International Journal of Antimicrobial Agents 61 (2023) 106806

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Bloodstream infections due to Gram-negative bacteria in patients with hematologic malignancies: updated epidemiology and risk factors for multidrug-resistant strains in an Italian perspective survey

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ARTICLE INFO

Article history:
Received 16 January 2023
Accepted 28 March 2023

Editor: Professor Jian Li

Keywords:
Antimicrobial resistance
Gram-negative bacteria
Bloodstream infections
Epidemiology
Hematological cancer
Multidrug resistance

ABSTRACT

Bloodstream infections (BSI) caused by Gram-negative bacteria (GNB) in patients with hematological malignancies (HM) have been associated with high mortality rates, particularly with infections caused by antibiotic-resistant strains.

A multicenter cohort study including all consecutive episodes of GNB BSI in HM patients was conducted to update the epidemiology and antibiotic resistance patterns (compared to our previous survey conducted between 2009 and 2012) and investigate risk factors for GNB BSI due to multidrug-resistant (MDR) isolates.

A total of 834 GNB were recovered in 811 BSI episodes from January 2016 to December 2018. Compared to the previous survey, there was a significant reduction in use of fluoroquinolone prophylaxis and a significant recovery in susceptibility rates to ciprofloxacin among *Pseudomonas aeruginosa*, *Escherichia*

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https://doi.org/10.1016/j.ijantimicag.2023.106806

coli and *Enterobacter cloacae* isolates. In addition, there was a shift to a significantly increased susceptibility of *P. aeruginosa* isolates to ceftazidime, meropenem, and gentamicin. A total of 256/834 (30.7%) isolates were MDR. In multivariable analysis, MDR bacteria culture-positive surveillance rectal swabs, previous therapy with aminoglycosides and carbapenems, fluoroquinolone prophylaxis, and time at risk were independently associated with MDR GNB BSI.

In conclusion, despite the persistence of a high prevalence of MDR GNB, there was a shift to a reduced use of fluoroquinolone prophylaxis and increased rates of susceptibility to fluoroquinolones in almost all isolates and to almost all antibiotics tested among *P. aeruginosa* isolates, compared to our previous survey. Fluoroquinolone prophylaxis and previous rectal colonization by MDR bacteria were independent risk factors for MDR GNB BSI in the present study.

1. Introduction

Improvements in the medical field have ensured better treatment for patients with hematological malignancies (HM). However, the use of invasive devices (e.g., urinary catheters, central venous catheters), and the deep depression of the immune system secondary to the underlying malignancy and chemotherapy, renders these patients particularly susceptible to severe infections. Among these, bloodstream infections (BSI) remain the most common and life-threatening infectious complications, with a described prevalence ranging from 11% to 38% and a crude mortality rate that reaches 40% [1].

Gram-negative bacteria (GNB) have recently been reported to be a more frequent cause of BSI than Gram-positive bacteria in HM patients, particularly in South-Eastern Europe and Asia, and high mortality rates have been reported in patients with GNB BSI, especially those caused by antibiotic-resistant strains [1–8].

Results from our previous Italian survey, conducted between 2009 and 2012, on the epidemiology and antimicrobial resistance patterns of bacterial BSI in patients with HM, showed that GNB were isolated in 52.8% of episodes of bacterial BSI [9]. In addition, a worrisome trend of decreasing susceptibility rates to the main antibiotic drugs among GNB was observed. In particular, only 20.1% of GNB were susceptible to ciprofloxacin, 36.9% of Enterobacteriaceae isolates were resistant to third-generation cephalosporins, carbapenem-resistant *K. pneumoniae* amounted to 34.9%, and multidrug-resistant (MDR) *P. aeruginosa* isolates accounted for 69.7% [9].

Herein is reported a prospective multicenter cohort study that was conducted between 2016 and 2018 to update the epidemiology and antimicrobial resistance patterns of GNB causing BSI in HM patients compared to our previous survey [9], and to investigate risk factors for MDR GNB BSI Fig. 1.

2. Methods

A prospective cohort study was conducted in Italy in 15 hematological wards of tertiary care centers or university hospitals taking part in the Hematologic Malignancies Associated Bloodstream Infections Surveillance (HeMABIS) registry–Sorveglianza Epidemiologica InFezioni nelle EMopatie (SEIFEM) group from January 2016 to December 2018. All consecutive episodes of BSI caused by Gram-negative bacteria (GNB-BSI) occurring in hospitalized HM adult patients were included.

Antimicrobial prophylaxis was administered to high-risk patients according to the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) criteria [10]. Of note, many hematology centers belonging to our registry autonomously decided to discontinue the administration of fluoroquinolone prophylaxis due to previous outbreaks of carbapenem-resistant BSI, as reported elsewhere [5].

A rectal swab was performed on all HM patients admitted to participating centers at admission as a screening for colonization by multidrug-resistant (MDR) bacterial isolates; each hospital conducted rectal swab screening according to its own protocols, as described elsewhere [11]. Only isolated MDR GNB strains (see definition below) were included for study purposes. Antimicrobial resistance was defined according to the EUCAST clinical breakpoint guidelines for each year of the study period.

Data collected from the hospital charts and the laboratory database included general patient characteristics, including age, sex and comorbidities (including diabetes mellitus, chronic obstructive pulmonary disease, chronic hepatic failure, and chronic renal failure); length of hospital stay (total and as time at risk, i.e., days from hospital admission and the date of GNB BSI onset); indwelling urethral catheter or central venous catheter, total parenteral nutrition, previous therapy with corticosteroids, antibiotics (i.e., carbapenems, cephalosporins, β-lactams/β-lactamases inhibitors, aminoglycosides) and/or monoclonal antibodies, chemotherapy and/or radiotherapy; neutropenia (as defined below); HM disease and disease stage at time of BSI, including type of hematopoietic stem cell transplantation (HSCT; autologous or allogeneic); previous MDR bacteria culture-positive surveillance rectal swabs; fluoroquinolone prophylaxis; outcome of infection, and, for each bacterial isolate, the antimicrobial susceptibility results.

All the information was entered into case report forms and then recorded in a specific database. The pool of variables considered for multivariable analysis of risk factors for MDR GNB BSI and of those for 30-day mortality was selected based on prior studies and clinical knowledge.

The ethics committee of each participating site approved the use of the HeMABIS-SEIFEM registry, upon approval of the ethics committee of the study coordinating center (Fondazione Policlinico Universitario Agostino Gemelli IRCCS – Università Cattolica del Sacro Cuore). Written informed consent was obtained from each patient.

Patients with BSI caused by multidrug-resistant (MDR) GNB were compared with those with non-MDR GNB to identify risk factors for infection by MDR isolates. Furthermore, survivors and non-survivors were compared to investigate risk factors for 30-day mortality.

2.1. Definitions

The following terms were defined prior to data analysis:

GNB BSI was defined as an infection manifested by the presence of at least 1 blood culture that grew a Gram-negative strain.

GNB isolates were considered MDR according to definitions of Magiorakos et al. (i.e., acquired non-susceptibility to at least one agent in three or more antimicrobial categories) [12].

Neutropenia was defined as an absolute neutrophil count (ANC) $<$500 neutrophils/μL at the onset of BSI; neutropenia was considered severe if ANC was $<$100 neutrophils/μL.

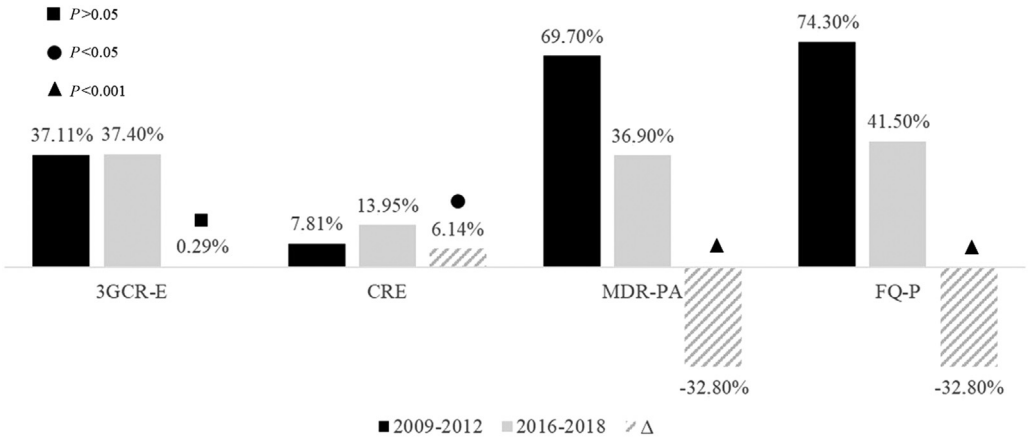

Figure 1. Correlation between percentages of third-generation cephalosporin-resistant Enterobacterales (3GCR-E), carbapenem-resistant Enterobacterales (CRE), MDR *P. aeruginosa* (MDR-PA) isolates and fluoroquinolone prophylaxis (FQ-P) during 2009-2012 [9] and 2016-2018 and their percentage differences (Δ).

Table 1
Antimicrobial susceptibility profiles of all Gram-negative bacteria and the most frequently isolated and multidrug-resistant bacterial isolates.

Microorganisms	Total							MDR isolates
		Ceftazidime	Ciprofloxacin	Meropenem	Amikacin	Gentamicin	Piperacillin/tazobactam	
Total	834	516 (61.9)	335 (40.2)	675 (80.9)	663 (79.5)	606 (72.6)	555 (66.5)	256 (30.7)
Escherichia coli	440	315 (71.6)	147 (33.4)	436 (99.1)	394 (89.6)	355 (80.7)	360 (81.8)	75 (17.1)
Klebsiella pneumoniae	160	57 (35.6)	48 (30.0)	78 (48.7)	100 (62.5)	86 (53.7)	55 (34.4)	101 (63.1)
Pseudomonas aeruginosa	122	81 (66.4)	73 (59.8)	80 (65.6)	89 (72.9)	86 (70.5)	74 (60.6)	45 (36.9)
Enterobacter cloacae	31	23 (74.2)	24 (77.4)	29 (93.5)	31 (100)	28 (90.3)	23 (74.2)	4 (12.9)
Acinetobacter baumannii	14	1 (7.1)	5 (35.7)	5 (35.7)	5 (35.7)	6 (42.8)	3 (21.4)	9 (64.3)
Stenotrophomonas maltophilia[a]	14	NA	NA	NA	NA	NA	NA	14 (100)
Others	53	39 (73.6)	38 (71.7)	47 (88.7)	44 (83.2)	45 (84.9)	40 (75.5)	8 (15.1)
MDR isolates	256	20 (7.8)	7 (2.7)	99 (38.7)	106 (41.4)	72 (28.1)	36 (14.1)	-

Values are n (%).

GNB BSI was considered hospital-acquired if the index culture had been collected >48 h after admission and signs and symptoms of infection were absent at admission [13].

Time at risk was defined as the time (days) elapsed between hospital admission and the date of index blood culture collection.

Patients with polymicrobial GNB BSI were considered as diagnosed with MDR GNB BSI if at least one GNB isolate causing the BSI was MDR.

2.2. Statistical analysis

Continuous variables were compared using the Student's t test for normally distributed variables and the Mann–Whitney U test for non-normally distributed variables. Categorical variables were evaluated using the $\chi 2$ or two-tailed Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the strength of any association that emerged. Values are expressed as means ± standard deviation (SD) (continuous variables), or as percentages of the group from which they were derived (categorical variables). A logistic regression model was used to identify independent risk factors for MDR GNB BSI and 30-day mortality. The multivariable models were chosen according to the Akaike information criterion. Subsequently, the final multivariable model of risk factors for MDR GNB BSI was adjusted taking into account the hospital effect, and that of risk factors for 30-day mortality was adjusted for presence of septic shock at BSI onset. Two-tailed tests were used to determine statistical significance; a *P*-value of <0.05 was considered significant. All the statistical analyses were performed using the Stata program, version 16, for Windows (Stata Corporation, College Station, Texas, USA).

3. Results

A total of 811 episodes of GNB BSI were included in the registry during the study period. Of these, 23 (2.8 %) had a polymicrobial GNB BSI. Therefore, a total of 834 GNB were isolated. The most frequently isolated Gram-negative bacterial species are reported in Table 1. *Escherichia coli* was the most common bacterial species (440/834, 52.7%), followed by *Klebsiella pneumoniae* (160/834, 19.2%), *Pseudomonas aeruginosa* (122/834, 14.6%), *Enterobacter cloacae* (31/834, 3.7%), *Acinetobacter baumannii* (14/834, 1.7%), and *Stenotrophomonas maltophilia* (14/834, 1.7%).

3.1. Antimicrobial resistance profiles of GNB isolates

The antimicrobial susceptibility rates to ceftazidime, ciprofloxacin, meropenem, amikacin, gentamicin and piperacillin/tazobactam of all GNB and of the most frequently isolated bacterial species are reported in Table 1. Overall, 517/834 (61.9%) GNB displayed susceptibility to ceftazidime, 335/834 (40.2%) to ciprofloxacin, 675/834 (80.9%) to meropenem, 663/834 (79.5%) to amikacin, 606/834 (72.6%) to gentamicin and 555/834 (66.5%) to piperacillin/tazobactam. The rate of susceptibility to meropenem among *K. pneumoniae* isolates was 48.7%, whereas a total of 395/631 (62.6%) Enterobacterales strains (including *E. coli, K. pneumoniae* and *E. cloacae*) were susceptible to ceftazidime. The rates of susceptibility to antibiotics of Enterobacterales and *P. aeruginosa* isolates in 2016-2018 are compared to those reported in our previous survey conducted in 2009-2012 in Table 2.

A total of 256 of 834 (30.7%) GNB isolates were classified as MDR. The rate of MDR strains was higher among *A. baumannii* (64.3%; all these strains were extensively drug-resistant [XDR]) and *K. pneumoniae* isolates (63.1%), followed by *P. aeruginosa* (36.9%), *E.*

Table 2
Susceptibility percentages to antibiotics in 2016-2018 compared to 2009-2012 [9] for Enterobacterales and *Pseudomonas aeruginosa* isolates.

Microorganisms	Ceftazidime			Ciprofloxacin			Meropenem			Amikacin			Gentamicin			Piperacillin/tazobactam		
	2016-2018	2009-2012	Δ (%)	2016-2018	2009-2012	Δ (%)	2016-2018	2009-2012	Δ (%)	2016-2018	2009-2012	Δ (%)	2016-2018	2009-2012	Δ (%)	2016-2018	2009-2012	Δ (%)
Escherichia coli	315/440 (71.6%)	131/187 (70%)	+1.6	147/440 (33.4%)	18/187 (9.6%)	+23.8*	436/440 (99.1%)	184/187 (98.4%)	+0.7	394/440 (89.6%)	183/187 (97.9%)	-8.3*	355/440 (80.7%)	155/187 (82.9%)	-2.2	360/440 (81.8%)	156/187 (83.4%)	-1.6
Klebsiella pneumoniae	57/160 (35.6%)	18/43 (41.9%)	-6.3	48/160 (30.0%)	13/43 (30.2%)	-0.2	78/160 (48.7%)	28/43 (65.1%)	-15.4	100/160 (62.5%)	25/43 (58.1%)	+4.4	86/160 (53.7%)	29/43 (67.4%)	-13.7	55/160 (34.4%)	19/43 (44.2%)	-9.8
Enterobacter cloacae	23/31 (74.2%)	12/26 (46.1%)	+28.1°	24/31 (77.4%)	13/26 (50%)	+27.4°	29/31 (93.5%)	24/26 (92.3%)	+1.2	31/31 (100%)	23/26 (88.5%)	+11.5°	28/31 (90.3%)	23/26 (88.5%)	+1.8	23/31 (74.2%)	12/26 (46.1%)	+28.1°
Pseudomonas aeruginosa	81/122 (66.4%)	30/66 (45.4%)	+21.0°	73/122 (59.8%)	13/66 (19.7%)	+40.1*	80/122 (65.6%)	19/66 (28.8)	+36.8*	89/122 (72.9%)	43/66 (65.1%)	+7.8	86/122 (70.5%)	15/66 (22.7%)	+47.8*	74/122 (60.6%)	38/66 (57.6%)	+3

* $P<0.001$; ° $P<0.05$.

coli (17.1%), and *E. cloacae* (12.9%). All 14 *S. maltophilia* isolates were considered MDR; of these, all isolates except one (13/14, 92.8%) were susceptible to trimethoprim/sulfamethoxazole.

In terms of antibiotic resistance, 37.4% (236/631) and 13.9% (88/631) of Enterobacterales (including *E. coli, K. pneumoniae* and *E. cloacae*) were resistant to third-generation cephalosporins and carbapenems, respectively, and 34.4% (42/122) of *P. aeruginosa* and 64.3% (9/14) of *A. baumannii* isolates were resistant to carbapenems.

3.2. Risk factors for MDR GNB BSI

The epidemiologic and clinical characteristics of the cohort patients according to MDR phenotype of GNB isolates causing BSI are reported in Table 3.

In univariate analysis, patients with MDR GNB BSI had a higher mean length of hospital stay and mean time at risk for a BSI episode compared with patients with non-MDR GNB BSI. Patients with MDR GNB BSI were more likely to have an indwelling urinary catheter, to have undergone total parenteral nutrition, to have received previous MDR bacteria culture-positive surveillance rectal swabs, to have received antibiotic prophylaxis with fluoroquinolones, and to have undergone any previous antibiotic therapy, particularly with carbapenems, β-lactams/β-lactamases inhibitors, and/or aminoglycosides. Non-MDR GNB BSI was more frequent in patients who had undergone autologous HSCT and in those with multiple myeloma.

In multivariable analysis, as reported in Table 4, the following variables were found to be independently associated with MDR GNB BSI: MDR bacterial culture-positive surveillance rectal swabs (OR 3.38, $P<0.001$), fluoroquinolone prophylaxis (OR 2.38, $P<0.001$), previous aminoglycoside therapy (OR 2.29, $P=0.02$), previous carbapenem therapy (OR 2.33, $P=0.008$), and increased time at risk (days, OR 1.01, $P=0.02$).

3.3. Outcome variables and mortality

The overall 30-day mortality rate was 16.3% (132/811). Mortality rate was significantly higher in patients who had a BSI caused by an MDR GNB than in those who had a non-MDR GNB BSI (88/256, 34.4% vs. 44/555, 7.9%; $P<0.001$), and in patients who experienced septic shock than in those who did not (87/181, 48.1% vs. 45/630, 7.1%; $P<0.001$). In multivariable analysis, MDR GNB BSI (OR 5.02, 95% CI 3.21-7.84; $P<0.001$) and chronic renal failure (OR 4.18, 95% CI 1.91-9.15; $P<0.001$) were independent risk factors for 30-day mortality, whereas autologous HSCT was independently associated with survival (OR 0.16, 95% CI 0.05-0.44; $P<0.001$).

4. Discussion

In the present, large, multicenter Italian cohort, epidemiological, microbiological, and clinical characteristics of HM patients diagnosed with GNB BSI were recorded. The most common GNB species was *E. coli* (52.7%), followed by *K. pneumoniae* (19.2%) and *P. aeruginosa* (14.6%). Together, these three bacterial species accounted for 86.5% of GBN causing BSI in the cohort (Table 1). This is in line with the results of a previous study conducted by our group between 2009 and 2012, and with the results of recent studies conducted in other countries [14–16].

Interestingly, there were some important differences in the antibiotic susceptibility patterns of GNB isolates in the present study compared with our previous survey [9]. First, there was a significant recovery in the susceptibility of GNB isolates (overall) to ciprofloxacin of 20.1% (40.2% during 2016-2018 vs. 20.1% during 2009-2012). During the 2016-2018 vs. 2009-2012 periods, the susceptibility rates to ciprofloxacin for single bacterial species

Table 3
Univariate analysis of risk factors for BSI caused by MDR GNB.

Variables	Total GNB BSI (n=811)	BSI caused by MDR GNB (n = 256)	BSI caused by non-MDR GNB (n = 555)	OR (95% CI)	*P*-values
Demographic information					
Male	475 (58.5)	153 (59.7)	322 (58.1)	1.07 (0.78-1.47)	0.63
Age (year [mean±SD])	56±13	55±14	57±13	-	0.08
Length of hospital stay (days [mean±SD])	32±21	35±23	31±20	-	0.01
Time at risk (days [mean±SD])	15±13	19±15	14±12	-	<0.001
Characteristics of BSI					
Polymicrobial	23 (2.8)	7 (2.7)	16 (2.8)	0.94 (0.35-2.47)	0.90
Hospital-acquired	691 (85.2)	227 (88.7)	464 (83.6)	1.53 (0.96-2.49)	0.06
Patients' clinical characteristics and comorbidities					
Chronic obstructive pulmonary disease	80 (9.8)	32 (12.5)	48 (8.6)	1.50 (0.90-2.48)	0.08
Diabetes mellitus	106 (13.1)	34 (13.3)	72 (12.9)	1.02 (0.64-1.61)	0.90
Chronic hepatic failure	48 (5.9)	11 (4.3)	37 (6.6)	0.62 (0.28-1.28)	0.18
Chronic renal failure	41 (5.1)	13 (5.1)	28 (5.1)	1.01 (0.47-2.05)	0.98
Indwelling central venous catheter	687 (84.7)	210 (82.1)	477 (85.9)	0.74 (0.49-1.13)	0.15
Indwelling urinary catheter	152 (18.7)	68 (26.5)	84 (15.1)	2.202 (1.38-2.95)	<0.001
Total parenteral nutrition	145 (17.8)	58 (22.6)	87 (15.7)	1.57 (1.06-2.31)	0.01
PMN < 500/mmc	707 (87.2)	228 (89.1)	479 (86.3)	1.29 (0.80-2.13)	0.27
PMN < 100/mmc	653 (80.5)	208 (81.2)	445 (80.2)	1.07 (0.72-1.59)	0.72
Corticosteroid treatment	431 (53.1)	137 (53.5)	294 (52.9)	1.02 (0.75-1.39)	0.88
Chemotherapy	667 (82.2)	201 (78.5)	466 (83.9)	0.69 (0.47-1.03)	0.06
Radiotherapy	42 (5.2)	10 (3.9)	32 (5.8)	0.66 (0.28-1.41)	0.26
Therapy with monoclonal antibodies	166 (20.5)	46 (17.9)	120 (21.6)	0.79 (0.53-1.17)	0.23
Previous MDR bacteria culture-positive surveillance rectal swabs	166 (20.5)	88 (34.4)	78 (14.1)	3.20 (2.21-4.62)	<0.001
Hematological malignancy					
Acute myeloid leukemia/ myelodysplastic syndrome	397 (48.9)	140 (54.7)	257 (46.3)	1.39 (1.02-1.90)	0.02
Chronic myeloid leukemia	10 (1.2)	3 (1.1)	7 (1.2)	0.92 (0.15-4.10)	0.91
Acute lymphatic leukemia	90 (11.1)	25 (9.7)	65 (11.7)	0.81 (0.48-1.35)	0.41
Lymphomas/ chronic lymphoid leukemia	244 (30.1)	74 (28.9)	170 (30.6)	0.92 (0.65-1.28)	0.61
Multiple myeloma	70 (8.6)	14 (5.5)	56 (10.1)	0.51 (0.26-0.96)	0.02
Hematopoietic stem cell transplantation	248 (30.6)	75 (29.3)	173 (31.2)	0.91 (0.65-1.27)	0.59
Autologous	121 (14.9)	26 (10.2)	95 (17.1)	0.54 (0.33-0.88)	0.009
Allogeneic	127 (15.6)	49 (19.1)	78 (14.1)	1.46 (0.96-2.21)	0.05
Fluoroquinolones prophylaxis	337 (41.5)	132 (51.6)	205 (36.9)	1.81 (1.33-2.47)	<0.001
Previous antibiotic therapy with:*					
Carbapenems	71 (8.7)	43 (16.8)	28 (5.1)	3.79 (2.23-6.51)	<0.001
Cephalosporins	39 (4.8)	11 (4.3)	28 (5.1)	0.84 (0.37-1.78)	0.64
β-lactams/ β-lactamases inhibitors	149 (18.4)	65 (25.4)	84 (15.1)	1.90 (1.30-2.78)	<0.001
Aminoglycosides	48 (5.9)	28 (10.9)	20 (3.6)	3.28 (1.74-6.27)	<0.001

Values are n (%) unless otherwise noted.
* During the last 30 days from the date of BSI onset.

Table 4
Multivariable analysis of risk factors for BSI caused by MDR GNB, adjusted for hospital effect.

Variables	OR	(95% CI)	*P*-values
Previous MDR bacteria culture-positive surveillance rectal swabs	3.38	(2.20-5.19)	<0.001
Fluoroquinolone prophylaxis	2.38	(1.53-3.70)	<0.001
Previous aminoglycosides therapy	2.29	(1.12-4.65)	0.02
Previous carbapenems therapy	2.33	(1.24-4.39)	0.008
Time at risk (days)	1.02	(1.01-1.03)	0.02

increased among *P. aeruginosa* (+40.1%), *E. coli* (+23.8%) and *E. cloacae* (+27.4%) isolates, whereas no difference in susceptibility rate was found among *K. pneumoniae* isolates [9]. Second, there was a shift to an increased susceptibility of *P. aeruginosa* isolates to all the antibiotics tested, particularly to ceftazidime (+21%), ciprofloxacin (+40.1%), meropenem (+36.8%), and gentamicin (+47.8%). Third, the rate of meropenem-susceptible *K. pneumoniae* isolates in the present study was lower than that reported in the study conducted in 2009-2012 (-16.4%) [9]. However, in another study by our group in January 2010 to June 2014 focusing on BSI caused by. *K. pneumoniae* in HM patients, the rate of resistance to carbapenems increased from 21.4% in 2010 to 75.9% in 2013, and was 61.1% during the first six months of 2014 [5]. Therefore, resistance to carbapenems among *K. pneumoniae* isolates was lower in the present study compared with these more recent data. Furthermore, the number of patients who had received prophylaxis with fluoroquinolones in the present study was 32.8% lower than in our previous survey [9]. Previous exposure to fluoroquinolones has been widely recognized as one of the most important risk factors for infections due to fluoroquinolone-resistant GNB, third-generation cephalosporin Enterobacterales, carbapenem-resistant *K. pneumoniae*, and MDR *P. aeruginosa* in both general and hematological populations [6,17-26]. Interestingly, Hakki et al. analysed 55 episodes of *P. aeruginosa* BSI in HM patients and demonstrated that the receipt of fluoroquinolone prophylaxis was independently associated with BSI caused by isolates displaying resistance to meropenem, which was due to the upregulation of efflux pumps and transcriptional downregulation of OprD in all these isolates [27]. These findings suggest that fluoroquinolones could promote the development of resistance to other antibiotic classes in *P. aeruginosa* isolates. Therefore, the reduction in use of fluoroquinolone prophylaxis may have played a key role in the increased susceptibility patterns observed in the present study compared with our previous epidemio-

logical survey, particularly with regard to *P. aeruginosa* isolates [9].

The role of fluoroquinolone prophylaxis in high-risk onco-hematological patients is a widely debated and controversial issue. In 2017, the European Conference on Infections in Leukemia (ECIL) conducted a meta-analysis of randomized controlled trials and observational studies published between 2006 and 2014 to evaluate the role of fluoroquinolone prophylaxis in onco-hematological patients. The authors found that fluoroquinolone prophylaxis did not have an effect on mortality, whereas it was associated with a lower rate of BSI and episodes of febrile neutropenia. Although the authors did not find a clear correlation between the use of fluoroquinolone prophylaxis and an increase in antimicrobial resistance, they concluded that the possible benefits of fluoroquinolone prophylaxis cannot be held applicable to regions with a high prevalence of resistant pathogens, such as Italy [28]. Interestingly, Clerici et al. conducted a single-center cohort study on 136 autologous HSCT and 223 allogeneic HSCT patients from January 2018 to December 2020, in which administration of fluoroquinolone prophylaxis was discontinued during the study period (from February 2019). Comparing antimicrobial resistance among GNB in allogeneic HSCT patients, the authors found a significantly higher proportion of GNB isolates that were susceptible to piperacillin/tazobactam (71% vs. 30%), fluoroquinolones (49% vs. 10%) and carbapenems (95% vs. 50%) in patients who had not received fluoroquinolone prophylaxis compared with those who had [29].

In the present study cohort, 31.5% (256/811) of the patients were diagnosed with a BSI caused by at least one MDR GNB. This is in line with the findings of Averbuch et al., who reported a rate of 35.2% of MDR GNB in their cohort of 655 HM patients (recruited in 25 countries in Europe, Australia, and Asia) who had undergone HSCT and experienced GNB BSI [16]. The analysis of risk factors for MDR GNB BSI in the present study cohort showed that fluoroquinolone prophylaxis was independently associated with MDR GNB BSI, increasing the risk of MDR GNB BSI by two times. This finding supports the role of reduction in fluoroquinolone prophylaxis use in improving antibiotic resistance patterns of GNB causing BSI in the present study HM patients. Of note, in the study by Averbuch et al., the rate of MDR GB BSI was significantly higher in HSCT patients treated in centers providing fluoroquinolone prophylaxis compared with those treated in centers that did not provide prophylaxis [16]. Of note, there were no changes in protocols for infection control measures and/or antimicrobial therapy in any participating hospitals during the study period (2016-2018). Furthermore, only one of 15 participating centers had a change in hospital infection control guidelines in the interval between the two study periods (i.e., 2013-2015). This supports the hypothesis that reduction in the use of fluoroquinolone prophylaxis might be considered one of the main reasons for the increase in antibiotic susceptibility found in the GNB isolates in this study.

Besides fluoroquinolone prophylaxis, the most important risk factors for MDR GNB BSI in the present study cohort were previous rectal colonization by MDR bacteria and previous exposure to carbapenems and aminoglycosides. These have been widely demonstrated as risk factors for infections caused by MDR GNB, particularly in HM patients, but also in the general population [11,18,30].

Finally, in the present study cohort, MDR GNB BSI was the most important independent risk factor associated with mortality. This is in line with previous studies evaluating the clinical impact of infections caused by antimicrobial resistant GNB in HM patients [1], and highlights that many efforts are needed to reduce the burden of antibiotic resistance in this clinical setting.

The present study has some limitations. First, the study was conducted in a country with a high prevalence of infections caused by antibiotic-resistant GNB [31]; therefore, the results may not be generalized to countries with a different epidemiology of antimicrobial resistance. In this regard, in a recently reported cross-sectional study conducted in 14 US cancer centers, including 343 onco-hematological febrile neutropenic patients diagnosed with BSI, the susceptibility rates to fluoroquinolones, cefepime, piperacillin-tazobactam, and carbapenems of GNB isolates were 49%, 84%, 88%, and 96%, respectively [32]. In addition, outcome variables (including requirement for mechanical ventilation, vasopressor, or death within 30 days) did not correlate with fluoroquinolone prophylaxis, organism type, initial antibiotics, or adequacy of coverage. The authors concluded that empirical antibiotic treatment for such infections with cefepime or piperacillin-tazobactam (in accordance with US guidelines) remains effective, maintaining high GN pathogen susceptibility [32]. This reinforces the concept of the importance of local epidemiology, and the adoption of different empirical therapy protocols according to different settings. Second, molecular characterization of the mechanisms of resistance, particularly to carbapenems, was not investigated. Third, with regard to previous antibiotic treatments as risk factors for MDR GNB BSI, antibiotic use as a binary variable without accounting for the timing/duration or the appropriateness of the antibiotics was considered and longer-term effects (i.e., use of antibiotics more than one month prior to infection onset) were not taken into account.

In conclusion, the results of the present study confirm a high prevalence of MDR strains among GNB causing BSI in HM patients. However, there were increased rates of susceptibility to fluoroquinolones in almost all bacterial species isolated and to almost all antibiotics tested among *P. aeruginosa* isolates compared with our previous survey conducted between 2009 and 2012. There was also a significant reduction in the use of fluoroquinolone prophylaxis compared with our previous survey, and this was an independent risk factor for MDR GNB BSI in the present study. Further studies are necessary to better understand the role of fluoroquinolone prophylaxis in countries such as Italy with a high prevalence of infections due to fluoroquinolone-resistant and MDR GNB. Furthermore, awareness of changes in antibiotic resistance patterns and in risk factors for MDR GNB BSI may have a favorable impact on the efficacy of empirical treatment protocols.

Declarations

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests: The authors declare that they have no conflicts of interest related to the submitted work. EMT has been a speaker at accredited educational courses funded by unrestricted grants from Pfizer, Angelini and Menarini. LP was a board member of Gilead Science, MSD, Pfizer, Basilea, Janssen, Novartis, Jazz Pharmaceutical, and Cidara, and has been a speaker for Gilead Sciences, MSD, Pfizer Pharmaceuticals, Astellas Pharma, Novartis, and Jazz Pharmaceutical, as well as a consultant for Menarini and Cidara. AB has received honoraria from Gilead Sciences, Merck, Pfizer Pharmaceuticals and Jazz Pharmaceuticals, has been a speaker for Gilead Sciences, Merck, Pfizer Pharmaceuticals, DiaSorin, GSK, Basilea and Novartis, and has been part of an Advisory Board for Takeda, Gilead and Pfizer. MT has been part of an Advisory Board for Menarini, MSD, and Shionogi, and has received honoraria from Pfizer.

Ethical Approval: The ethics committee of each participating site approved the use of the HeMABIS-SEIFEM registry, upon approval of the ethics committee of the study coordinating center (Fondazione Policlinico Universitario Agostino Gemelli IRCCS – Univer-

tà Cattolica del Sacro Cuore). Written informed consent was obtained from each patient.

Sequence Information: Not applicable

Availability of data and material

Data may be made available upon reasonable request.

Author contributions

EMT wrote the original draft; all the authors reviewed and edited the manuscript; the conceptualization was conducted by EMT, LP and MT; all the authors contributed to the investigations; the methodology was devised by EMT and so was the formal analysis. All the authors read and approved the final version.

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