Multidrug-resistant bacterial infections in patients with decompensated cirrhosis and with ACLF across Western Europe

Javier Fernández [1,2], Verónica Prado[1], Alex Amoros[2], Thierry Gustot[3], Reiner Wiest[4], Carme Deulofeu[2], Juan Acevedo[5], Valentin Furhman[6], Jonel Trebicka[2,7], ……., Marco Pavesi[2], Rajiv Jalan[9], Pere Ginès[1,2], Paolo Angeli[10], Vicente Arroyo[2] and the European Foundation for the Study of Chronic Liver Failure (EF Clif)

[1]Liver ICU, Liver Unit, Hospital Clinic, University of Barcelona, Barcelona, Spain,

[2]European Foundation of Chronic Liver Failure (EF-Clif), Barcelona, Spain

[3]Liver Transplant Unit, Erasme Hospital, Brussels, Belgium,

[4]Department of Medicine and Surgery, Inselspital, University of Bern, Bern, Switzerland

[5]South West Liver Unit, Derriford Hospital, UK

[6]University Medical Center Hamburg-Eppendorf, Germany

[7]University of Bonn, Germany

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[9]ILDH, Division of Medicine, University College London Medical School, London, United Kingdom

[10]University of Padova, Padova, Italy

Address for correspondence: J. Fernández, MD, PhD. Liver Unit, Hospital Clínic, Villarroel 170, 08036, Barcelona. Spain. Phone: 34-93-2275400 3329; Fax: 34-93-4515522; E-mail: Jfdez@clinic.ub.es.

Word count: 4293 without references

Number of figures and tables: 2 figures; 6 tables, 4 Supplementary tables, 1 Supplementary figure

Keywords: epidemiology; prevalence; prognosis; antibiotic resistance; antibiotic strategies

List of Abbreviations: ACLF: acute-on-chronic liver failure; AD: acute decompensation; CANONIC: chronic liver failure (CLIF) Acute-on-Chronic Failure in Cirrhosis; SBP: spontaneous bacterial peritonitis; UTI: urinary tract infection; SSTI: skin and soft tissue infections; MDROs: multidrug-resistant organisms; PMN: polymorphonuclear; SBE: spontaneous bacterial empyema; XDR: extensively-drug resistance; PDR: pandrug resistance; ESBL: extended-spectrum beta-lactamase; MRSA: methicillin-resistant *Staphylococcus aureus*; VSE: vancomycin-susceptible *Enterococcus*; VRE: vancomycin-resistant *Enterococcus;* CDI: *Clostridium difficile* infection; SIRS: systemic inflammatory response syndrome; ICU: intensive care unit; HCA: healthcare-associated; CA: community-acquired.

Financial support: The study was supported by the European Foundation for the Study of Chronic Liver Failure (EF-Clif). EF-Clif received unrestricted donations from Grifols and Cellex Foundations and is partner or contributor in several projects of the EU Horizon 2020 research program

Disclosures:

The EASL-CLIF Consortium is a network of 101 European University hospitals supported by the EF-Clif. EF-Clif is a private non-profit organization aimed at improving clinical and translational research in cirrhosis. The scientific agenda of the EASL-CLIF Consortium and the specific research protocols are made exclusively by the Steering Committee members without any participation of pharmaceutical companies.

Conflicts of interest

Pere Ginès has received speaker honorarium and research funding from Grifols, served on the scientific advisory board for Ferring and Sequena and received research funding from Sequena. Vicente Arroyo and Javier Fernandez have received grant and research support from Grifols. All other authors declare that they have no conflict of interest.

Author contributions: JF, VP, JA, AA, CD, MP and VA participated in data analysis and interpretation. JF, TG, RW, PG and VA participated in the writing group. VA was responsible for obtaining funding and overall project collaboration.

Abstract

Antibiotic resistance has been increasingly reported in decompensated cirrhosis in different unicentric studies. Prospective investigations reporting broad epidemiological data are scarce.

Patients: 1288 patients from the Canonic study prospectively evaluated in 29 centers/12 countries from Western Europe, 417 with acute-on-chronic liver failure (ACLF) and 871 with acute decompensation (AD). Data on epidemiology, clinical characteristics of bacterial infections, microbiology and empirical antibiotic schedules were assessed.

Results: 508 patients developed 578 bacterial infections (38%). SBP (n=140), UTI (n=124), and pneumonia (n=90) were the most frequent proved infections. Nosocomial infections predominated in the series, being more frequent in North than in South Europe (59% vs. 46% in the South; p=0.002). Severity of the patient at diagnosis of infection was also significantly higher in North Europe. Forty-eight percent of the infections were culture-positive (n=280) and 77 of them were caused by multidrug-resistant organisms (MDROs: 27.5%). Prevalence of MDR bacterial infections was similar between North and South Europe (30% vs. 25% in culture-positive infections), but differed markedly among countries and centers. Extended-spectrum beta-lactamase-producing *Escherichia coli* was the most frequent multiresistant strain reported (n=19), followed by vancomycin-susceptible *Enterococcus faecium* (n=15) and methicillin-resistant *Staphylococcus aureus* (n=12). However, the pattern of antibiotic resistance significantly differed among countries and centers. Antibiotic resistance was associated to poor prognosis and to failure of first line antibiotic strategies based on third-generation cephalosporins or quinolones. Nosocomial infection (OR: 2.96; 95% CI: 1.58-5.53; p<0.001**)** and recent hospitalization (OR: 1.92; 95% CI: 1.04-3.55; p=0.036**)** were identified as independent predictors of MDR infection.

Conclusion: MDR bacterial infections constitute a prevalent and complex healthcare problem in decompensated cirrhosis and ACLF across Western Europe and negatively impact prognosis. Strategies aimed at preventing the spread of antibiotic resistance in cirrhosis should be urgently evaluated.

INTRODUCTION

Bacterial infections constitute a frequent complication of patients with decompensated cirrhosis and the most frequent trigger of ACLF in Western countries.[1-5] Patients with cirrhosis and acute decompensation (AD) are prone to develop spontaneous and secondary bacterial infections, risk that magnifies at short-term in patients with acute-on-chronic fiver failure (ACLF).[1,5,6] It is well known that bacterial infection has a critical relevance in the clinical course of decompensated cirrhosis, increasing 2 to 4 fold short-term mortality.[7,8] Recent data derived from the Canonic series also show that bacterial infections are severe and associated with intense systemic inflammation, poor clinical course and high mortality in patients with ACLF.[6]

Early diagnosis and adequate empirical antibiotic treatment of bacterial infections is therefore key in the management of patients with cirrhosis.[1,9] However, epidemiology of bacterial infections in cirrhosis is nowadays much more complex than in the past.[9] The efficacy of classical empirical antibiotic strategies based on the administration of third-generation cephalosporins or amoxicillin clavulanic-acid has markedly decreased in the last decade due to the emergence of multidrug-resistant (MDR) bacteria.[9-13] Resistance to antibiotics in pathogenic bacteria is nowadays a major global public health problem,[14] and is particularly serious in patients with decompensated cirrhosis. These patients frequently accumulate several risk factors for MDR organisms (MDROs) including recurrent hospitalizations, invasive procedures and repeated exposition to prophylactic or therapeutic antibiotics.[9] Antibiotic overuse and failure of control measures to prevent the spread of resistant bacteria in the healthcare setting seem to have magnified antimicrobial resistance in cirrhosis. Therefore, the characterization of these epidemiological changes and the

identification of the MDROs that are nowadays infecting our cirrhotic patients are of clinical relevance. The great majority of the epidemiological data on antibiotic resistance in cirrhosis derive from unicentric studies,[2,4,10-13,15-20] multicentric studies performed in specific countries,[21] or multicentric studies assessing a specific type of infection.[22] However, at present no study has been reported in patients with cirrhosis and all type of infections, exploring the epidemiology of MDROs in large geographical, multinational regions. This type of studies are the most relevant to understand the global impact of antibiotic resistance and could help to identify effective prophylactic and therapeutic strategies.

Therefore, the current study was designed to assess the prevalence of MDR bacterial infections in cirrhosis across Western Europe, potential epidemiological differences among countries and centers, the characteristics of these infections, their impact on prognosis, risk factors for MDR and type and efficacy of empirical antibiotic treatment using information from the Canonic Study database. The study consisted of a large prospective observational study in 1288 patients hospitalized for the treatment of an episode of AD in which data on bacterial infection were carefully collected.[5]

PATIENTS AND METHODS

Study population and aims of the study

In the current investigation, all patients included in the Canonic series (February to September 2011) were considered. Only 61 subjects with incomplete data at diagnosis of infection were excluded. Therefore, 1288 patients were analyzed, 417 with ACLF (302 diagnosed at enrolment and 115 during hospitalization) and 871 with AD (no ACLF). Data on epidemiology, clinical characteristics of infections, microbiology and empirical and final antibiotic schedules were prospectively recorded.

The aim of the study was to assess the epidemiology of bacterial infections across Western Europe and potential differences in the prevalence and type of MDROs among geographical areas, countries and centers. Three different strategies for the analysis of the data were used. First, infections developing in the whole region and in North Europe (Austria, Belgium, Czech Republic, Denmark, Germany, Ireland, Switzerland, UK and The Netherlands) and South Europe (France, Italy and Spain) were compared. Second, comparisons were performed among countries (n= 12) and centers (n=29). Finally, the third objective was to perform a comprehensive assessment of the impact and risk factors of MDR bacterial infections and to evaluate the type and efficacy of empirical antibiotic strategies used in the whole region.

Definitions

Diagnostic criteria of bacterial infections were the following: spontaneous bacterial peritonitis (SBP): polymorphonuclear (PMN) cell count in ascitic fluid ≥250/mm^3; urinary tract infection (UTI): abnormal urinary sediment (>10 leukocytes/field) and

positive urinary culture or uncountable leukocytes per field if negative cultures; spontaneous bacteremia: positive blood cultures and no cause of bacteremia; secondary bacteremia: a) catheter-related infection (positive blood and catheter cultures), b) bacteremia occurring within 24h after an invasive procedure; pneumonia: clinical signs of infection and new infiltrates on chest x-ray; bronchitis: clinical features of infection, no radiographic infiltrates and positive sputum culture; skin and soft tissue infections (SSTI): clinical signs of infection associated with swelling, erythema, heat and tenderness in the skin; cholangitis: cholestasis, right upper quadrant pain and/or jaundice and radiological data of biliary obstruction; spontaneous bacterial empyema (SBE): PMN count in pleural fluid ≥250/mm^3; secondary peritonitis: PMN count in ascitic fluid ≥250/mm^3 and evidence (abdominal CT/ surgery) of an intraabdominal source of infection; *Clostridium difficile* infection (CDI): positive stool toxin in a patient with diarrhea; unproved bacterial infection: presence of fever and leukocytosis requiring antibiotic therapy without any identifiable source. The criteria used to define the site of acquisition of infection have been previously described.[6,10]

MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial category. Extensively-drug resistance (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and pandrug-resistant (PDR) as non-susceptibility to all currently available agents.[23] The following bacteria were considered MDR in the current study: ESBL (mainly *Escherichia coli* and *Klebsiella pneumoniae)* or desrepressed chromosomic AmpC β-lactamase-producing *Enterobacteriaceae* (*Enterobacter or Citrobacter spp),* carbapenem-resistant *Klebsiella pneumoniae,* carbapenem-resistant *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia,* carbapenem-resistant *Acinetobacter*

baumanii, *Burkholderia cepacia*, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-susceptible and vancomycin-resistant *Enterococcus faecium* (VSE, VRE).

ACLF at infection diagnosis was defined according to the CLIF consortium criteria.[5] Patients were considered to have SIRS (sepsis) if they fulfilled at least two of the following criteria: (a) core temperature > 38ºC or < 36ºC; (b) heart rate > 90 beats/minute; (c) respiratory rate > 20 breaths/minute in the absence of hepatic encephalopathy; and (d) white blood cell count > 12.000 or < 4000 /mm^3, or differential count showing ≥ 10% immature PMN neutrophils. Severe sepsis was defined by the presence of SIRS and at least one acute organ failure. Septic shock was diagnosed by the presence of data compatible with SIRS and need of vasopressor drugs in the setting of hypotension (mean arterial pressure below 60 mmHg).[24] Recently defined sepsis criteria (sepsis-3 and q-SOFA) were not applied in the current study as they were proposed after the end of the Canonic Study.[25]

Infections were considered cured when all clinical signs of infection disappeared and on the presence of: a) urinary infections: normal urine sediment and negative urine culture; b) spontaneous or secondary bacteremia: negative control cultures after antibiotic treatment; c) pneumonia: normal chest X-ray and negative control cultures if positive at diagnosis; d) bronchitis: negative bronchial aspirate/sputum culture; e) cellulitis: normal physical exam of the skin and negative control cultures if positive at diagnosis; f) cholangitis: improvement of cholestasis, resolution of clinical symptoms and negative control cultures if positive at diagnosis; g) SBP and SBE: PMN cell count in ascitic/pleural fluid < 250/mm³ and negative control cultures if positive at diagnosis. Resolution of the rest of infections was based on conventional clinical criteria.

Definitions on antibiotic therapy

Two types of empirical antibiotic strategies were considered: 1) “Classical” strategies: those including one to third-generation cephalosporins, amoxicillin clavulanic-acid/cloxacillin or quinolones and 2) MDR strategies: regimens using piperacillin-tazobactam, carbapenems or ceftazidime/cefepime ± glycopeptides (or linezolid/daptomycin).

The criteria used to consider an initial antibiotic therapy appropriate were the following: 1) For culture positive infections if an antibiotic with an in vitro activity appropriate for the isolated pathogen or pathogens was administered at diagnosis of infection; 2) For culture-negative infections, when the antibiotic strategies administered at the time of infection diagnosis solved the infection without need for further escalation. Otherwise, the initial therapy was considered inappropriate.[6] We decided not to use the fulfillment of international guidelines criteria because there were no broadly accepted norms for empiric management of bacterial infections in cirrhosis at the time of performing the Canonic study. Time to antibiotic therapy administration after diagnosis of infection was not recorded in the study.

Statistical analysis

Results are presented as frequencies and percentages for categorical variables, means and SDs for normally distributed continuous variables and median and interquartile range for not normally distributed continuous variables. In univariate analyses, Chi-square test was used for categorical variables, Student’s t-test or ANOVA for normal continuous variables and Mann-Whitney or Kruskal Wallis test for not normally distributed continuous variables. To identify predictors of infection

caused by MDROs, logistic regression models were carried out. Factors showing a clinically and statistically significant association to the outcome in univariate analyses were selected for the initial model. The final models were fitted by using a step-wise forward method based on Likelihood Ratios with the same significance level ($p<0.05$) for entering and dropping variables. Binary logistic regression models were used to identify independent predictors of MDROs. In all statistical analyses, significance was set at $p<0.05$. Analyses were done with SPSS (version 23.0; SPSS, Inc. Chicago, IL) and SAS (version 9.4; SAS Institute Inc.; Cary, NC) statistical packages.

RESULTS

Overall bacterial infections

Table 1 shows the prevalence, type, clinical and epidemiological characteristics of bacterial infections diagnosed in the whole series and in patients from North and South Europe. A total of 508 patients (38%) developed 578 bacterial infections during the study period with no differences in the prevalence of infection between North and South Europe. Sixty-three patients developed 2 or more infections. The majority of infections were diagnosed outside the ICU (80%). Regular ward (n=122; 47%) was the most frequent site of hospitalization at infection diagnosis in North Europe and emergency department (n=140; 47%) in South Europe (p<0.001). SBP (n=140), UTI (n=124), and pneumonia (n=90) were the most frequent proved infections in the whole series and in patients from both, North and South Europe. SSTI was significantly more frequent in North Europe (11% vs. 6%, p=0.03) while unproved infections were more prevalent in the South (18% vs. 12%, p=0.03). No other differences in the type of infections were observed between groups. Nosocomial infections predominated in the whole series (n=302; 52%), being more frequent in North Europe (59% vs. 46%; p=0.002). Severity of the patient at diagnosis of infection was also significantly higher in North Europe with a higher prevalence of severe sepsis/shock (18% vs. 11%, p=0.03) and of ACLF (53% vs. 47.5%, p=0.001).

Bacteria isolated in the whole series, in North and South Europe and per country

A total of 303 bacteria were isolated in 280 culture-positive infections (48.4%). Isolation rate was ~~not~~ significantly higher in North Europe (53% vs. 44%;

p=0.035). Bacterial isolation was ~~also~~ similar in nosocomial, healthcare-associated (HCA) and community-acquired (CA) infections (51% vs. 43% vs. 47%; p=0.331). The rate of positive cultures was 73% in UTI, 46% in SSTI, 50% in SBP and 43% in pneumonia.

Supplementary Table 1 shows all bacteria isolated in the whole series, in North and South Europe and per country. *Escherichia coli* was the most frequently isolated organism (36%), followed by *Staphylococcus aureus* (11%), *Enterococcus faecalis* (10%), *Klebsiella pneumoniae* (7%) and *Streptococcus viridans* and *Enterococcus faecium* (5% each).

Eighty out of the 303 organisms isolated in the study (26.4%) were MDROs. They were isolated in 77 infections (13.3% of all infections, 27.5% of culture-positive infections) from 72 patients (14%). As a whole, ESBL-producing *Escherichia coli* was the most frequent multiresistant strain reported (n=19), followed by vancomycin-susceptible *Enterococcus faecium* (n=15), MRSA (n=12) and ESBL-producing *Klebsiella pneumoniae* (n=9) (Table 2). The total number of isolated MDROs was significantly higher in infections occurring in North Europe [43 (17%) vs. 37 (12%); p=0.04]. Prevalence of MDROs also differed significantly among countries ranging from 0% in Switzerland, Czech Republic and Denmark, 7 to 9% in Spain and Italy, respectively, 21% in UK, 25% in Ireland and 34% in France (p <0.001) [Table 2, Figure 1 (panel A)].

Type of isolated MDROs also differed among countries [Table 2, Figure 1 (panels B and C)] and regions (North vs. South Europe, Table 2, Suppl Figure 1). ESBL and Amp-C producing *Enterobacteriaceae* were more frequent in France (18%), followed by UK and The Netherlands (12% each), Austria (3.8%), Belgium (3.4%) and Spain (3%). VSE predominated in France and Austria (8% each) and MRSA in infections

occurring in The Netherlands (6%), UK and Ireland (5% each). Infections by XDR bacteria were infrequent and heterogeneously distributed. Carbapenem-resistant *Klebsiella pneumoniae* was reported in 2 patients from North Europe (<1%, 1 in Germany and in 1 UK*)* while carbapenem-resistant *Pseudomonas aeruginosa* was reported in 4 cases in South Europe (1.3%; 1 in Italy, 1 in Spain and 2 in France). VRE was also infrequent (n=3) and only diagnosed in North Europe (1.2%; 1 in Germany, 1 in UK and 1 in Ireland). When comparing MDR isolations between North and South Europe only MRSA was significantly more frequent in the North (3.5% vs. 1%, respectively; p=0.04). No PDR bacteria was reported in this study.

Suppl Table 2 shows the MDR bacteria isolated in the different centers participating in the study. Nineteen out of the 29 centers (66%) reported infections caused by MDROs. Remarkable differences were observed in the prevalence and type of MDR strains among these centers. Frankfurt (41%), Clichy (39%), Villejuif (30%) and London (King's College, 27%) showed the highest prevalence of MDROs while no resistant strains were reported in Aarhus, Hvidovre, Berna, Bologna, Graz, Ghent, Madrid (Ramon y Cajal), Prague and Turin. No culture-positive infections were reported in Vienna. ESBL-*E. coli* predominated in Clichy, Frankfurt, Barcelona (St. Pau), Padua, London (King's College) and Leuven and ESBL-*Klebsiella pneumoniae* in London (UC) and Hamburg. A heterogeneous distribution of the rest of MDROs was observed in the other centres, even in those located in the same geographical region and city (Figure 2).

Infections caused by MDROs

Table 3 shows the prevalence, type, clinical and epidemiological characteristics of bacterial infections caused by MDROs in the whole series and in North and South

Europe. Prevalence of MDR bacterial infections was 12% if all infections are considered and 27.5% in culture-positive episodes. No significant differences in the prevalence of MDROs were observed between North and South Europe (all infections: 14.7% vs. 9.7%; culture-positive infections: 30% vs. 25%). MDROs were more frequently isolated in bacteremia (25%), pneumonia (22.2%), and UTI (18.5%) in the whole series, although differences were not statistically significant. The rate of isolation of MDROs was similar among specific infections in North and South Europe except for pneumonia, which was more frequently caused by resistant bacteria in the North (27.1% vs. 16.7%, p=0.01). MDR bacteria were also more frequently isolated in ICU (18.4% vs. 12.3%; p=0.09) and in nosocomial infections (19.2% vs. 7.5% and 5.8% in CA and HCA infections, respectively; p<0.001). This finding was also observed when infections were analyzed separately in North and South Europe. Finally, MDROs were more frequently isolated in infections causing severe sepsis or shock (30.3% vs. 11%, p<0.001) or ACLF (18.7% vs. 8.3%, p<0.001), feature also observed when infections were analyzed separately in North or South Europe.

Type and efficacy of first line antibiotic strategies

Two main factors influenced the type of first line antibiotic schemes: the site of acquisition of infection and severity (Suppl Table 3). Classical antibiotic strategies were used more frequently in CA infections as first line therapy, both in the whole series (70.1%) and in North (65.4%) and South Europe (73.4%), while strategies covering MDROs were prescribed more frequently in nosocomial episodes (63.4%, 62.8% and 64.0%, respectively). Both strategies were similarly used for the empirical treatment of HCA infections. Remarkably, patients with severe sepsis or shock received more frequently broad-spectrum antibiotics covering MDROs as first line

antibiotic therapy in the whole series and in North and South Europe (69.3%, 65.1%, and 75.0%, respectively). However, significant geographical differences in antibiotic prescription were observed in patients with sepsis, with patients from North Europe receiving more frequently MDR covering strategies (71% vs. 38%, p<0.001)

The efficacy of classical and MDR empirical antibiotic strategies is shown in Table 4. In the whole series, empirical MDR covering strategies were more effective (higher infection resolution rate or higher adequacy to the microbiological susceptibility) than empiric classical schemes in nosocomial infections (83.5% vs. 69.5%, respectively, p=0.009), severe sepsis/shock (82.7% vs. 60.9%, p=0.04) and in infectious episodes with or without sepsis (85.4% vs. 77.4%, p=0.04). This higher efficacy of MDR covering strategies was observed in North but not in South Europe. Inadequacy of first line antibiotic strategies had a negative impact on short-term survival in both, AD and ACLF patients (Suppl. Table 4).

Impact of antibiotic resistance on clinical outcome

Table 5 shows the clinical outcome of infections caused by MDROs in comparison to that observed in infections caused by susceptible bacteria or with no microbiological isolation in the whole series and in patients from North and South Europe. Resolution of infection was significantly lower in episodes caused by MDROs (71.4% vs. 87.8%, p<0.001), especially in those reported in South Europe (65.7% vs. 76.2%, p=0.038). Infections caused by MDR strains showed a higher prevalence of severe sepsis/septic shock (31.9% vs. 11.7%, p<0.001), ACLF (67.5% vs. 45.1%, p<0.001) and higher 28-d mortality (35.1% vs. 18.2%, p= p<0.001). The negative impact on clinical outcome of antibiotic resistance was confirmed in both, North and South Europe.

Risk factors for MDR bacterial infection

Table 6 shows the risk factors associated with the development of infections caused by MDROs in the univariate analysis in the whole series. Nosocomial infection (OR: 2.96; 95% CI: 1.58-5.53; p<0.001**)** and recent hospitalization (OR: 1.92; 95% CI: 1.04-3.55; p=0.036**)** were identified as independent predictors of MDR infection. Mechanical ventilation (OR: 3.63; 95% CI: 1.63-8.13; p=0.002**)** was the only factor independently associated with MDR infection in nosocomial episodes. No independent predictors of MDR infection were identified for community-acquired and HCA infections.

DISCUSSION

The current investigation reports for the first time the epidemiology of MDR bacterial infections in decompensated cirrhosis and ACLF across Western Europe. The study analyzes information prospectively recorded from the Canonic Study and includes 508 patients with bacterial infection enrolled in 29 centers from 12 countries. From a geographically point of view, the study constitutes the broadest epidemiological assessment of bacterial infections ever performed in cirrhosis. Our investigation confirms that MDR bacterial infections constitute a global and growing healthcare problem in Hepatology. MDR were reported in 2 every three liver units and 9 out of the 12 countries participating in the Canonic Study. Both, North and South Europe showed a similar prevalence of MDR bacterial infections, feature that is in line with the epidemiological data reported in the general population. The pattern of antibiotic resistance was highly heterogeneous, with marked differences in the type of MDROs among countries and centers.

The overall prevalence of MDR bacterial infections in the whole cohort of culture-positive infections was 27.5% (12% if all infections are considered). This figure is similar to that reported in some unicentric investigations performed in European countries. Studies so far published report a prevalence of MDROs in culture-positive infections ranging from 8% in Turkey, 19-21% in Greece, 14-24% in Sweden-Germany and 21-31% in Spain to 31% in France and 27-46% in Italy.[6,12,13,15,20,26-31] It is important to remark that in the current study, marked differences in the prevalence of MDROs were observed among countries. MDROs isolation rate ranged from 0% in Switzerland, Czech Republic and Denmark and 7% in Spain and 9% in Italy to 21% in UK, 25% in Ireland and 34% in France. Belgium, Germany, The Netherlands and Austria showed intermediate rates of MDROs. Differences in the prevalence of

MDROs were also observed among the participant centers, even among those located in the same geographical region or city. Frankfurt, Clichy, Villejuif and King's College of London showed the highest prevalence of MDROs meanwhile other centers reported no resistant strains or intermediate MDR rates. The low number of infections recorded in centers from Switzerland, Czech Republic and Denmark could explain the absence of MDROs isolation in these countries (17 infections in total). On the other hand, this study extended just for 7 months, feature that could have limited our capacity to evaluate with precision the real prevalence of MDROs in the different countries and centers. This short duration of the investigation could explain the discrepancies observed in the prevalence of MDROs between our study and other investigations (i.e. Spain and Italy).[6,12,21]

As a whole, ESBL-producing *Enterobacteriaceae* was the MDRO more frequently isolated in the study followed by VSE and MRSA. However, the type of resistant strains significantly differed across countries and centers. ESBL and Amp-C producing *Enterobacteriaceae* were more frequently isolated in France, UK and The Netherlands; VSE predominated in France and Austria and MRSA in infections occurring in The Netherlands, UK and Ireland. The relevant differences observed in the type of MDROs isolated among countries and centers underline the importance of having surveillance programs at a local level that investigate the prevalence and epidemiological pattern of MDROs at each hospital. Global epidemiological data are informative but are not applicable to a specific center or population.[32]

Infections by XDR bacteria were infrequent and heterogeneously distributed. Carbapenem-resistant *Klebsiella pneumoniae* and VRE were reported sporadically in North Europe while carbapenem-resistant *Pseudomonas aeruginosa* was only isolated in South Europe. No PDR bacteria were reported. This low prevalence of

XDR and PDR strains is probably due to the time in which the investigation was conducted. In 2011, these superbugs were exceptional in the majority of the European regions in the general population (except for Italy and Greece).[32] The current epidemiological scenario has probably changed with an increase in the prevalence of these difficult to treat bacteria. In fact, recent studies performed in Italy report an increasing prevalence of XDR or PDR bacteria ranging from 3% to 14%.[12]

MDR bacteria were more frequently isolated in bacteremia, pneumonia and UTI, in the ICU and in nosocomial episodes. MDR bacterial infections were more severe (higher rate of severe sepsis/shock and ACLF at diagnosis and during follow-up) and associated to lower resolution rate and higher mortality at 28-d in the whole cohort and in both, North and South Europe. Our results, therefore, confirm previous studies in decompensated cirrhosis showing that antibiotic resistance is associated to poor prognosis and high short-term mortality.[10,13,17,20-22] This poor prognosis of infections caused by MDROs has also been reported in patients with solid or hematological malignancies and in critical care in the general population.[33-35]

Nosocomial origin of infection and recent hospitalization in the previous 3 months were the only independent risk factors for MDR bacterial infections identified in the whole cohort, finding that underlines the key relevance of hospitalization in determining the epidemiological risk of antibiotic resistance in the cirrhotic population. Instrumentation, exposition to broad-spectrum antibiotics and possibly in-hospital colonization by MDR bacteria could account for this finding. In contrast to previous studies, long-term norfloxacin prophylaxis[10] and HCA infections were not identified as risk factors of MDR in the current series. The low number of patients on long-term quinolone prophylaxis in our study (n=7) prevented us from evaluating adequately this potential risk factor. Our finding on the low risk of antibiotic

resistance in HCA infections differs from recent publications from Italy[4,12] and is probably related to differences in epidemiological risk factors between countries and centers. Mechanical ventilation, a parameter reflecting both organ support and high degree of instrumentation, was the only factor independently associated with MDR infection in nosocomial episodes. Regretfully, we were unable to identify risk factors for MDR in infections developing within the first 48h of hospitalization.

The current study also describes for the first time the type and efficacy of empirical antibiotic strategies used across Europe. Classical antibiotics, those based on third-generation cephalosporins and quinolones, were mainly used in CA infections while schemes covering MDROs were prescribed more frequently in nosocomial episodes and in severe sepsis or shock. As a whole, MDR covering strategies were more effective than classical schemes. However, this higher efficacy of MDR covering strategies was only observed in infections reported in North Europe, finding that cannot be justified by differences in the prevalence of MDROs. Importantly, inadequacy of first line antibiotic strategies had a negative impact on short-term survival, both in AD and in ACLF patients. Our findings support therefore the current recommendations on empirical antibiotic strategies in decompensated cirrhosis. Broad schemes covering all potential pathogens should be empirically used in the nosocomial setting and in severe sepsis or shock and should be followed by rapid de-escalation strategies to avoid a further spread of antibiotic resistance.[1,9,36,37] First line antibiotic strategies should be decided locally together with the infectious disease specialists and should consider the specific epidemiological pattern of antibiotic resistance, a feature highly heterogeneous according to the results of the current investigation. Two recent studies demonstrate the efficacy of adapting the empirical antibiotic strategies to the local pattern of resistance.[38,39]

Our investigation confirms the increasing prevalence and negative impact of MDR bacterial infections in cirrhosis in many of the European centers participating in the Canonic study. This observation demands the urgent evaluation of new strategies aimed at preventing the spread of antibiotic resistance in the cirrhotic population. Clinical impact and cost/effectiveness of measures such as epidemiological surveillance (regular assessment of potential carriers of MDROs through rectal and nasal swabs during hospitalization)[40,41], rapid microbiological tests (micro-arrays or multiplex PCR techniques capable of detecting gene targets specific of MDROs and MALDI-TOF MS),[42,43] and antibiotic stewardship programs deserve further evaluation. [9,44,45]

In conclusion, our study demonstrates that MDR bacterial infections constitute a global and growing healthcare problem in cirrhosis across Western Europe. The pattern of antibiotic resistance was highly heterogeneous, with marked differences in the type of MDROs among countries and centers. Antibiotic resistance was associated to poor prognosis and to failure of first line antibiotic strategies based on third-generation cephalosporins or quinolones.

FIGURE LEGENDS

Figure 1.

Rate of infections caused by MDROs (Panel A), ESBL and Amp-C producing *Enterobacteriaceae* (Panel B) and MRSA (Panel C) across Western Europe. Marked differences were observed among countries.

Figure 2

Overall rate of MDROs isolation in the different European centres participating in the study. Marked differences in the type and prevalence of MDROs were observed among centres. The size of the circles correlates with the overall prevalence of MDROs at each center. Different colours represent different MDR bacteria.

Suppl. Figure 1

Number of different types of MDROs isolated in the study in North (blue bars) and South Europe (orange bars). The total number of MDROs isolations was higher in North Europe as occurred with the total number of MDR GNB and GPC.

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