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Liver Transplantation

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Multidrug-resistant bacterial infections after liver transplantation: Prevalence, impact, and risk factors

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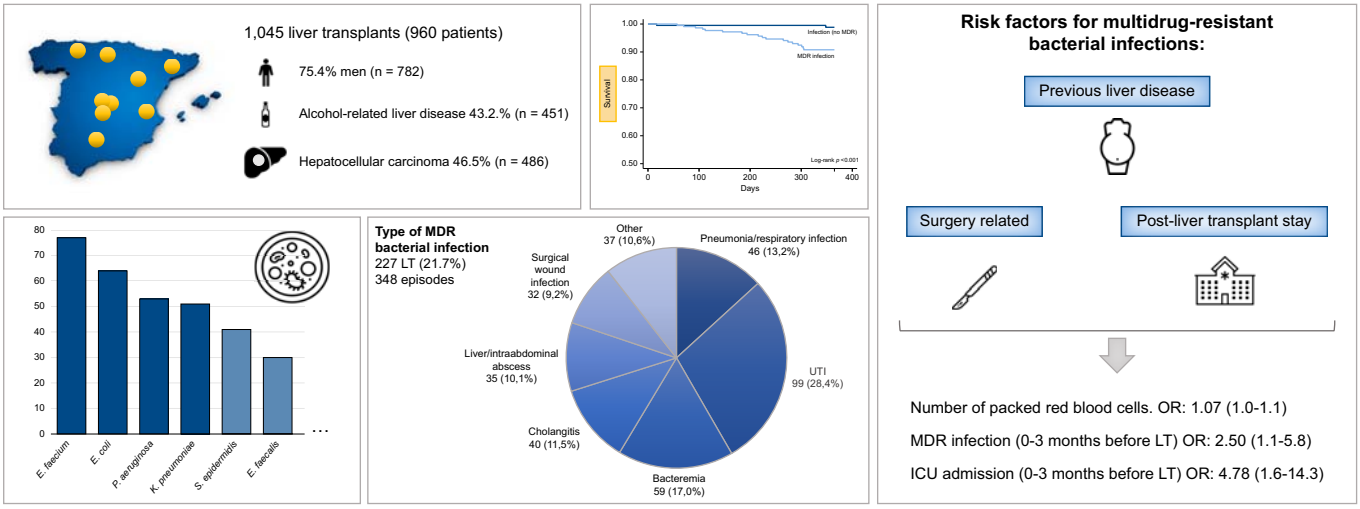
Graphical abstract



Highlights

- MDRB infections are frequent in patients with cirrhosis after LT and are associated with increased mortality.
- Urinary tract infections are the most common MDRB infections, followed by bacteremia and respiratory infections.
- Gram-negative bacteria are the most frequently isolated, but infections caused by gram-positive enterococci are increasing.
- Recent ICU admission or MDRB infection (0-3 months before LT) and RBC transfusions are risk factors for post-LT MDRB infection.

Impact and implications

Multidrug-resistant bacterial infections have a deep impact on morbidity and mortality after liver transplantation. Strategies aimed at improving prophylaxis, early identification, and empirical treatment are paramount. Our study unveiled the prevalence and main risk factors associated with these infections, and demonstrated that gram-positive bacteria, particularly *Enterococcus faecium*, are frequent in this clinical scenario. These findings provide valuable insights for the development of prophylactic and empirical antibiotic treatment protocols after liver transplantation.

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Multidrug-resistant bacterial infections after liver transplantation: Prevalence, impact, and risk factors

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Background & Aims: Infections by multidrug-resistant bacteria (MDRB) are an increasing healthcare problem worldwide. This study analyzes the incidence, burden, and risk factors associated with MDRB infections after liver transplant(ation) (LT).
Methods: This retrospective, multicenter cohort study included adult patients who underwent LT between January 2017 and January 2020. Risk factors related to pre-LT disease, surgical procedure, and postoperative stay were analyzed. Multivariate logistic regression analysis was performed to identify independent predictors of MDRB infections within the first 90 days after LT.
Results: We included 1,045 LT procedures (960 patients) performed at nine centers across Spain. The mean age of our cohort was 56.8 ± 9.3 years; 75.4% (n = 782) were male. Alcohol-related liver disease was the most prevalent underlying etiology (43.2%, n = 451). Bacterial infections occurred in 432 patients (41.3%) who presented with a total of 679 episodes of infection (respiratory infections, 19.3%; urinary tract infections, 18.5%; bacteremia, 13.2% and cholangitis 11%, among others). MDRB were isolated in 227 LT cases (21.7%) (348 episodes). *Enterococcus faecium* (22.1%), *Escherichia coli* (18.4%), and *Pseudomonas aeruginosa* (15.2%) were the most frequently isolated microorganisms. In multivariate analysis, previous intensive care unit admission (0-3 months before LT), previous MDRB infections (0-3 months before LT), and an increasing number of packed red blood cell units transfused during surgery were identified as independent predictors of MDRB infections. Mortality at 30, 90, 180, and 365 days was significantly higher in patients with MDRB isolates.
Conclusion: MDRB infections are highly prevalent after LT and have a significant impact on prognosis. *Enterococcus faecium* is the most frequently isolated multi-resistant microorganism. New pharmacological and surveillance strategies aimed at preventing MDRB infections after LT should be considered for patients with risk factors.

Introduction

Bacteria resistant to at least one agent from three or more antimicrobial families are classified as multidrug-resistant bacteria (MDRB). Infections caused by these bacteria are a leading cause of mortality worldwide, accounting for 4.95 (3.62-6.57) million deaths in 2011.[1] The most common MDRB are extended-spectrum β-lactamase-producing Enterobacteriaceae (*i.e.*, *Escherichia coli* [*E. coli*] and *Klebsiella pneumoniae* [*K. pneumoniae*]), AmpC β-lactamase–producing Enterobacteriaceae (*i.e.*, *Enterobacter* and *Citrobacter* spp.), carbapenemase-producing Enterobacteriaceae (*i.e.*, *Klebsiella* spp. and *E. coli*), methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant enterococci (*i.e.*, *Enterococcus faecium [E. faecium]*).[2]

Bacterial infections are more frequent and severe in patients with advanced liver disease.[3] The risk of infection is increased by diverse factors, such as frequent hospitalizations, multiple invasive procedures, nutritional deficits and sarcopenia, proton pump inhibitor overuse, and the many prophylactic and therapeutic antibiotic regimens that these patients often receive.[2] The susceptibility to infections parallels the cirrhosis stage, which increases with the severity of cirrhosis-associated immune dysfunction and comprises different stages of systemic inflammation and immune exhaustion.[4] In addition, several studies have analyzed the prevalence of MDRB infections in patients with decompensated cirrhosis and acute-on-chronic liver failure,[3,5] and have shown a significant negative impact on prognosis and survival.[5]

Infections are a leading cause of morbidity and mortality during the short-term post-liver transplant(ation) (LT) period.[6] LT recipients are at particularly high risk of colonization and infection by MDRB due to previous immune dysfunction, the

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surgical procedure, and the subsequent hospital stay. In addition, they receive immunosuppressive drugs that significantly dampen the host immune response and contribute to increased risk of infections.[7] Therefore, prophylaxis, early detection, and empirical treatment of MDRB infections are particularly relevant in this clinical setting.[8,9]

To date, the impact and risk factors associated with MDRB infections after LT have not been fully explored. However, a better understanding and characterization of this problem may aid in guiding the prophylactic and therapeutic antibiotic strategies required in this challenging scenario, ultimately contributing to improved outcomes and survival.[10] This study aims to assess the incidence of MDRB infections during the first 90 days after LT, the epidemiological and microbiological features, the associated risk factors, and the impact on patient and graft survival.

Patients and methods

Patients and study design

This was a retrospective multicenter cohort study that included adult patients who underwent LT from deceased donors between January 2017 and January 2020. Risk factors related to the pre-LT disease, the surgical procedure, and the postoperative stay were analyzed (Fig. 1). Comparisons were made between patients presenting with multidrug-resistant (MDR) infections and those with non-MDR infections during the first 90 days after transplantation. The development of fungal and viral infections is associated with other particular risk factors; therefore, they were considered out of the scope of the study and were not analyzed. Data were collected and managed using REDCap (Research Electronic Data Capture) tools hosted at the Instituto Ramón y Cajal de Investigación Sanitaria in Madrid.[11,12] The results are reported following the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) recommendations.[13]

Definitions

MDR was defined as resistance to at least one agent in three or more antimicrobial categories. Extensively drug-resistant bacteria were defined as non-susceptible to at least one agent in all but two or fewer antimicrobial categories, and pandrug-resistant bacteria were defined as non-susceptible to all currently available agents.[14] These criteria are based on the consensus definitions jointly proposed by the European Center for Disease Prevention and Control, and the Centers for Disease Control and Prevention in 2012.[14] This epidemiological classification establishes different antimicrobial categories depending on whether they are prescribed against *Enterobacteriaceae, Pseudomonas aeruginosa* (*P. aeruginosa*) or *Acinetobacter spp*., but do not consider the intrinsic resistance patterns of the different microorganisms.

The following types of infections were analyzed using diagnostic criteria previously published elsewhere.[5,15] Of note, only infections clinically suspected and treated accordingly were included in our study:

Urinary tract infection (UTI): abnormal urinary sediment (>10 leukocytes/field) and positive urinary culture, or uncountable leukocytes per field if negative cultures.

Respiratory infection (pneumonia or upper tract): clinical signs of infection and new infiltrates on chest x-ray. Clinical signs of infection and positive sputum or bronchoalveolar lavage cultures.

Hepatic or intra-abdominal abscess: clinical signs of infection, and radiological evidence of intra-abdominal or liver non-solid infected collections.

Surgical wound/skin and soft tissue infection: clinical signs of infection associated with swelling, erythema, heat, and tenderness in the skin.

Bacteremia (spontaneous or catheter-related): positive blood cultures.

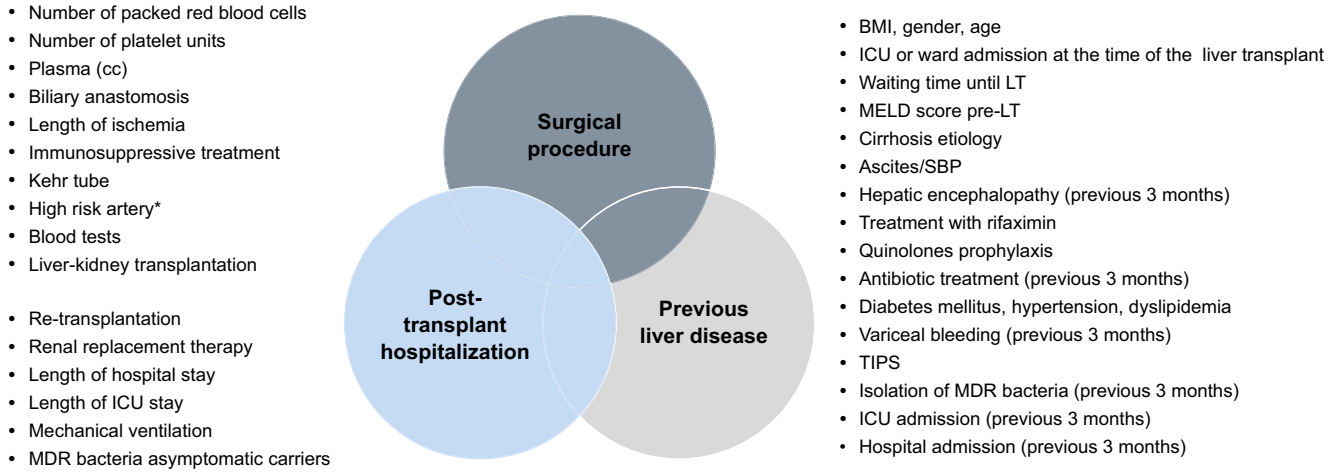

Fig. 1. Risk factors related to the pre-LT disease, the surgical procedure, and the postoperative stay. *Factors associated with a higher risk of hepatic artery thrombosis: Arterial anatomical anomalies, complex arterial reconstructions, poor arterial wall quality or a caliber less than 3 mm, ischemia time exceeding 12 h, increased anticardiolipin antibodies, use of fresh frozen plasma in high-risk patients (ABO incompatible), and acquired protein S deficiency. ICU, intensive care unit; LT, liver transplant; MDR, multidrug-resistant; MELD, model for end-stage liver disease; SBP, spontaneous bacterial peritonitis; TIPS, transjugular intrahepatic portosystemic shunt.

Cholangitis: cholestasis, right upper quadrant pain and/or jaundice and radiological signs of biliary obstruction.

Clostridioides difficile infection: positive stool toxin in a patient with diarrhea.

Unproven bacterial infection: fever ≥38 °C and white blood cell count ≥12,000/mm^3 requiring antibiotic therapy without any identifiable source.

MDR colonization status was defined by positive rectal and/or respiratory swabs when obtained per protocol in asymptomatic patients during the postoperative period. National programs for the surveillance of ICU-acquired infections are activated upon admission across centers. This includes obtaining microbiological samples (rectal and respiratory swabs) on day 0 and weekly thereafter. Once the patient is transferred to a regular ward, most centers continue to perform weekly screening for carriage of MDRB, but this is not a mandatory standard and depends on local policies. Only a minority of the centers obtain microbiological samples to detect asymptomatic carriage of MDRB in individuals on the waiting list.

Infections diagnosed 48 h after admission were classified as nosocomial, and those present at admission or that developed within the first 48 h after hospitalization were classified as community acquired. The term healthcare-associated infections was used for patients hospitalized for at least 2 days in the previous 90 days, living in a long-term care facility or receiving chronic hemodialysis, who presented an infection at admission or during the first 48 h after hospitalization.[16]

Statistical analysis

Statistical analyses were performed with Stata version 17.0 (StataCorp LP, College Station, TX). Graphs were generated with Stata and GraphPad Prism software version 8.2.1 (GraphPad Software Inc., San Diego, CA).

Continuous variables are described as mean ± SD and were compared using Student's *t* test or one-way analysis of variance if normally distributed. Non-normally distributed continuous variables are reported as the median and IQR and were compared with the Mann-Whitney *U* test or Kruskal-Wallis test. Categorical variables are described as absolute numbers and percentages and compared with the chi-square test. We also performed a multivariate logistic regression analysis to identify independent predictors of MDRB infections within the first 90 days after LT. The model was constructed with variables significantly associated with the primary outcome (MDRB infection) on the univariate analysis, and other parameters associated with an increased risk of infections in prior publications if deemed appropriate. Survival analysis was performed using Kaplan-Meier curves and compared with the log-rank test. We did not use any imputation method for missing data due to the low rate of missing values.

The study adhered to the principles of the Declaration of Helsinki and European Union regulation 2016/679. The research protocol (MDR2019. Protocol Version 1.2 July 2021) was approved by the Ethics Committees for Clinical Research of the Hospital Universitario Ramón y Cajal and all the participating centers.

Results

Whole cohort

Our study included 1,045 LT procedures (960 patients) performed at nine tertiary hospitals across Spain (Fig. S1). Mean age was 56.8 ± 9.3 years, 75.4% (n = 782) were male, and the most frequent causes of cirrhosis were alcohol-related liver disease (43.2% [n = 451]) and chronic hepatitis C (25.4% [n = 265]). Regardless of the etiology, 486 patients (46.5%) had a diagnosis of hepatocellular carcinoma at the time of the LT, and 14 (1.34%) were HIV positive. The median time on the waiting list until LT was 65.5 days (IQR 18-165). Other clinical baseline relevant data for the whole cohort are shown in Table 1.

Table 1. Whole cohort baseline characteristics.

	No infection (n = 613)	Infection (MDR and no MDR) (n = 432)	Total (N = 1,045 LT)
Age (years), mean ±SD	57.2 ± 9.0	56.2 ± 9.7	56.8 ± 9.3
Sex (male), n (%)	482 (79.4)	300 (69.7)	782 (75.4)
Etiology, n (%)			
Alcohol	270 (44.1)	181 (41.9)	451 (43.2)
HCV	163 (26.6)	102 (23.6)	265 (25.4)
HBV	42 (6.9)	29 (6.7)	71 (6.8)
MAFLD	46 (7.5)	38 (8.8)	84 (8.0)
PBC	21 (3.4)	17 (3.9)	38 (3.6)
PSC	7 (1.1)	11 (2.6)	18 (1.7)
Hemochromatosis	1 (0.2)	1 (0.2)	2 (0.2)
Autoimmune hepatitis	22 (3.6)	13 (3.0)	35 (3.35)
Wilson	4 (0.7)	1 (0.2)	5 (0.5)
Cryptogenic	21 (3.4)	10 (2.3)	31 (3.0)
Other	56 (9.1)	36 (8.3)	92 (8.8)
Hepatocellular carcinoma, n (%)	306 (49.9)	180 (41.7)	486 (46.5)
HIV, n (%)	7 (1.1)	7 (1.6)	14 (1.3)
Re-transplantation, n (%)	41 (7.2)	44 (10.8)	85 (8.7)
Weight (kg), mean ±SD	77.8 ± 14.9	76.1 ± 16.2	77.1 ± 15.5
Height (cm), mean ±SD	169.5 ± 8.1	167.6 ± 9.6	168.7 ± 8.8
Body mass index (kg/m^2), mean ±SD	27.0 ± 4.4	27.0± 5.0	27.0 ± 4.7
MELD score (biochemical), mean ±SD	14.7 ± 7.0	16.4± 7.8	15.4± 7.4
Days on the waiting list, median (IQR)	75 (19-185)	60 (17-141)	66 (18-165)

MAFLD, metabolic dysfunction-associated fatty liver disease; MDR, multidrug-resistant; MELD, model for end-stage liver disease; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis. Continuous variables are reported as mean ± SD or median and IQR and categorical variables as absolute numbers and relative frequencies.

Bacterial infections

Bacterial infections were diagnosed in 432 LT cases (41.3%) during the first 90 days after the surgical procedure. In total, 679 infections were analyzed since 160 patients (15.3%) developed ≥2 episodes throughout the study period. The most common type were respiratory infections (n = 130; 19.3%), followed by UTIs (n = 125; 18.5%) (Fig. 3). The rate of culture-positive infections was 75% (n = 509). Most of the culture-negative episodes were respiratory infections (n = 69), unproved bacterial infections (n = 41), cholangitis (n = 18) and surgical wound/skin and soft tissue infections (n = 14). Overall median time to infection was 10 days (IQR: 4-21).

Among those who developed post-LT bacterial infections, 47 (14.1%) were hospitalized at a regular ward at the time of the LT, whereas 32 (7.9%) were admitted to an intensive care unit (ICU). In addition, 203 patients had been recently hospitalized (3 months prior to the LT), and 45 had been admitted to the ICU. Regarding the site of acquisition, nosocomial infections were more frequent than community-acquired infections (n = 504; 78.6% *vs.* n = 119; 18.6%). Although all patients underwent a systematic infection screening before LT, most of the community-acquired infections were diagnosed once the patient was discharged from the initial LT stay (median time to diagnosis of community-acquired infection: 24 days [IQR 2-54]). Overall, the group who developed infections had a significantly higher median biochemical model for end-stage liver disease (MELD) score pre-LT than the group without infections (13 [9-19] *vs.* 15 [10-21] $p \leq 0.001$).

MDRB infections

MDR pathogens were isolated in 227 patients after LT (prevalence 21.6% in the whole cohort, 52.5% in the subgroup of infections). Overall, 348 episodes of infections caused by resistant microorganisms were identified, which represents 68.4% of all culture-positive infections. The most frequent were UTIs (n = 99, 28.4%), followed by bacteremia (n = 59, 17.0%) and respiratory infections (n = 46; 13.2%) (Fig. 2). There were no significant differences regarding age, sex, time on the waiting list, or cardiovascular risk factors (diabetes, hypertension, dyslipidemia, or obesity) between patients with MDRB infections and those with susceptible bacteria infections or no microbiological isolation (Table 2). The prevalence of MDRB infections according to each participating center is detailed in Fig. S2.

The main MDRB found in our cohort are shown in Fig. 3. Interestingly, a Gram-positive coccus, *E. faecium*, was the most frequently isolated (77 episodes, 22.1%). In particular, multi-resistant *E. faecium* was found in microbiological cultures from 19 (24.7%) patients with liver/intra-abdominal abscess, 12 (15.6%) with bacteremia, 17 (22.1%) with cholangitis, 13 (16.9%) with UTI, 10 (13.0%) with surgical wound infections, 1 (1.3%) with respiratory infections and 5 (6.5%) with other types of infection. In 28 cases (36.4%) this microorganism was isolated in polymicrobial cultures. AmpC β-lactamase production and quinolone resistance mechanisms were predominantly involved, whereas vancomycin resistance was only present in 12 cases of *E. faecium* antibiotic resistance.

Resistant *E. coli* was the second most isolated pathogen (64, 18.4%), followed by *P. aeruginosa* (53, 15.2%) and resistant *K. pneumoniae* (51, 14.7%). Production of extended spectrum β-lactamases was detected in 32 and 30 cases of *E. coli* and *K. pneumonia* infection, respectively. On the other hand, production of carbapenemases predominated as the mechanism of *P. aeruginosa* resistance (31/53) and, less frequently, in MDR *K. pneuominae* infections (17/51). A detailed table specifying the different isolated MDRB and the main mechanisms of resistance is presented in Table S1. No cases of pandrug-resistant bacteria were reported.

Median time to first non-MDRB infection was 9 days (IQR 4-20), and median time to first MDRB infection was 11 days (IQR 4-22). Differences in this regard were not statistically significant ($p = 0.305$).

Risk factors related to the previous liver disease

Impact of antibiotic exposure before LT

Antibiotic prophylaxis up to 3 months prior to the LT was examined in detail. Among patients who developed infections, 50 (11.6%) had received prophylaxis for spontaneous bacterial peritonitis (SBP), with norfloxacin being the most frequently prescribed (n = 43, 86%). In addition, 109 (27.3%) patients had received rifaximin to prevent encephalopathy. Interestingly, these prophylactic regimens were associated with a non-

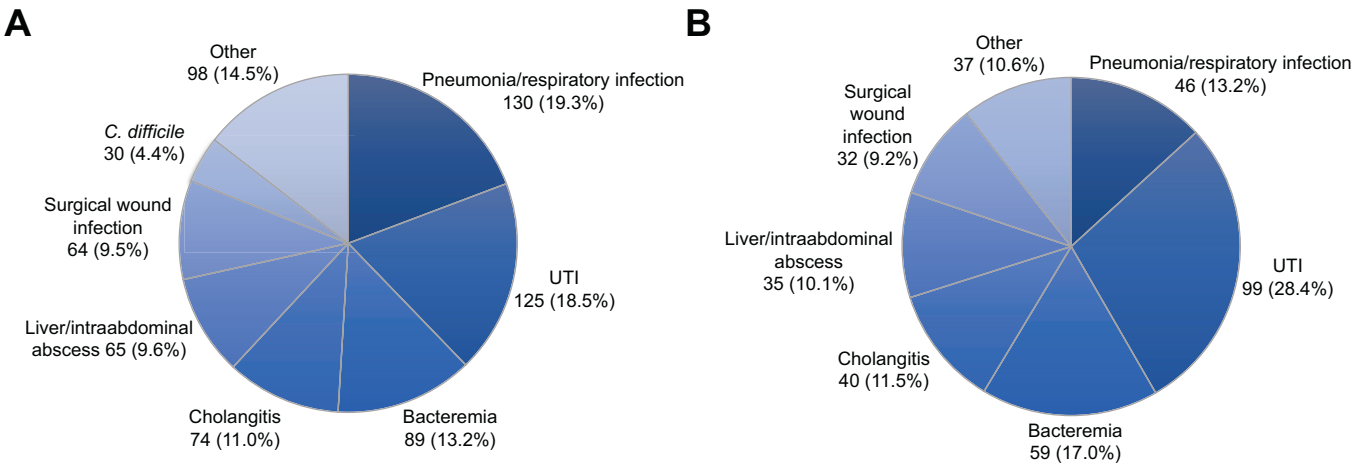

Fig. 2. Types of infections. (A) Overall bacterial infections, (B) MDR infections. MDR, multidrug-resistant; UTI, urinary tract infection.

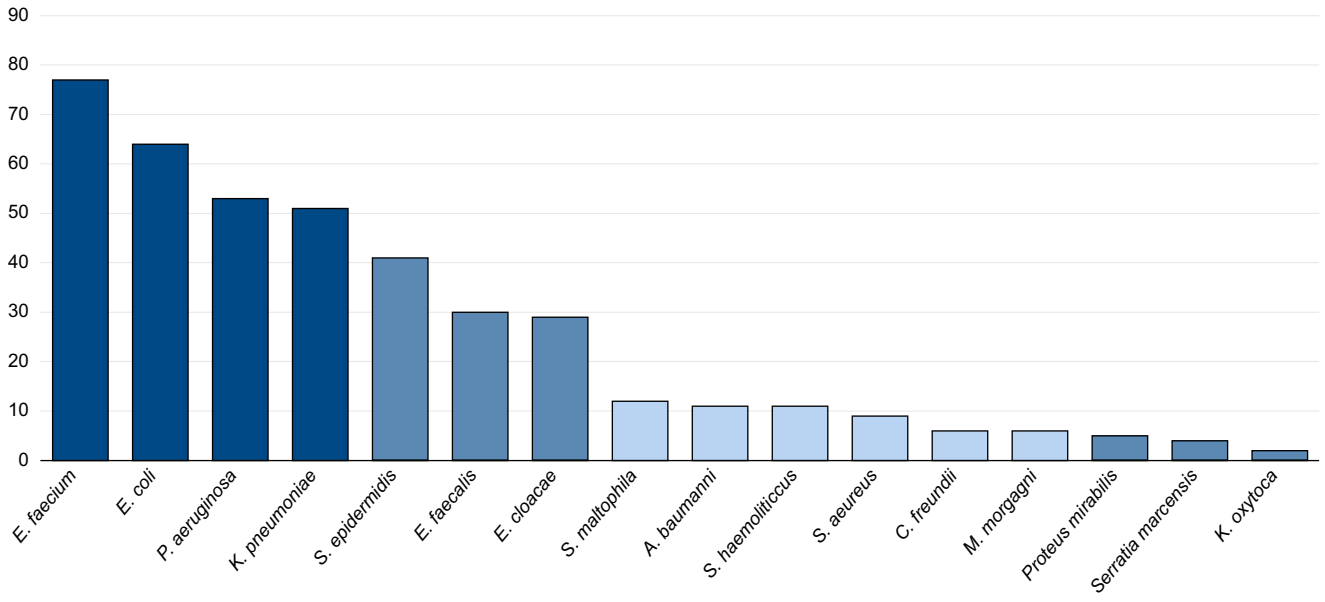

Fig. 3. The most frequent MDR bacteria isolated in the whole cohort. MDR, multidrug-resistant.

significant increase in the prevalence of MDRB in the univariate analysis (SBP prophylaxis: 51.6% *vs.* 60%, p = 0.262, and rifaximin: 50.7% *vs.* 56.9%, p = 0.270) (Table 2). Table S2 shows the different periprocedural antibiotic prophylaxis protocols for LT candidates followed at the nine participant centers. Although strategies containing amoxicillin-clavulanic acid are the most common, other schemes are used according to local epidemiological patterns and individual risk factors.

In addition to antibiotic prophylaxis, we also analyzed the effect of antibiotic treatments for any type of infection 3 months

Table 2. Risk factors for bacterial infections 90 days after liver transplantation.

	No MDRB (n = 205 LT)	MDRB (n = 227 LT)	Total (n = 432 LT)	*p* value
Diabetes mellitus, n (%)	58 (34.5)	62 (37.5)	120 (35.8)	0.619
Dyslipidemia, n (%)	29 (17.3)	22 (13.3)	51 (15.2)	0.308
High blood pressure, n (%)	54 (32.1)	52 (31.1)	106 (31.6)	0.843
Weight (kg), mean ±SD	76.5 ± 15.7	75.8 ± 16.6	76.1 ± 16.2	0.650
Height (cm), mean ±SD	168.0 ± 10.1	167.2 ± 9.3	167.6 ± 9.7	0.421
Body mass index, mean ±SD	27.0 ± 4.9	27.0 ± 5.1	27.0 ± 5.0	0.982
MELD score (biochemical), mean ±SD	15.4 ± 7.6	17.3 ± 7.8	16.4 ± 7.8	0.014
Days on the waiting list, median (IQR)	66 (18-147)	54.5 (16-138)	59.5 (17-141)	0.555
Age (years), mean ±SD	56.2 ± 9.7	56.1 ± 9.7	56.2 ± 9.7	0.929
Sex (male), n (%)	143 (69.7)	156 (68.7)	300 (69.4)	0.773
Hepatocellular carcinoma, n (%)	95 (46.3)	85 (37.4)	180 (41.7)	0.061
HIV, n (%)	2 (1.0)	5 (2.2)	7 (1.6)	0.313
Re-transplantation, n (%)	20 (9.7)	24 (10.6)	44 (10.2)	0.273
Previous variceal bleeding, n (%)	34 (17.8)	51 (24.8)	85 (21.1)	0.118
Previous encephalopathy, n (%)	64 (33.3)	96 (45.5)	160 (39.7)	0.013
Previous ascites, n (%)	96 (50.8)	127 (59.6)	223 (55.8)	0.075
Previous SBP, n (%)	27 (14.3)	40 (19.0)	67 (16.8)	0.212
TIPS, n (%)	20 (10.4)	23 (10.8)	43 (10.6)	0.914
Entero-biliary anastomosis, n (%)	10 (4.9)	21 (9.25)	31 (7.2)	0.090
Kehr tube, n (%)	63 (32.3)	89 (40.5)	152 (36.6)	0.086
Packed red blood cells, median (IQR)	2 (0-4)	3 (1-6)	2 (0-5)	0.000
Platelets (units), median (IQR)	1 (0-1)	1 (0-2)	1 (0-2)	0.417
Plasma (cc), median (IQR)	1,000 (0-3,000)	1,000 (0-2,800)	1,000 (0-3,000)	0.642
Cold ischemia (mins), median (IQR)	285 (210-360)	244 (170-330)	268 (181-350)	0.003
Warm ischemia (mins), median (IQR)	39 (29-47)	36.5 (25-45)	38 (29-45)	0.422
Renal replacement therapy, n (%)	13 (7.03)	37 (18.1)	50 (12.85)	0.001
Endotracheal tube (days), median (IQR)	1 (1-1)	1 (1-1)	1 (1-1)	0.580
Rifaximin, n (%)	47 (24.7)	62 (29.7)	109 (27.3)	0.270
Prophylaxis with norfloxacin, n (%)	17 (8.3)	26 (11.5)	43 (9.95)	0.262
Carriers of MDRB, n (%)	18 (9.6)	50 (24.3)	68 (17.3)	0.000

(*continued on next page*)

Table 2. (*continued*)

	No MDRB (n = 205 LT)	MDRB (n = 227 LT)	Total (n = 432 LT)	*p* value
Induction with basiliximab, n (%)	98 (42.6)	132 (57.4)	230 (53.2)	0.031
Antibiotics (0-3 months before LT), n (%)	135 (65.9)	159 (70.0)	294 (68.1)	0.351
MDRBIs (0-3 months before LT), n (%)	17 (9.19)	55 (26.1)	72 (18.18)	0.000
Hospitalization (0-3 months before LT), n (%)	84 (43.8)	119 (55.6)	203 (50.0)	0.017
ICU admission (0-3 months before LT), n (%)	6 (3.1)	39 (18.2)	45 (11.1)	0.000
Hospital stay (days), median (IQR), n (%)	20 (12-30)	23 (14-37.5)	21 (13-34)	0.035
ICU stay (days), median (IQR), n (%)	4 (3-8)	4 (3-9)	4 (3-8)	0.530
Hospitalized at the time of the LT, n (%)	16 (9.58)	31 (18.6)	47 (14.1)	0.018
At the ICU at the time of the LT, n (%)	10 (5.24)	22 (10.3)	32 (7.9)	0.058

ICU, intensive care unit; LT, liver transplant; MDRB, multidrug-resistant bacteria; MDRBI, multidrug-resistant bacterial infections; MELD, model for end-stage liver disease; SBP, spontaneous bacterial peritonitis; TIPS, transjugular intrahepatic portosystemic shunt. Continuous variables are reported as mean ± SD or median and IQR. Categorical variables are reported as absolute numbers and relative frequencies. Chi-square test was used for comparisons applying Fisher exact test when required in categorical variables. Student's *t* test was applied for continuous normal variables. Non-normally distributed continuous variables are compared with Mann-Whitney *U* test. *p* values <0.05 are considered significant.

prior to the LT. We found that 294 patients had received antibiotics, which resulted in a non-significant increase in the number of MDR events (49.3% *vs.* 54.1%, $p = 0.351$). Interestingly, prior MDR infections and being an asymptomatic carrier of MDRB, had a deep impact on the prevalence of MDR events after LT (48.1% *vs.* 76.4%, $p = 0.000$, and 47.9% *vs.* 73.5%, $p \leq 0.001$, respectively).

Other factors associated with MDRB infections

Hospitalization during the 90 days prior to the LT led to a significant increase in the prevalence of MDRB infections (46.8% *vs.* 58.62%, $p = 0.017$). Moreover, this rate was significantly higher in the group of patients admitted to the ICU (48.5% *vs.* 86.7%, $p \leq 0.001$). Overall, the median MELD score was significantly higher in patients with MDRB in comparison to those with susceptible/culture-negative infections ($14^{9–19}$ *vs.* $16,^{11–22}$ $p = 0.007$). When analyzing the impact of the different types of decompensations, we found that a previous episode of SBP, ascites or variceal bleeding, did not imply a higher risk of MDRB infections. However, a previous history of encephalopathy significantly increased this rate (47.3% *vs.* 60%, $p = 0.013$). Lastly, we examined the impact of various etiologies of liver disease, and specifically alcohol-related liver disease, on the MDR infection rate. We found no significant differences in patients with alcohol- *vs*. non-alcohol-related cirrhosis in terms of overall or MDRB infections post-LT (41.9% *vs.* 58.1%, and 42.7% *vs.* 57.3%, respectively).

Risk factors related to the surgical procedure

Operative LT reports were analyzed to identify technical aspects that could have an impact on the risk of infection. The biliary reconstruction was duct-to-duct anastomosis in 92.5% patients, and Roux-en-Y choledochojejunostomy in the remaining cases. The prevalence of MDR infections was higher in the group with a bilioenteric anastomosis, but the differences were not statically significant (67.7% *vs.* 51.8%, $p = 0.088$). A T-tube was inserted in 36.6% of patients with no impact on the prevalence of MDRB episodes (no-Kehr 49.8% *vs.* Kehr 58.6%, $p = 0.086$). The median warm ischemia and cold ischemia times were 38 (29-45) minutes and 268 (181-350) minutes, respectively. Intraoperative blood transfusion requirements were similar in both groups, except for the median number of packed red blood cells, which was significantly higher in the group developing MDRB infections (median 2 *vs.* 3, $p \leq 0.001$).

Risk factors related to the post-LT hospital stay

In univariate analysis, another factor significantly associated with MDRB was the use of renal replacement therapy post-LT (7.03% *vs.* 18.14%, $p = 0.001$). Conversely, respiratory support, evaluated as the length of endotracheal intubation, was not associated with a higher risk (median 1 (IQR 1-1) for both groups, $p = 0.580$). The hospital stay was longer in the group of patients with MDRB infections (median 20 [12-30] days *vs.* 23 [14-37.5] days, $p = 0.035$), whereas the ICU stay was not significantly different (median $4^{3–8}$ days *vs.* $4^{3–9}$ days; $p = 0.530$).

Finally, we explored the potential consequences of differences in immunosuppression protocols across participating hospitals on the risk of MDRB infections. All regimens were based on calcineurin inhibitors (specifically tacrolimus) in combination with mycophenolate mofetil and corticosteroids. Additionally, some centers prescribed basiliximab, allowing for a delayed introduction of tacrolimus. A detailed description of the different protocols can be found in Table S3. Our analysis showed that the duration of corticosteroid treatment (less than 1 month *vs.* up to 3 months) did not have a significant impact on the risk of overall or MDRB infections (41.8% *vs.* 40.9%, $p = 0.78$ and 54.3% *vs.* 48.7%, $p = 0.27$, respectively). Interestingly, we observed an increase in MDRB infections among patients who received basiliximab (47.0% *vs.* 57.3%, $p = 0.031$), albeit only at the univariate level. All analyzed risk factors are specified in Table 2.

In multivariate analysis, independent predictors of MDRB infections were previous ICU admission (0-3 months before LT) (odds ratio [OR] 4.78, 95% CI 1.60-14.29, $p = 0.005$), previous MDRB infections (0-3 months before LT) (OR 2.50, 95% CI 1.08-5.83, $p = 0.033$), and an increasing number of packed red blood cell units transfused during surgery (OR 1.07, 95% CI 1.01-1.14, $p = 0.022$) (Table 3). Of note, we additionally investigated the role of the variable "Institution" as a potential confounding factor influencing the outcomes. Interestingly, this variable did not change the multivariable analysis results and was not an independent predictor of MDRB infections (OR 0.91, 95% CI 0.809–1.043, $p = 0.190$).

Clinical impact

During the first 90 days after LT, 40 patients who presented ≥1 episode of infection died. The mortality rate was significantly higher in those with MDRB *vs.* susceptible bacteria infections (7.6% *vs.* 1.62%, $p \leq 0.001$). In addition, mortality at 30, 180 and

Table 3. Multivariate analysis of risk factors for the development of MDR bacterial infections.

	Multivariate OR (95% CI)	*p* value
MELD score (biochemical) pre-LT	0.94 (0.94-1.02)	0.271
Number of packed red blood cells	1.07 (1.01-1.14)	0.022
Carriers of MDRB	1.26 (0.58-2.77)	0.55
Renal replacement therapy after LT	1.58 (0.62-4.08)	0.339
MDRBIs (0-3 months before LT)	2.50 (1.08-5.83)	0.033
ICU admission (0-3 months before LT)	4.78 (1.60-14.29)	0.005
Hospitalization (0-3 months before LT)	0.86 (0.48-1.53)	0.606
Induction with basiliximab	1.26 (0.75- 2.09)	0.384
Cold ischemia	1.00 (0.996-1.00)	0.106
Previous encephalopathy	1.43 (0.84-2.43)	0.192
Hospital stay	1.00 (0.99-1.01)	0.897

ICU, intensive care unit; LT, liver transplant; MDRB, multidrug-resistant bacteria; MDRBIs, multidrug-resistant bacterial infections; MELD, model for end-stage liver disease; OR, odds ratio. Logistic regression analysis.

365 days was also significantly higher when a resistant infection occurred (0.46% *vs.* 2.08%, $p = 0.049$; 2.08% *vs.* 9.03%, $p \leq 0.001$; and 3.24% *vs.* 10.42%, $p \leq 0.001$, respectively). These findings were further supported by Kaplan-Meier curves that showed a significant decrease in survival when comparing MDR infections *vs*. infections by susceptible bacteria or without microbiological isolation (Fig. 4A), and when comparing MDR infections *vs.* the rest of the cohort (susceptible bacteria or no microbiological isolation *vs.* no infections) (Fig. 4B). As expected, there were no significant differences in the mortality risk across the institutions (log-rank test at 90, 180 and 365 days: $p = 0.707$, $p = 0.753$ and $p = 0.339$, respectively).

Finally, we performed a 90-day survival analysis, which revealed that MDRB infections after LT were an independent predictor of mortality, increasing the risk of death by up to 8.11 times (Table 4).

Table 4. Independent predictors of 90-day mortality.

	Multivariable OR (95% CI)	*p* value
Age	1.05 (1.00-1.11)	0.047
MELD score (biochemical) pre-LT	1.01 (0.95-1.07)	0.840
BMI	0.99 (0.91-1.07)	0.715
Antibiotics (0-3 months before LT)	3.00 (1.02-8.9)	0.047
Asymptomatic carriers of MDRB	1.25 (0.45-3.48)	0.673
ICU stay (days)	1.05 (1.02-1.07)	0.001
Re-transplantation	3.78 (1.09-13.00)	0.036
MDRB infection	8.11 (2.56-25.70)	0.000
ICU admission (0-3 months before LT)	0.37 (0.09-1.55)	0.175
Hospitalization (0-3 months before LT)	0.68 (0.24-1.92)	0.464
Multiple infections after LT (≥2)	0.96 (0.41-2.25)	0.922

ICU, intensive care unit; LT, liver transplant; MELD, model for end-stage liver disease; MDRB, multidrug-resistant bacteria; OR, odds ratio. Logistic regression analysis.

Discussion

MDRB infections are an increasing concern worldwide as they are associated with high mortality rates, prolonged hospital stay, and increased healthcare costs.[14] This is especially relevant in immunosuppressed patients, such as LT recipients, who are at an increased risk.[17] Our study, conducted in a large cohort of patients, revealed that MDRB infections are frequent (21.6% in the whole cohort, 52.5% in the subgroup with infections) and severe, with a significant impact on survival after LT. One of our primary aims was to identify risk factors associated with MDRB infections during the first 90 days after LT. We performed a comprehensive analysis of variables that could potentially promote these infections, including factors related to the surgical procedure, immediate hospital stay, and pre-LT

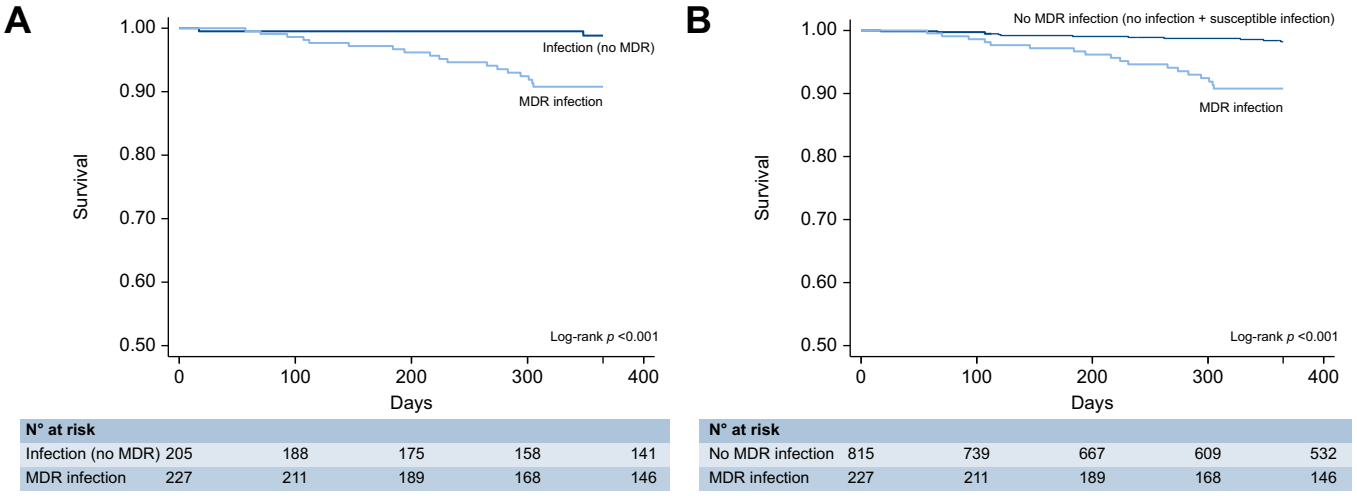

Fig. 4. One-year overall survival analysis. (A) Stratified analysis according to the development of MDR bacterial infections *vs.* infections by susceptible bacteria or no microbiological isolation. (B) Stratified analysis comparing the group with MDR bacterial infections *vs.* the rest of the cohort (susceptible bacteria or no microbiological isolation *vs.* no infections). Survival analysis. Log-rank test. MDR, multidrug-resistant.

disease. Key risk factors included previous ICU admission or MDRB infections and high transfusion requirements during the surgical procedure. In addition, we identified a gram-positive bacterium, *E. faecium*, as the most frequently isolated MDR microorganism. Therefore, our study provides valuable insights that could lead to improved prophylactic and empirical treatment protocols for LT recipients at high risk of developing MDRB infections.

The impact of MDRB infections in patients with decompensated cirrhosis and acute-on-chronic liver failure as been previously described by J. Fernández *et al.*[6] The authors found an increasing prevalence across Europe over the last decade, and confirmed its negative impact on prognosis and survival. They found that nosocomial infections, ICU admission, and recent hospitalization were independent risk factors for MDRB infections. Our data are in line with these findings and highlight the relevance of previous ICU hospitalizations and MDRB infections as determining factors. Interestingly, patients with previous MDRB infections but not asymptomatic carriers of MDRB were at increased risk in our study. This may be related to the fact that patients with pre-LT MDRB infections have received broad-spectrum antibiotics, which contribute to drug resistance selection.

The surgical procedure and the subsequent immunosuppressive regimens pose additional challenges for LT recipients. Increasing intraoperative red blood cell transfusion requirements were previously recognized as a risk factor for bacterial infections in LT recipients.[18] We found that this is also true for multi-resistant infections, although the median number of transfused RBCs was significantly lower in our study than in other publications.[7,19] We did not find significant differences in the prevalence of MDRB infections when comparing the immunosuppression protocols of the participating centers. The addition of basiliximab did not increase the risk of MDRB infections in the multivariate analysis. This finding may be attributed to the fact that basiliximab is typically prescribed only in cases of renal dysfunction in most centers, which may introduce potential confounding factors that are overcome by the logistic regression. Moreover, different prophylactic antibiotic protocols were applied; however, the different prophylactic regimens did not lead to different infection outcomes, probably because the protocols are adapted to local policies and resistances, and they may be modified, if required, on a case-by-case basis.

Gram-negative bacteria were the predominant MDROs in our cohort, however, the most frequent single isolation was *E. faecium,* an emerging gram-positive microorganism in healthcare settings.[20] Considering that most of the antibiotic protocols followed by the centers included are especially active against gram-negative bacteria, this finding should be considered for future empirical treatments and prophylactic strategies. We found that 75% of the infections were confirmed microbiologically (culture-positive infections). This rate is higher than the ≈50% reported in other studies analyzing bacterial infections in cirrhosis.[3,5,21–23] It can be argued that patients immunosuppressed and hospitalized after LT are closely monitored for infection, and, in addition, microbiological samples are readily available in this context, which increases the probabilities of microbial detection. Finally, previous publications have suggested that long-term prophylaxis with norfloxacin is associated with the development of MDRB infections.[21] In our study, we could not confirm this association, which is in consonance with recent studies on patients with cirrhosis,[22,24] and supports the current indication for norfloxacin in selected patients.

Our study has limitations that need to be acknowledged. First, the retrospective design may impact the reporting of a certain number of infection episodes, although this would be, if any, a random error affecting both susceptible and resistant infections. Another concern is related to the analysis of mortality risk factors. We found that MDRB infections are associated with increased short- and long-term mortality rates. However, the study was not specifically designed to analyze this outcome, and other variables with a significant impact on long-term survival are likely missing. Additionally, MDRB infections during the first 90 days after LT have little direct impact on mortality beyond this period. However, they act as surrogate markers of patient complexity, performance status, and resource consumption, which have a more lasting influence on long-term events. Therefore, results regarding the role of MDRB infections in mortality after LT should be interpreted with caution, as further specific studies are needed to confirm their effect.

In conclusion, our study provides evidence of the high impact of MDRB infections after LT, and confirms the increasing role of gram-positive bacteria in this clinical scenario. These results may aid in the design of future prophylactic and empirical treatment protocols for patients at high risk of MDRB infections during the immediate post-LT stay.

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Abbreviations

E. coli, Escherichia coli; *E. faecium, Enterococcus faecium*; ICU, intensive care unit; *K. pneumoniae, Klebsiella pneumoniae;* LT, liver transplant(ation); MAFLD, metabolic dysfunction-associated fatty liver disease; MDRB, multidrug-resistant bacteria/organisms; MELD, model for end-stage liver disease; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; SBP, spontaneous bacterial peritonitis.

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Conflicts of interest

The authors have no conflict of interest with regards to this study.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study concept and design: RM, AA. Acquisition of data: all authors. Analysis and interpretation of data: RM, AA. Writing of the manuscript: RM, AA. Critical revision of the manuscript for important intellectual content: RM, LMA, OL, ICV, MB, AA. All authors approved the final manuscript prior to submission.

Data availability statement

Data will be made available to other researchers upon request to the corresponding and/or to the first author (rosam.martinma@salud.madrid.org) of the manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2024.02.023.

References

[1] Murray CJ, Ikuta KS, Sharara F, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet 2022;399:629–655. https://doi.org/10.1016/S0140-6736(21)02724-0.

[2] Jalan R, Fernandez J, Wiest R, et al. Bacterial infections in cirrhosis: a position statement based on the EASL Special Conference 2013. J Hepatol 2014;60:1310–1324. https://doi.org/10.1016/j.jhep.2014.01.024.

[3] Piano S, Singh V, Caraceni P, et al. Epidemiology and effects of bacterial infections in patients with cirrhosis worldwide. Gastroenterology 2019;156:1368–1380.e10. https://doi.org/10.1053/j.gastro.2018.12.005.

[4] Albillos A, Martin-Mateos R, Van der Merwe S, et al. Cirrhosis-associated immune dysfunction. Nat Rev Gastroenterol Hepatol 2022;19:112–134. https://doi.org/10.1038/s41575-021-00520-7.

[5] Fernández J, Prado V, Trebicka J, et al. Multidrug-resistant bacterial infections in patients with decompensated cirrhosis and with acute-on-chronic liver failure in Europe. J Hepatol 2019;70:398–411. https://doi.org/10.1016/j.jhep.2018.10.027.

[6] Fishman JA. Infection in solid-organ transplant recipients. N Engl J Med 2007;357:2601–2614. https://doi.org/10.1056/NEJMra064928.

[7] Sun HY, Cacciarelli TV, Singh N. Identifying a targeted population at high risk for infections after liver transplantation in the MELD era. Clin Transpl 2011;25:420–425. https://doi.org/10.1111/j.1399-0012.2010.01262.x.

[8] Aguado JM, Silva JT, Fernández-Ruiz M, et al. Management of multidrug resistant Gram-negative bacilli infections in solid organ transplant recipients: SET/GESITRA-SEIMC/REIPI recommendations. Transpl Rev 2018;32:36–57. https://doi.org/10.1016/j.trre.2017.07.001.

[9] Campos-Varela I, Blumberg EA, Giorgio P, et al. What is the optimal antimicrobial prophylaxis to prevent postoperative infectious complications after liver transplantation? A systematic review of the literature and expert panel recommendations. Clin Transpl 2022;36:e14631. https://doi.org/10.1111/ctr.14631.

[10] Fernández A, Díez-Picazo C, Iglesias Sobrino C, et al. Implementation and impact of an antibiotic control program and multidrug-resistant bacterial colonization in a liver transplant unit. Revista Espanola de Enfermedades Digestivas 2022. https://doi.org/10.17235/reed.2022.8810/2022.

[11] Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: building an international community of software platform partners. J Biomed Inform 2019;95:103208. https://doi.org/10.1016/j.jbi.2019.103208.

[12] Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42:377–381. https://doi.org/10.1016/j.jbi.2008.08.010.

[13] von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol 2008;61:344–349. https://doi.org/10.1016/j.jclinepi.2007.11.008.

[14] Magiorakos AP, Srinivasan A, Carey RBB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.

[15] Bajaj JS, Kamath PS, Reddy KR. The evolving challenge of infections in cirrhosis. New Engl J Med 2021;384:2317–2330. https://doi.org/10.1056/nejmra2021808.

[16] Friedman ND, Kaye KS, Stout JE, et al. Health care–associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med 2002;137:791–797. https://doi.org/10.7326/0003-4819-137-10-200211190-00007.

[17] Zhang W, Wang W, Kang M, et al. Bacterial and fungal infections after liver transplantation: microbial Epidemiology, risk factors for infection and death with infection. Ann Transpl 2020;25:e921591. https://doi.org/10.12659/AOT.921591.

[18] Chen C, Chen B, Yang J, et al. Development and validation of a practical machine learning model to predict sepsis after liver transplantation. Ann Med 2023;55:624–633. https://doi.org/10.1080/07853890.2023.2179104.

[19] Benson AB, Burton JR, Austin GL, et al. Differential effects of plasma and red blood cell transfusions on acute lung injury and infection risk following liver transplantation. Liver Transpl 2011;17:149–158. https://doi.org/10.1002/lt.22212.

[20] O'Toole RF, Leong KWC, Cumming V, et al. Vancomycin-resistant Enterococcus faecium and the emergence of new sequence types associated with hospital infection. Res Microbiol 2023:104046. https://doi.org/10.1016/j.resmic.2023.104046.

[21] Fernández J, Acevedo J, Castro M, et al. Prevalence and risk factors of infections by multiresistant bacteria in cirrhosis: a prospective study. Hepatology 2012;55:1551–1561. https://doi.org/10.1002/hep.25532.

[22] Marciano S, Gutierrez-Acevedo MN, Barbero S, et al. Norfloxacin prophylaxis effect on multidrug resistance in patients with cirrhosis and bacterial infections. Eur J Clin Microbiol Infect Dis 2023. https://doi.org/10.1007/s10096-023-04572-2.

[23] Martínez J, Hernández-Gea V, Rodríguez-de-Santiago E, et al. Bacterial infections in patients with acute variceal bleeding in the era of antibiotic prophylaxis. J Hepatol 2021;75:342–350. https://doi.org/10.1016/j.jhep.2021.03.026.

[24] Moreau R, Elkrief L, Bureau C, et al. Effects of long-term norfloxacin therapy in patients with advanced cirrhosis. Gastroenterology 2018;155:1816–1827.e9. https://doi.org/10.1053/j.gastro.2018.08.026.