.	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*
Meteorology				
Temperature (°C)	1.01 (0.90–1.14)	**1.19 (1.07–1.33)**[b]	1.10 (0.97–1.25)	**1.20 (1.07–1.34)**[b]
Relative humidity (%)	1.00 (0.97–1.03)	**1.04 (1.01–1.07)**[b]	1.02 (0.99–1.05)	1.02 (0.99–1.05)
Other factors				
Renal disease	**3.98 (1.43–11.08)**[b]	2.09 (0.81–5.39)	1.05 (0.33–3.37)	**2.85 (1.09–7.47)**[a]
Charlson Comorbidity Index	**0.78 (0.67–0.92)**[b]	0.91 (0.80–1.04)	0.91 (0.78–1.05)	0.93 (0.81–1.06)
Time at risk	**1.05 (1.01–1.09)**[b]	**1.04 (1.01–1.07)**[a]	1.03 (1.00–1.07)	0.99 (0.95–1.02)
Admission to ICU	**3.16 (1.24–8.09)**[a]	**3.10 (1.38–6.94)**[b]	8.35 (3.04–22.91)[c]	**2.46 (1.11–5.45)**[a]
Use of immunosuppressing drugs	1.54 (0.13–18.43)	9.62 (0.80–72.79)	7.98 (0.70–90.49)	0.89 (0.08–10.37)
Use of carbapenems	1.30 (0.32–5.24)	2.50 (0.72–8.65)	2.92 (0.79–10.83)	**12.76 (3.86–42.17)**[c]
Use of anti-anaerobic drugs	**0.35 (0.12–0.99)**[a]	**0.38 (0.16–0.91)**[a]	0.39 (0.14–1.06)	1.24 (0.56–2.77)

ICU, intensive care unit; anti-anaerobic drugs, clindamycin and metronidazole; OR, odds ratio; CI, confidence interval.
Note: *Escherichia coli* is the reference category. Significant results are presented in bold type. [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.

for analysis using temperature on the day of diagnosis or the moving average of the seven previous days [21].

This study also had several strengths. To the authors' knowledge, this is the first study to analyse the interplay of traditional risk factors, season and weather parameters on the epidemiology of HCAI. Extensive chart review was performed and rigorous analytic models were used.

In conclusion, this study identified the impact of season and weather on the aetiology of GNB-BSI. The impact was independent of demography, comorbidities, invasive procedures or use of antimicrobials. It was detected in wards with and wards without climate control. While several doubts remain regarding the mechanisms underlying seasonality of GNB, these findings reinforce the need for strengthening infection control measures during summer and in warmer periods.

Table VII
Final multi-variable model for overall imipenem resistance in Gram-negative bacilli bloodstream infections

Factor	OR (95% CI)	*P*-value
Temperature	1.13 (1.04–1.22)	0.006
Time at risk	0.96 (0.94–0.98)	<0.001
Pressure ulcer	2.27 (1.28–4.01)	0.005
Cephalosporins, third/fourth generation	2.45 (1.40–4.28)	0.002
Carbapenems	7.49 (4.03–13.84)	<0.001
Anti-anaerobic drugs	2.33 (1.28–4.22)	0.005

OR, odds ratio. CI, confidence interval.

Conflict of interest statement
None declared.

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References

[1] Richet H. Seasonality in Gram-negative and healthcare-associated infections. Clin Microbiol Infect 2012;18:934—40.

[2] McDonald LC, Banerjee SN, Jarvis WR. Nosocomial Infections Surveillance System. Seasonal variation of *Acinetobacter* infections: 1987—1996. Clin Infect Dis 1999;29:1133—7.

[3] Perencevich EN, McGregor JC, Shardell M, Furuno JP, Harris AD, Morris Jr JG, et al. Summer peaks in the incidences of Gram-negative bacterial infection among hospitalized patients. Infect Control Hosp Epidemiol 2008;29:1124—31.

[4] Anderson DJ, Richet H, Chen LF, Spelman DW, Hung YJ, Huang AT, et al. Seasonal variation in *Klebsiella pneumoniae* bloodstream infection on 4 continents. J Infect Dis 2008;197:752—6.

[5] Kolonitsiou F, Papadimitriou-Olivgeris M, Spiliopoulou A, Stamouli V, Papakostas V, Apostolopoulou E, et al. Trends of bloodstream infections in a university Greek hospital during a three-year period: incidence of multidrug-resistant bacteria and seasonality in Gram-negative predominance. Pol J Microbiol 2017;66:171—80.

[6] Schwab F, Gastmeier P, Meyer E. The warmer the weather, the more Gram-negative bacteria — impact of temperature on clinical isolates in intensive care units. PLoS One 2014;9:e91105.

[7] Fortaleza CM, Caldeira SM, Moreira RG, Akazawa RT, Corrente JE, de Souza LdoR, et al. Tropical healthcare epidemiology: weather determinants of the etiology of bloodstream infections in a Brazilian hospital. Infect Control Hosp Epidemiol 2014;35:85—8.

[8] Fisman D, Patrozou E, Carmeli Y, Perencevich E, Tuite AR, Mermel LA, et al. Geographical variability in the likelihood of bloodstream infections due to Gram-negative bacteria: correlation with proximity to the equator and health care expenditure. PLoS One 2014;9:e114548.

[9] Eber MR, Shardell M, Schweizer ML, Laxminarayan R, Perencevich EN. Seasonal and temperature-associated increases in Gram-negative bacterial bloodstream infections among hospitalized patients. PLoS One 2011;6:e25298.

[10] Safdar N, Maki DG. The commonality of risk factors for nosocomial colonization and infection with antimicrobial-resistant *Staphylococcus aureus*, enterococcus, Gram-negative bacilli, *Clostridium difficile*, and *Candida*. Ann Intern Med 2002;136:834—44.

[11] Falagas ME, Kopterides P. Risk factors for the isolation of multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: a systematic review of the literature. J Hosp Infect 2006;64:7—15.

[12] van Loon K, Voor In 't Holt AF, Vos MC. A systematic review and meta-analyses of the clinical epidemiology of carbapenem-resistant Enterobacteriaceae. Antimicrob Agents Chemother 2017;62:e01730—17.

[13] Cohen AL, Calfee D, Fridkin SK, Huang SS, Jernigan JA, Lautenbach E, et al. Recommendations for metrics for multidrug-resistant organisms in healthcare settings: SHEA/HICPAC position paper. Infect Control Hosp Epidemiol 2008;29:901—13.

[14] Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373—83.

[15] Biesheuvel CJ, Vergouwe Y, Steyerberg EW, Grobbee DE, Moons KG. Polytomous logistic regression analysis could be applied more often in diagnostic research. J Clin Epidemiol 2008;61:125—34.

[16] Greenland S. Modeling and variable selection in epidemiologic analysis. Am J Public Health 1989;79:340—9.

[17] Harris AD, Karchmer TB, Carmeli Y, Samore MH. Methodological principles of case—control studies that analyzed risk factors for antibiotic resistance: a systematic review. Clin Infect Dis 2001;32:1055—61.

[18] Fisman DN. Seasonality of infectious diseases. Annu Rev Public Health 2007;28:127—43.

[19] Fisman D. Seasonality of viral infections: mechanisms and unknowns. Clin Microbiol Infect 2012;18:946—54.

[20] Altizer S, Dobson A, Hosseini P, Hudson P, Pascual M, Rohani P. Seasonality and the dynamics of infectious diseases. Ecol Lett 2006;9:467—84.

[21] Caldeira SM, Cunha AR, Akazawa RT, Moreira RG, Souza Ldo R, Fortaleza CM. Weather parameters and nosocomial bloodstream infection: a case-referent study. Rev Saude Publ 2015;49:19.

[22] Kadri SS, Hohmann SF, Orav EJ, Bonne SL, Moffa MA, Timpone JG, et al. Tracking colistin-treated patients to monitor the incidence and outcome of carbapenem-resistant Gram-negative infections. Clin Infect Dis 2015;60:79—87.

[23] Ergönül Ö, Aydin M, Azap A, Başaran S, Tekin S, Kaya Ş, et al. Healthcare-associated Gram-negative bloodstream infections: antibiotic resistance and predictors of mortality. J Hosp Infect 2016;94:381—5.

[24] Lastoria LC, Caldeira SM, Moreira RG, Akazawa RT, Maion JC, Fortaleza CM. Ecological competition and the incidence of *Acinetobacter baumannii* bloodstream infections in a teaching hospital in Southeastern Brazil. Rev Soc Bras Med Trop 2014;47:583—8.