Original article

Efficacy of prophylactic antibiotics in the adjuvant treatment of alcohol-related liver disease (ALD): A systematic review and meta-analysis Prophylactic antibiotics in ALD

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ABSTRACT

Introduction and Objectives: This research aims to evaluate the efficacy and safety of prophylactic antibiotics in patients with alcohol-related liver disease (ALD).
Materials and Methods: We systematically searched databases including PubMed, Embase, Cochrane, and Web of Science up to October 2023. Our scope encompassed the influence of prophylactic antibiotics on all-cause mortality, infection, variceal bleeding, hepatic encephalopathy (HE), hepatorenal syndrome (HRS), adverse events (AE), fungal infection, clostridioides difficile infection (CDI), and multidrug-resistant (MDR) bacterial infection. Additionally, total bilirubin, creatinine, platelet counts, and plasma endotoxin levels were also analyzed.
Results: After comprehensive selection, 10 studies with 974 participants were included for further analysis. The study demonstrated that prophylactic antibiotic therapy was associated with reductions in infection rates, HE incidence, variceal bleeding, and all-cause mortality. The treatment did not increase the incidence of AE, fungal infection, and CDI, but it did raise the MDR bacteria infection rate. The analysis revealed no significant protective effect of antibiotic prophylaxis on total bilirubin and creatinine levels. Furthermore, the administration of antibiotics led to marginal increases in platelet counts, a minor reduction in endotoxin concentrations, and a subtle enhancement in HRS; however, these changes did not reach statistical significance.
Conclusions: Prophylactic antibiotic therapy was an effective and safe treatment for advanced ALD. To mitigate the risk of MDR bacterial infections, a strategy of selective intestinal decontamination could be advisable. Future investigations should prioritize varied ALD patient populations with extended follow-up periods and assorted antibiotic regimens to solidify the efficacy and safety of ALD treatments.

1. Introduction

In 2018, the World Health Organization estimated that alcohol consumption was a contributing factor in approximately three million fatalities worldwide [1]. Additionally, over 75 million individuals have been identified with alcohol use disorder, placing them at an elevated risk of developing alcohol-related liver disease (ALD) [2]. ALD encompasses a continuum of hepatic conditions that include fatty liver, steatohepatitis, progressive fibrosis, cirrhosis and its associated complications, and potentially hepatocellular carcinoma. Among those affected by ALD, a subset may experience alcoholic hepatitis (AH)—the most critical form of ALD, characterized by rapid onset jaundice and a precipitous decline in hepatic function, which notably escalates mortality risk [3]. The incidence of AH is on an upward trajectory, with a staggering 50% of patients succumbing within eight weeks of onset [4]. Thus, effective interventions are urgent to ameliorate adverse outcomes.

Excessive alcohol consumption can increase bacterial populations and cause gut permeability impairment, which facilitates the translocation of bacterial products such as lipopolysaccharides (LPS), endotoxins, bacterial DNA, and pathogen-associated molecular patterns (PAMPs) into the portal circulation [5]. Toxic metabolites such as PAMPs can stimulate Kupffer cells to elicit proinflammatory molecules and initiate inflammatory cascades, which leads to hepatocellular injury [6]. The relationship between gut and liver, or gut−liver axis, is pivotal in the pathogenesis of ALD. Therapeutic strategies,

including the administration of antibiotics and probiotics, aim at modulating this axis to combat ALD. Antibiotics can reduce bacterial populations, decrease LPS release, and diminish the associated inflammatory responses [5]. Numerous studies in recent years have explored the efficacy of prophylactic antibiotics in reducing mortality, infection rates, and complications associated with ALD. For instance, rifaximin has been reported to lower endotoxemia and improve alcoholic cirrhosis-related thrombocytopenia [7], as well as decrease the incidence of infections, liver-related complications, and mortality rates [8]. In contrast, a study involving 284 patients with severe AH demonstrated that combining amoxicillin-clavulanate with prednisolone did not enhance two-month survival rates when compared to sole prednisolone treatment, although it did substantially decrease infection rates [9]. Therefore, the use of prophylactic antibiotics to treat ALD remains controversial.

Although a multitude of randomized controlled trials (RCTs) and cohort investigations have probed the role of prophylactic antibiotics in combating ALD, the field lacks a holistic, systematic review and meta-analysis focused on appraising the efficacy and safety profiles of such interventions in patients with ALD. To bridge this gap, we conducted an exhaustive systematic review and meta-analysis, incorporating the most recent scholarly contributions to furnish contemporary evidence-based insights for the management of ALD.

2. Material and Methods

2.1. Literature search

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (Supplementary Table S1). Our study's protocol garnered registration within the International Prospective Register of Systematic Reviews (PROSPERO), bearing the identifier CRD42024497645. Relevant articles were searched electronically by using Embase, PubMed, Cochrane Central Register of Controlled Trials, and Web of Science up to October 2023. The search harnessed both MeSH terms and text keywords, specifically: ("Hepatitis, Alcoholic" OR "Liver Diseases, Alcoholic") AND ("Anti-Bacterial Agents"). A comprehensive presentation of our search strategy can be found in Supplementary Table S2. All included studies were evaluated independently by two researchers, Xiuyan Wang and Endian Zheng, with any contentions in study selection resolved by the arbitration of a third reviewer, Yi Huang.

2.2. Identification and selection of eligible studies

The inclusion criteria for this study were as follows: (1) studies adopting randomized controlled, cohort, or case-control designs; (2) patients aged over 18 years with a medical diagnosis of ALD, including AH, alcoholic cirrhosis (AC); (3) a confirmed diagnosis of AH or AC, substantiated by liver biopsy or clinical and laboratory assessments, coupled with a history of excessive alcohol consumption and the absence of mechanical or obstructive causes of cholestasis. Additionally, most of the candidates were screened for the presence of hepatitis B surface antigen or RNA of the hepatitis C virus, autoimmune liver diseases, and liver tumors; (4) intervention of antibiotics was primarily given before infection. (5) publications providing adequate data enabling the computation of odds ratios (ORs) or weighted mean differences (WMDs). (6) articles were published in the English language. We excluded non-English texts, reviews, case reports, editorials, letters, conference abstracts, and any works not formally published.

2.3. Data extraction and quality assessment

Assessment of quality, level of evidence, and data extraction were carried out independently by two researchers, Xiuyan Wang and

Endian Zheng, with any divergences reconciled through consultation with a third reviewer, Yi Huang. The following data were extracted: (1) study characteristics (first author, year of publication, research duration, country where the study was conducted, design of the study, population, sample size, age, registration number of RCTs, and mean follow-up); (2) intervention characteristics (type of drugs, duration of treatment, dosage, and any additional treatments given as an adjunct); (3) Outcomes of interest included all-cause mortality, infection, any adverse event, the change of the liver function, the incidence rate of each complication including variceal bleeding, HE, HRS between antibiotic group versus control group. All-cause mortality was the primary outcome; the others were secondary outcomes. Continuous variables presented as median with range, standard error, or interquartile range were converted into means ± standard deviations via statistical formulas [10,11]. The risk of bias within RCTs and cohort studies was appraised by employing the Cochrane [12] and Newcastle−Ottawa Scale (NOS) [13], respectively.

2.4. Statistical analysis

Statistical analysis was performed in the Review Manager 5.4 version (Cochrane Collaboration, Oxford, UK). Dichotomous outcomes were displayed as ORs and 95 % confidence intervals (CIs), while continuous variables were expressed as WMDs and 95 % CI. Statistical heterogeneity was evaluated by the chi-squared (χ^2) test (Cochran's Q) and inconsistency index (I^2) [14]. In cases where substantial heterogeneity was identified (χ^2 p value ⟨ 0.05 or I^2⟩ 50 %), a random-effect model was utilized to aggregate WMDs or ORs. Conversely, the fixed-effect model was applied. Subgroup analyses were carried out to pinpoint potential sources of heterogeneity among the studies based on factors like study design, duration of follow-up, geographical region, the type of antibiotics and population. A leave-one-out sensitivity analysis was executed to assess whether any single study disproportionately influenced the aggregated results. Egger's regression tests (Stata 15 ver, Stata Corp College Station, TX, USA) [15] and funnel plots (Review Manager 5.4 version) were applied to assess publication bias. A p-value threshold of <0.05 was set for determining statistical significance.

2.5. Ethical declarations

All analyses were based on previously published studies; thus, no ethical approval or patient consent is required.

3. Results

3.1. Study search and selection

The flow of study identification and selection is illustrated in Fig. 1. Our initial search yielded a total of 2555 articles, from which 2552 were collected from four databases— PubMed (n = 772), Web of Science (n = 299), Embase (n = 1304), Cochrane Central Register of Controlled Trials (n = 177)— and three additional records retrieved by manual search. After duplicates were eliminated (n = 564), we meticulously screened 1991 titles and abstracts. This led to a full-text review of 22 articles, culminating in the inclusion of 10 studies encompassing 974 participants for the subsequent meta-analysis.

3.2. Study characteristics and baseline characteristics

The study demonstrated that half of the studies [7,8,16-18] were regarded as high quality. The details of the quality assessment are presented in Supplementary Table S3 and Supplementary Table S4. Regarding the study type, five articles were cohort studies [7,8,16-18], whereas the other five studies were RCTs [9,19-22]. In terms of antibiotic therapy, four studies used rifaximin [7,8,18,19],
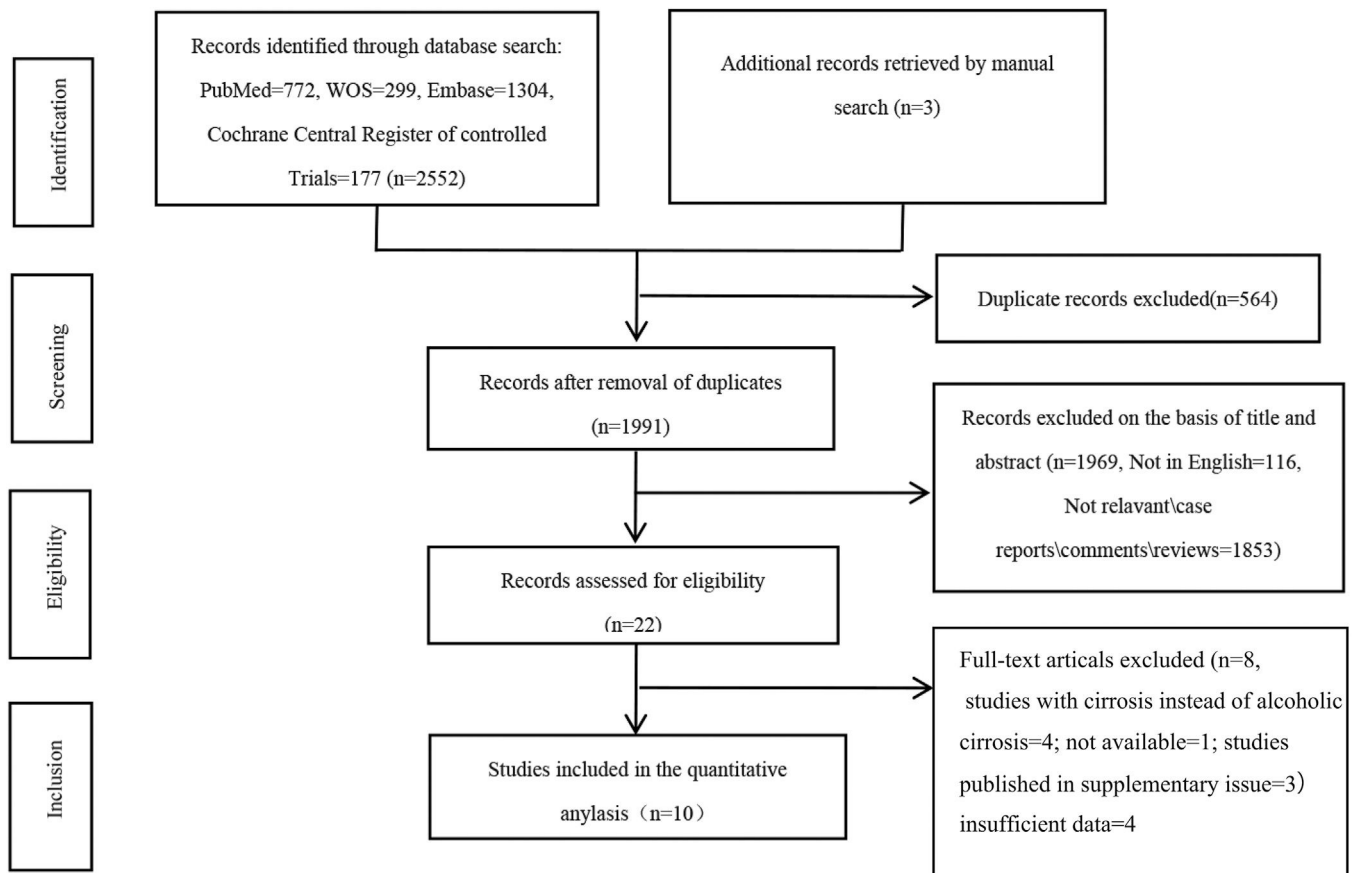
Fig. 1. The flowchat of study identification and selection process.

two studies used norfloxacin [21,22], four studies used another type of antibiotics including amoxicillin-clavulanate [9], multiple combined antibiotics (vancomycin, gentamycin and meropenem) [16], paromomycin sulfate [20], and non-defined antibiotics [17]. As for the study region, two studies were conducted in Asia [20,21], and others in Europe. The baseline characteristics are detailed in Table 1. No significant differences in sex (male/total, OR: 0.92; 95 % CI: 0.67, 1.27; $p = 0.63$) and age (WMD: -0.69; 95 % CI: -1.57, 0.19; $p = 0.12$) between the antibiotic group and control group were found.

3.3. All-cause mortality rate

Data pertaining to all-cause mortality was aggregated from eight studies [8,9,16,18-22]. The synthesis revealed that prophylactic antibiotics treatment was associated with a significant decrease in the all-cause mortality rate (OR: 0.63; 95 % CI: 0.45, 0.88; $p = 0.008$). There was an absence of heterogeneity among these studies ($I^2 = 0$ %, $p = 0.74$) as depicted in Fig. 2A, and no publication bias was detected as evidenced by the funnel plot (Fig. 3A) and Egger's test ($p = 0.381$). Subgroup analyses supported the primary outcome, indicating a statistically significant reduction in all-cause mortality within the RCT subgroup (OR: 0.68; 95 % CI: 0.48–0.99; $p = 0.049$), in studies with a follow-up duration greater than three months (OR: 0.65; 95 % CI: 0.44–0.96; $p = 0.03$), and within the European cohort (OR: 0.66; 95 % CI: 0.47–0.95; $p = 0.02$). Each of these subgroups exhibited negligible heterogeneity (Table 2). Conversely, no significant mortality benefit was observed in the cohort studies, those with a follow-up duration of three months or less, or within the Asian population. Furthermore, stratification by antibiotic type or specific populations such as AH or AC did not yield any substantial divergence in mortality outcomes between the antibiotic group and the control group (Table 2). Forest

plots depicting the subgroup analyses based on study design, follow-up duration, geographical region, the type of antibiotics, and population demographics are presented in Supplementary Figures S1-S5.

3.4. Complications

3.4.1. Infection rate

Pooled analysis from five studies[8,9,17,21,22] indicated that prophylactic antibiotics significantly diminished infection rate (OR: 0.6; 95 % CI: 0.44, 0.82; $p = 0.001$) (Fig. 2B) with mild-to-moderate heterogeneity ($I^2 = 33$ %, $p = 0.2$). The evidence of publication bias was not detected by the funnel plot (Fig. 3B) and Egger's test ($p = 0.425$). In addition, prophylactic antibiotics also reduced the incidence rate of spontaneous bacterial peritonitis (SBP) significantly [8,9,18,21] (OR: 0.33; 95 % CI: 0.13, 0.81; $p = 0.02$), with no heterogeneity ($I^2 = 0$, $p = 0.48$) (Fig. 2C) and no publication bias (Fig. 3C and Egger's test, $p = 0.455$).

3.4.2. Hepatic encephalopathy

The cumulative analysis of hepatic encephalopathy (HE) incidence, drawn from a total of five studies [8,18,20-22], delineated that the occurrence of HE was notably reduced in the antibiotic group compared to the control group (OR: 0.50; 95 % CI: 0.32–0.79; $p = 0.003$). This outcome indicates a substantial protective effect of prophylactic antibiotics against the development of HE. Additionally, the studies exhibited low heterogeneity ($I^2 = 21$ %, $p = 0.28$), as illustrated in Fig. 2D. Nevertheless, funnel plot (Fig. 3D) and Egger's test ($p = 0.023$) identified the presence of publication bias in these studies.

Table 1
Baseline characteristics of include studies.

Authors	Study period	Country	Study design	Patients (n) Antibiotics/Control	Median follow-up (months)	Intevention group	Control group	Mean age (years) Antibiotics/Control	Male(%) Antibiotics/Control
A. Louvet et al.	2015−2019	France	RCT	142/142	6	Amoxicillin-clavulanate1g/125mg, 3 times daily +40 mg/d of oral prednisolone for 30 days.	Placebo 3 times daily+40 mg/d of oral prednisolone for 30 days	52.8 ± 8.7/52.5 ± 9.7	71.8 %/73.2 %
N. Kimer et al.	2015−2018	Denmark	RCT	16/15	3	Rifaximin 550 mg, 3 times daily+ standard medical therapy for 28 days	Standard medical therapy(SMT)* for 4 weeks	51.5 ± 11.02/52±9.77	81.3 %/53.3 %
C. Jiménez et al.	2013−2019	Spain	Cohort study	21/42	3	Rifaximin 400 mg, 3 times daily +conventional therapy for 90 days.	conventional therapy** for 90 days	55±10.73/53.5 ± 14.58	76.2 %/66.7 %
S. Støy et al.		Denmark	Cohort study	14/17	3	Vancomycin 500 mg, gentamycin 40 mg, and meropenem 500 mg, once daily +standard supportive medical care for 7 days.	Standard supportive medical care***		64.3 %/52.9 %
R. Parker et al.	2009−2014	UK	Cohort study	50/50	12	Prophylactic antibiotics	No prophylactic antibiotics.	48.6 ± 3.3/49.9 ± 3.02	
C. Bode et al.		Japan	RCT	24/25	1	Paromomycin sulfate 1 g, three times daily with an interval of 8 hour for 3−4 weeks.	Placebo pulvis (1 g) was administered three times daily, with an interval of −8 hr for 21d-28d	48±7.7/48+7.13	100 %/100 %
Jiannis et al.	2004−2010	Greece	Cohort study	23/46	60	Long term administration of rifaximin in a daily dose of 1200 mg.	The same treatment apart from rifaximin administration	64.2 ± 9/64.1 ± 9.1	87 %/87 %
Anand et al.	2019−2022	India	RCT	20/13	3	Norfloxacin 400 mg once daily + standard medical therapy for 30 days.	Oral matched placebo once daily for 30 days with standard medical therapy****	39.85±7.22/40.38±10.1	100 %/100 %
R. Moreau et al.	2010−2014	France	RCT	144/147	12	Norfloxacin 400 mg daily for 6 months.	Placebo for 6 months	55.2 ± 8.5 /56.0 ± 9.5	65.3 %/73.5 %
Georgios et al.		Greece	Cohort study	13/10	1	Rifaximin 1200 mg/d for 4 weeks.	No treatment	58±10.82/ 54±9.49	92 %/90 %

SMT* included pentoxifylline 400 mg x 3 daily for 2−4 weeks. and nutrition, fluid therapy, systemic antibiotics and possibly Vitamin K, if suggested by the treating physician. In case of improvement, prednisolone 40 mg per day was administered. Conventional therapy ** included support nutrition, preventive therapy for withdrawal syndrome and corticosteroids if they did not exhibit any contraindications at the time of admission. Standard supportive medical care*** included nutrition therapy and prednisolone 40 mg/d if Glasgow AH Score ≥9. Standard medical therapy**** included diuretics, steroid therapy for alcohol-associated hepatitis (AAH), beta-blockers, lactulose, and nutritional support.
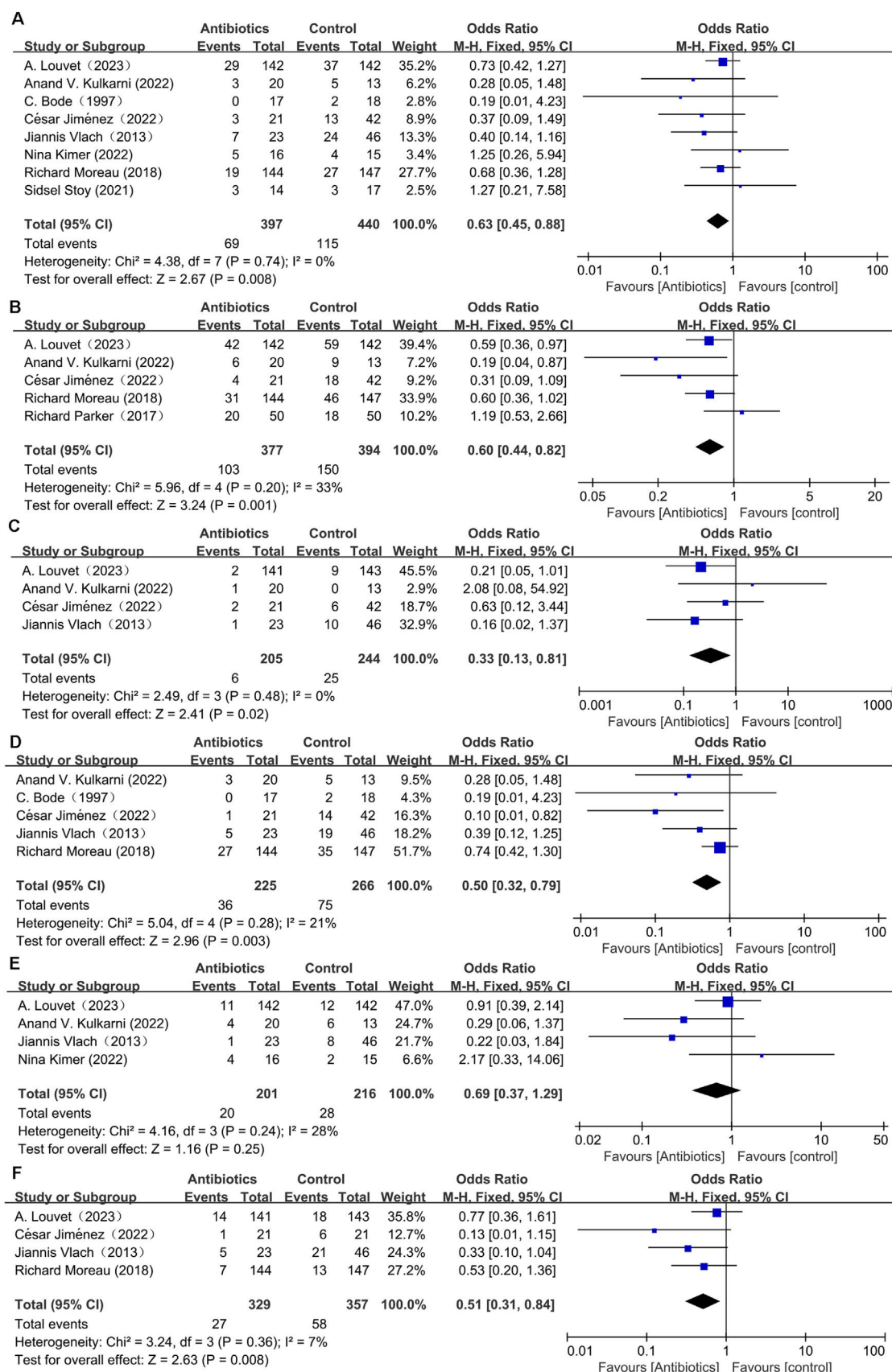
A

Study or Subgroup	Antibiotics Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
A. Louvet（2023）	29	142	37	142	35.2%	0.73 [0.42, 1.27]
Anand V. Kulkarni (2022)	3	20	5	13	6.2%	0.28 [0.05, 1.48]
C. Bode（1997）	0	17	2	18	2.8%	0.19 [0.01, 4.23]
César Jiménez（2022）	3	21	13	42	8.9%	0.37 [0.09, 1.49]
Jiannis Vlach（2013）	7	23	24	46	13.3%	0.40 [0.14, 1.16]
Nina Kimer (2022)	5	16	4	15	3.4%	1.25 [0.26, 5.94]
Richard Moreau (2018)	19	144	27	147	27.7%	0.68 [0.36, 1.28]
Sidsel Stoy (2021)	3	14	3	17	2.5%	1.27 [0.21, 7.58]
Total (95% CI)		**397**		**440**	**100.0%**	**0.63 [0.45, 0.88]**
Total events	69		115			

Heterogeneity: Chi² = 4.38, df = 7 (P = 0.74); I² = 0%
Test for overall effect: Z = 2.67 (P = 0.008)

Odds Ratio M-H, Fixed, 95% CI
0.01 0.1 1 10 100
Favours [Antibiotics] Favours [control]

B

Study or Subgroup	Antibiotics Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
A. Louvet（2023）	42	142	59	142	39.4%	0.59 [0.36, 0.97]
Anand V. Kulkarni (2022)	6	20	9	13	7.2%	0.19 [0.04, 0.87]
César Jiménez（2022）	4	21	18	42	9.2%	0.31 [0.09, 1.09]
Richard Moreau (2018)	31	144	46	147	33.9%	0.60 [0.36, 1.02]
Richard Parker（2017）	20	50	18	50	10.2%	1.19 [0.53, 2.66]
Total (95% CI)		**377**		**394**	**100.0%**	**0.60 [0.44, 0.82]**
Total events	103		150			

Heterogeneity: Chi² = 5.96, df = 4 (P = 0.20); I² = 33%
Test for overall effect: Z = 3.24 (P = 0.001)

Odds Ratio M-H, Fixed, 95% CI
0.05 0.2 1 5 20
Favours [Antibiotics] Favours [control]

C

Study or Subgroup	Antibiotics Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
A. Louvet（2023）	2	141	9	143	45.5%	0.21 [0.05, 1.01]
Anand V. Kulkarni (2022)	1	20	0	13	2.9%	2.08 [0.08, 54.92]
César Jiménez（2022）	2	21	6	42	18.7%	0.63 [0.12, 3.44]
Jiannis Vlach（2013）	1	23	10	46	32.9%	0.16 [0.02, 1.37]
Total (95% CI)		**205**		**244**	**100.0%**	**0.33 [0.13, 0.81]**
Total events	6		25			

Heterogeneity: Chi² = 2.49, df = 3 (P = 0.48); I² = 0%
Test for overall effect: Z = 2.41 (P = 0.02)

Odds Ratio M-H, Fixed, 95% CI
0.001 0.1 1 10 1000
Favours [Antibiotics] Favours [control]

D

Study or Subgroup	Antibiotics Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Anand V. Kulkarni (2022)	3	20	5	13	9.5%	0.28 [0.05, 1.48]
C. Bode（1997）	0	17	2	18	4.3%	0.19 [0.01, 4.23]
César Jiménez（2022）	1	21	14	42	16.3%	0.10 [0.01, 0.82]
Jiannis Vlach（2013）	5	23	19	46	18.2%	0.39 [0.12, 1.25]
Richard Moreau (2018)	27	144	35	147	51.7%	0.74 [0.42, 1.30]
Total (95% CI)		**225**		**266**	**100.0%**	**0.50 [0.32, 0.79]**
Total events	36		75			

Heterogeneity: Chi² = 5.04, df = 4 (P = 0.28); I² = 21%
Test for overall effect: Z = 2.96 (P = 0.003)

Odds Ratio M-H, Fixed, 95% CI
0.01 0.1 1 10 100
Favours [Antibiotics] Favours [control]

E

Study or Subgroup	Antibiotics Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
A. Louvet（2023）	11	142	12	142	47.0%	0.91 [0.39, 2.14]
Anand V. Kulkarni (2022)	4	20	6	13	24.7%	0.29 [0.06, 1.37]
Jiannis Vlach（2013）	1	23	8	46	21.7%	0.22 [0.03, 1.84]
Nina Kimer (2022)	4	16	2	15	6.6%	2.17 [0.33, 14.06]
Total (95% CI)		**201**		**216**	**100.0%**	**0.69 [0.37, 1.29]**
Total events	20		28			

Heterogeneity: Chi² = 4.16, df = 3 (P = 0.24); I² = 28%
Test for overall effect: Z = 1.16 (P = 0.25)

Odds Ratio M-H, Fixed, 95% CI
0.02 0.1 1 10 50
Favours [Antibiotics] Favours [control]

F

Study or Subgroup	Antibiotics Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
A. Louvet（2023）	14	141	18	143	35.8%	0.77 [0.36, 1.61]
César Jiménez（2022）	1	21	6	21	12.7%	0.13 [0.01, 1.15]
Jiannis Vlach（2013）	5	23	21	46	24.3%	0.33 [0.10, 1.04]
Richard Moreau (2018)	7	144	13	147	27.2%	0.53 [0.20, 1.36]
Total (95% CI)		**329**		**357**	**100.0%**	**0.51 [0.31, 0.84]**
Total events	27		58			

Heterogeneity: Chi² = 3.24, df = 3 (P = 0.36); I² = 7%
Test for overall effect: Z = 2.63 (P = 0.008)

Odds Ratio M-H, Fixed, 95% CI
0.01 0.1 1 10 100
Favours [Antibiotics] Favours [control]

Fig. 2. Forest plots of outcomes: (A) all-cause mortality rate (B) infection rate (C) SBP rate (D) HE rate (E) HRS rate (F) variceal bleeding rate.
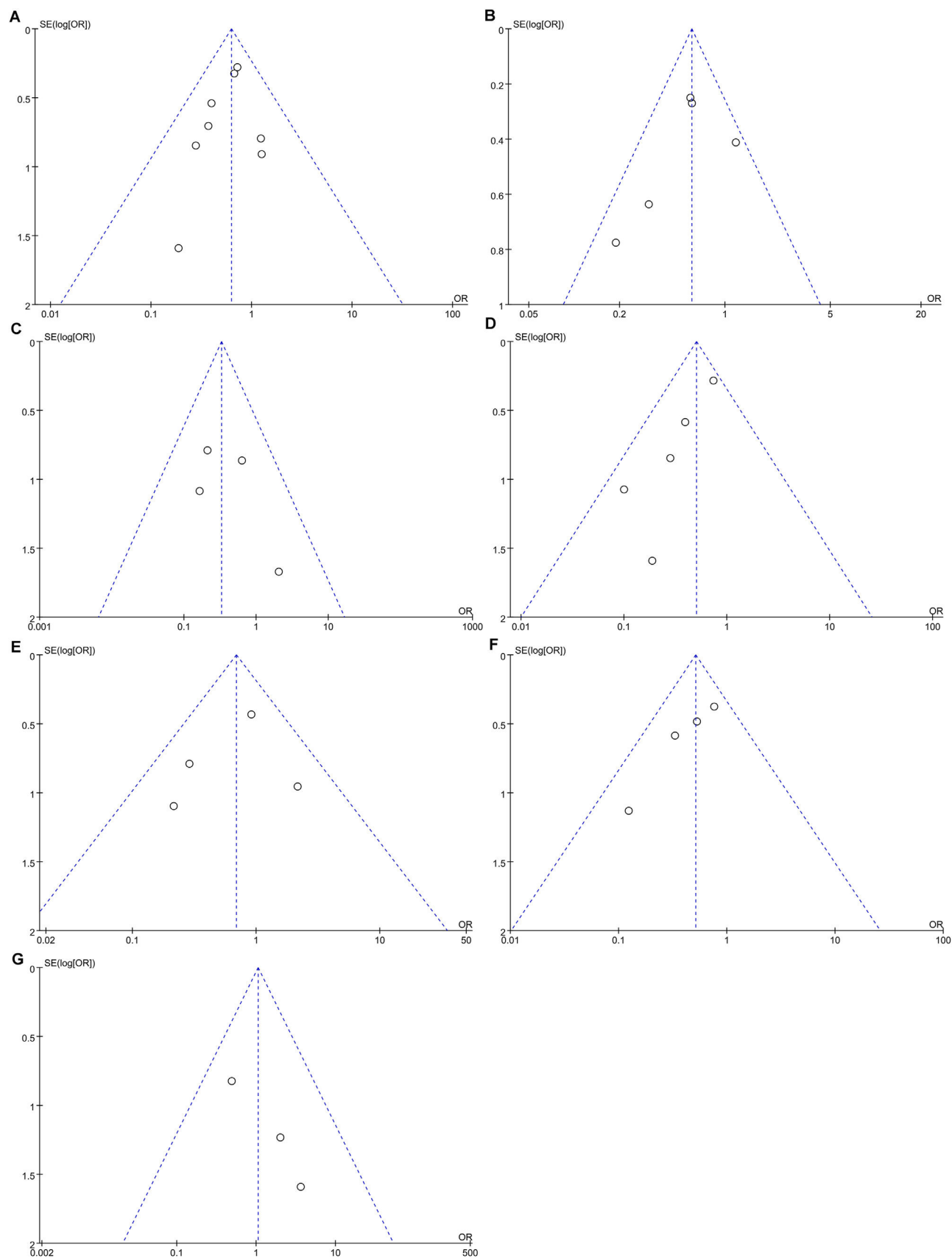
Fig. 3. Funnel plots of outcomes: (A) all-cause mortality rate (B) infection rate (C) SBP rate (D) HE rate (E) HRS rate (F) variceal bleeding rate (G) fungal infection rate.

Table 2
Subgroup analysis of antibiotics versus control for alcoholic liver disease.

Subgroup	All-cause motality			
	Study	OR [95 %CI]	P value	I^2
Total	8	0.63 [0.45, 0.88]	0.008	0 %
Study design				
RCT	5	0.68[0.68,0.99]	0.049	0 %
Cohort study	3	0.48 [0.23, 1.02]	0.05	0 %
Follow-up				
>3 months	3	0.65 [0.44, 0.96]	0.03	0 %
≤3months	5	0.55 [0.26, 1.14]	0.11	0 %
Region				
Asia	2	0.25 [0.06, 1.09]	0.07	0 %
Europe	6	0.66 [0.47, 0.95]	0.02	0 %
Antibiotics				
Rifaximin	3	0.50 [0.24, 1.04]	0.06	0 %
Norfloxacin	2	0.60 [0.33, 1.09]	0.1	0 %
Other	3	0.72 [0.43, 1.22]	0.22	0 %
Population				
AH	6	0.66 [0.42, 1.02]	0.06	0 %
AC	2	0.59 [0.34, 1.01]	0.05	0 %

OR, odds ratio; CI, confidence interval; AH, alcoholic hepatitis;AC, alcoholic cirrhosis.

3.4.3. Hepatorenal syndrome

The analysis from four studies [9,18,19,21] showed that prophylactic antibiotics could not control the incidence of hepatorenal syndrome (HRS) (OR: 0.69; 95 % CI: 0.37, 1.29; p = 0.25). Furthermore, heterogeneity was not detected in this study (I^2 = 28 %, p = 0.24) (Fig. 2E). Publication bias was not detected by funnel plot (Fig. 3E) or Egger's test (p = 0.634).

3.4.4. Variceal bleeding

The data on variceal bleeding rate were synthesized from three studies [8,9,18,22]. Pooled results revealed that prophylactic antibiotics reduced variceal bleeding incidence significantly when compared with the control group (OR: 0.51; 95 % CI: 0.31, 0.84; p = 0.008), without significant heterogeneity (I^2 = 7 %, p = 0.36) (Fig. 2F). However, publication bias was identified by funnel plot (Fig. 3F) and Egger's test (p = 0.036).

3.5. Adverse event

Adverse events considered included not only serious adverse events that resulted in death, were life-threatening, required inpatient hospitalization or an extension of existing hospitalization, led to persistent or significant disability or incapacity, were congenital anomalies/birth defects, or were deemed clinically significant by the investigator [9,22], but also nonserious adverse drug reactions. All prespecified liver-related complications were categorized as serious adverse events. Adverse events were classified using the Medical Dictionary for Regulatory Activities (www.meddra.org). The adverse event from two articles [9,22], was similar between the prophylactic antibiotic group and control group (OR: 1.1; 95 % CI: 0.76, 1.59; p = 0.63). Further analysis showed that heterogeneity was not observed in this study (I^2 = 0 %, p = 0.6) (Fig. 4A). Moreover, the meta-analysis showed that the incidence of clostridioides difficile infection (CDI) in the prophylactic antibiotic group was not significantly different from the control group (OR: 0.41; 95 % CI: 0.06, 2.64; p = 0.35). Heterogeneity was not observed (I^2 = 0 %, p = 0.74) (Fig. 4B).

The effect of prophylactic antibiotics on fungal infection was examined in three clinical trials [9,16,21]. Pooled results indicated that antibiotics increased fungal infection slightly, with no significant difference (OR: 1.05; 95 % CI: 0.34, 3.29; p = 0.93), and no heterogeneity was observed (I^2 = 0 %, p = 0.42) (Fig. 4C). Publication bias was not detected by funnel plot (Fig. 3G) and Egger's test (p = 0.113).

Regarding the effect of prophylactic antibiotics on MDR bacteria infection, the analysis from two articles [9,22] demonstrated that MDR bacteria infection rate increased significantly in the antibiotic group (OR: 4.57; 95 % CI: 2.06, 10.14; p = 0.0002). Heterogeneity was not observed in this study (I^2 = 0 %, p = 0.49) (Fig. 4D).

3.6. Change in plasma endotoxin concentrations

The influence of prophylactic antibiotics on alterations in plasma endotoxin concentrations was assessed across two clinical investigations [7,20]. Synthesis of the evidence revealed comparable decreases in plasma endotoxin levels between the antibiotic group and the control group (WMD: 0.03; 95 % CI: −0.33, 0.38; p = 0.89), with no significant heterogeneity (I^2 = 0 %, p = 0.67) (Supplementary Figure S6).

3.7. Change in platelet level

Changes in platelet counts were analyzed in two studies [7,20], with pooled data showing parallel increases in platelet levels between subjects receiving antibiotics and controls (WMD: 14.17; 95 % CI: −36.35, 64.69; p = 0.58). No significant heterogeneity was identified in this study (I^2 = 0 %, p = 0.75) (Supplementary Figure S7).

3.8. Total bilirubin and creatinine level

Two publications addressed fluctuations in serum total bilirubin [8,20] and creatinine concentrations [19,20]. Meta-analysis indicated that antibiotic-treated subjects exhibited comparable differential values of bilirubin and creatinine levels to those in the control group (WMD: 1.41; 95 % CI:−2.21, 5.03; p = 0.45). Heterogeneity assessments for both bilirubin (I^2 = 0 %, p = 0.99) (Supplementary Figure S8) and creatinine metrics (WMD: 4.94; 95 % CI: −32.10, 41.98; p = 0.79) (I^2 = 0 %, p = 0.32) demonstrated no significant variances (Supplementary Figure S9).

3.9. Sensitivity analysis

Leave-one-out sensitivity analysis procedures were employed to ascertain the influence on the cumulative OR of all-cause mortality, infection prevalence, HE, SBP, and variceal bleeding within each included study. The outcomes indicated that the exclusion of individual studies did not markedly impact the rates of all-cause mortality, infection prevalence, HE occurrences, or variceal bleeding (Fig. 5A, 5B, 5C, 5D). Contrarily, the significance of the aggregated OR for SBP incidence shifted from noteworthy to non-significant upon the sequential omission of either A. Louvet et al.'s or Jiannis et al.'s studies, signaling instability in these findings (Fig. 5E).

4. Discussion

The international debate surrounding the preventative use of antibiotics in patients with ALD is both vigorous and ongoing, as reflected by numerous studies [7-9,16,19]. In an effort to contribute to this conversation, we executed a comprehensive systematic review and meta-analysis, examining data from 974 participants across 10 studies, which spanned various ALD stages, such as AH and AC, and incorporated disparate antibiotic interventions.

The study demonstrated that prophylactic antibiotics were associated with a lower number of infections, non-infection complications (e.g., HE, variceal bleeding), and all-cause mortality. In addition, prophylactic antibiotics didn't increase the incidence rate of all adverse events, fungal infection, and CDI, but they raised the multidrug-resistant bacteria infection rate. Based on differential values of total bilirubin and creatinine levels, prophylactic antibiotics had no protective effect on the liver and renal function. Moreover, while there was a marginal improvement in HRS, a slight elevation in platelet counts,
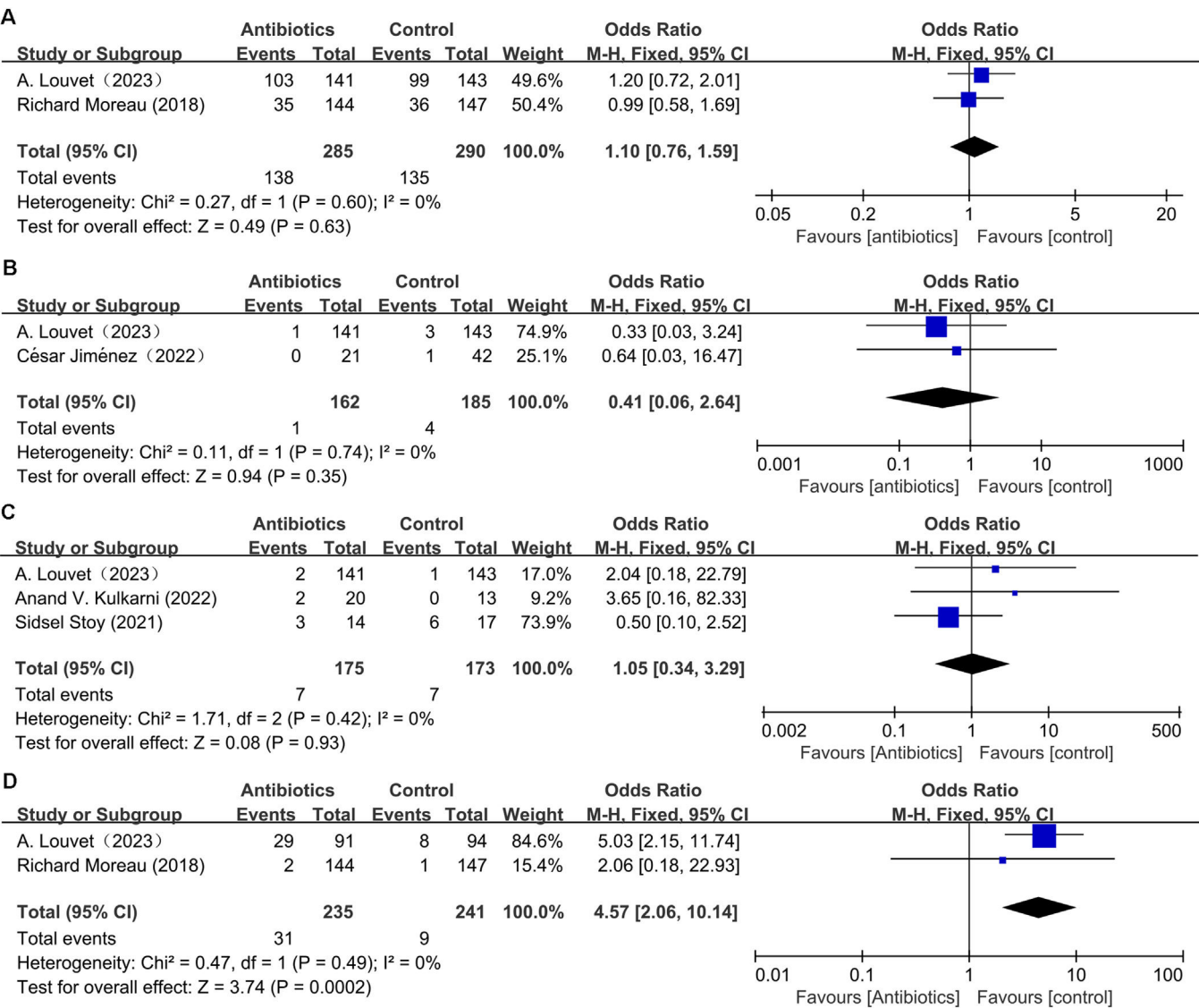
Fig. 4. Forest plots of outcomes: (A) any adverse event rate (B) CDI rate (C) fungal infection rate (D) MDR bacteria infection rate.

and a minor reduction in endotoxin levels attributed to prophylactic antibiotics, these alterations did not achieve statistical significance. Prophylactic antibiotic in ALD treatment act as selective intestinal decontamination agents to eliminate or inhibit the gut flora, modify the microbiome, inhibit the bacterial overgrowth, reduce bacterial translocation, lessen the infection resulting from pathogens from gastrointestinal flora, reduce systemic inflammation, and eventually alleviate susceptibility to liver injury.

Our findings, in concordance with other research [8,9,22], indicated that prophylactic antibiotic administration diminished infection rates among individuals with ALD. Nevertheless, Richard Parker *et al.*'s study [17] presented conflicting evidence. Potential reasons for these divergent outcomes could stem from distinct methodological approaches, with Parker's team utilizing a retrospective design and potentially inadequate control of disease severity variables. Additionally, our analysis suggested that the incidence of SBP was reduced in the prophylactic antibiotics group compared to controls. This aligns with several reports highlighting the beneficial impact of norfloxacin and rifaximin in managing primary and secondary SBP [23,24]. Another meta-analysis underscored the efficacy of rifaximin, showcasing a 47 % reduction in SPB risk versus no antibiotic regimen for primary prophylaxis, and a 74 % decrease when compared with

systemic antibiotics for secondary prophylaxis [25]. However, the variable outcomes from our leave-one-out sensitivity analyses regarding SBP call into question the robustness of these conclusions.

Our pooled analysis of non-infection complications revealed that prophylactic antibiotics could significantly lower the risk of variceal bleeding and HE. Prophylactic antibiotics have been shown to potentially decrease infection in variceal bleeding with cirrhosis [26], presumably by reducing hepatic venous pressure gradient (HVPG) [27]. A reduction of portal pressure by >20 % of baseline values or <12 mmHg could effectively protect against the risk of initial or recurrent variceal bleeding [28]. Current disease management guidelines recommend antibiotic prophylaxis in the management of upper gastrointestinal bleeding in cirrhosis [29]. A similar benefit of antibiotic prophylaxis was also confirmed in overt hepatic encephalopathy treatment [30]. In a pilot study [8], mere one out of 21 subjects undergoing rifaximin treatment developed mild HE, whereas the control arm saw 14 out of 42 participants affected. This suggests that rifaximin, when deployed in severe AH management, may be associated with a lower HE occurrence rate. Furthermore, rifaximin has been approved for use as a first-line therapy for prophylaxis of HE.

The intervention of prophylactic antibiotic in ALD patients significantly reduced the mortality rate caused by complications such as
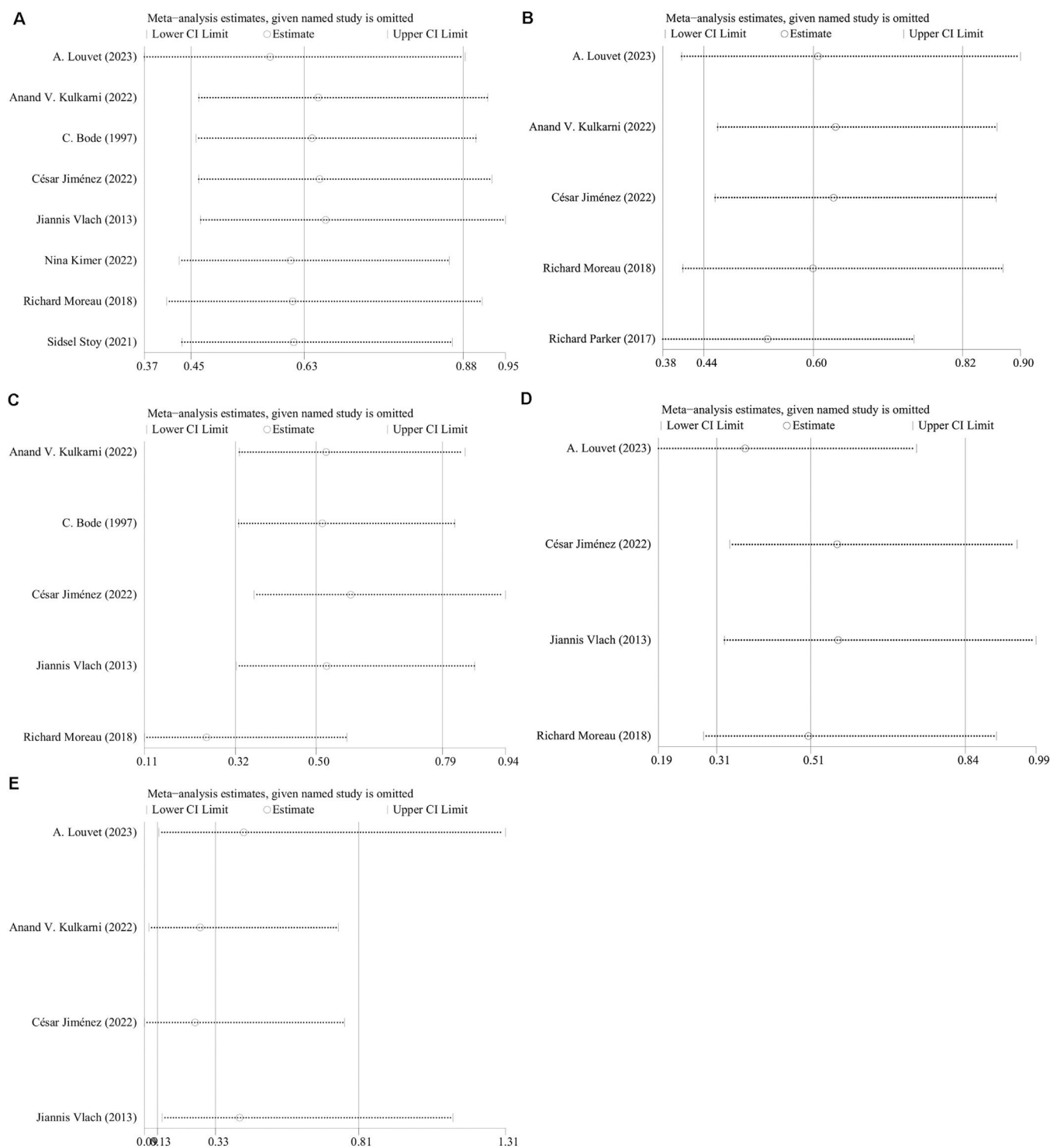
Fig. 5. Sensitivity analysis of (A) all-cause mortality (B) infection (C) HE (D) variceal bleeding (E) SBP.

infection, variceal bleeding, and HE. Nevertheless, this observation stands in contrast to the findings reported by A. Louvet [9] and S. Stoy [16], with potential discrepancies arising from various factors, including the distinct influence of different antimicrobials on gut microbiota. Subgroup analyses yielded consistent results supporting the efficacy of prophylactic antibiotics on all cause mortality within RCTs, in studies with follow-up duration exceeding three months, and within European subgroup. Conversely, no significant differences were detected between the two groups in the cohort studies, studies with duration of follow up ≤ 3months, and Asian population. In terms

of study design, the nonrandomized studies typically displayed more favorable findings and exhibited higher risks of bias. However RCTs represent the most robust and rigorous research methods, providing higher-quality evidence to evaluate the efficacy and safety of interventions [31]. Consequently, the positive outcomes derived from RCTs are considered more reliable. Regarding follow-up duration, our analysis indicated that longer follow-up periods were associated with more pronounced reductions in mortality rates due to prophylactic antibiotics. Intriguingly, studies with a follow-up duration of ≤ 3months pertained exclusively to the AH population. In contrast,

studies with longer follow-up periods primarily involved patients with AC. This divergence might be attributed to several factors: the variation in disease severity between AH and AC, the prevalent use of corticosteroids for severe AH which increases the risk of infectious complications due to defective lymphocyte signaling [32], and the potential for infections to act as a precipitator of acute-on-chronic liver failure, thereby elevating mortality risk in AH. The smaller sample size in the ≤3-month subgroup also contributed to less reliable outcomes. In terms of the types of antibiotics, the efficacy of various antibiotic treatments in reducing mortality rates was inconsistent, indicating variable outcomes across studies. Although the results were not statistically significant, rifaximin appeared more effective than norfloxacin in reducing mortality, as surported by a lower OR 0.5 [0.24,1.04] vs. 0.6 [0.33,1.09]) in subgroup analysis (Table 2). This might be due to the increasing prevalence of gram-positive, quinolone-resistant, and multi-drug-resistant (MDR) bacterial in recent years [23], and the extensive, long-term use of prophylactic quinolines exacerbating this trend. Gut microbiota dysbiosis and disruption of the gut—liver axis are implicated in the pathogenesis of various chronic liver diseases including ALD, contributing to liver inflammation, fibrosis, and portal hypertension [33]. While antibiotics generally have detrimental effects on the gut microbiota—including reduced species diversity, altered metabolic activity, and screening of antibiotic-resistant organisms—rifaximin has unique effects. It appears to increase populations of beneficial intestinal bacteria without significantly altering the overall composition of the gut microbiota, and is thought to aid in restoring the intestinal barrier, thereby potentially mitigating bacterial translocation and systemic endotoxemia in individuals with cirrhosis [33-36]. Besides, a RCT [37] comparing rifaximin and norfloxacin for secondary prophylaxis of SBP reported threefold higher encephalopathy-related deaths in the norfloxacin group, with fewer side effects observed in the rifaximin group. Therefore, rifaximin, a gut-selective antibiotic with low microbial resistance and broad antibacterial spectrum, has been suggested as an effective oral alternative to norfloxacin.

Drug safety is the primary concern in medical therapy and significantly influences the choice of medication for patients. In this study, prophylactic antibiotics have been shown not to significantly increase the incidence of all adverse effects, fungal infections, or CDI, yet they were associated with an increased rate of MDR infections. One RCT [9] identified a higher occurrence of MDR bacteria infection (29/91 or 31.9 %) in the amoxicillin-clavulanate group with 31.9 % (29/91) of participants affected, as opposed to only 8.5 % (8/94) in the placebo group. Moreover, systemic administration of amoxicillin-clavulanate is linked to systemic side effects, including hepatotoxicity, gastrointestinal disturbances, allergic reactions, enhanced bacterial resistance, and potential drug interactions [38]. J. Fernandez et al [39] reported that prolonged norfloxacin prophylaxis was an independent predictor of MDR bacteria infections. Thus, patients should receive the right drug and dose for the right duration to reduce or avoid the MDR bacteria infection. Nevertheless, we did not carry out subgroup analysis for the rate of MDR bacteria infection based on different types of antibiotic owing to the insufficient data.

In this study, we present a comprehensive and the most up-to-date meta-analysis by including publications from 1997 to 2023, which makes our study more credible. We also acknowledge several limitations in this study. Firstly, the research was exclusively conducted in Asian and European countries, which may introduce selection bias, thus the conclusion cannot be generalized to a wider population. Secondly, the research was exclusively conducted in individuals with advanced ALD. Future studies should enroll more patients from different ethnic backgrounds with long-term follow-up in different types antibiotics treatments, to validate the efficacy and safety of ALD therapy. Lastly, the number of our meta-analyses was limited, which may impact the reliability of the I^2 statistic in detecting study heterogeneity. Paul T. von Hippel *et al.* have highlighted that the widely utilized heterogeneity statistic, I^2, can be imprecise and biased when used in smaller meta-analyses. They suggested that calculating confidence intervals around I^2 could potentially mitigate this issue [40].

5. Conclusions

Prophylactic antibiotic was an effective and safe treatment for advanced ALD with improvements in all-cause mortality, infection, variceal bleeding, and HE, without additional risk of any adverse events, fungal infection, and CDI. The non-absorbable antibiotics and selective systemic antibiotics for bowel decontamination can be opted to reduce MDR bacterial infection. In future studies, it is essential to consider diversity in ALD patient recruitment with long-term follow-up and more antibiotic treatments to solidify the efficacy and safety of ALD therapy.

Availability of data

All data from this study are included in this published article and its supplementary files.

Declaration of interests

None.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.aohep.2024.101571.

References

[1] Hernández-Évole H, Jiménez-Esquivel N, Pose E, Bataller R. Alcohol-associated liver disease: epidemiology and management. Ann Hepatol 2024;29(1):101162. https://doi.org/10.1016/j.aohep.2023.101162.

[2] Shi JF, Liu Y, Wang Y, Gao R, Wang Y, Liu J. Targeting ferroptosis, a novel programmed cell death, for the potential of alcohol-related liver disease therapy. Front Pharmacol 2023;14:1194343. https://doi.org/10.3389/fphar.2023.1194343.

[3] Im GY. Acute alcoholic hepatitis. Clin Liver Dis 2019;23(1):81–98. https://doi.org/10.1016/j.cld.2018.09.005.

[4] Louvet A, Naveau S, Abdelnour M, Ramond MJ, Diaz E, Fartoux L, et al. The lille model: a new tool for therapeutic strategy in patients with severe alcoholic hepatitis treated with steroids. Hepatology 2007;45(6):1348–54. https://doi.org/10.1002/hep.21607.

[5] Sung H, Kim SW, Hong M, Suk KT. Microbiota-based treatments in alcoholic liver disease. World J Gastroenterol 2016;22(29):6673–82. https://doi.org/10.3748/wjg.v22.i29.6673.

[6] Stickel F, Datz C, Hampe J, Bataller R. Pathophysiology and management of alcoholic liver disease: update 2016. Gut Liver 2017;11(2):173–88. https://doi.org/10.5009/gnl16477.

[7] Kalambokis GN, Mouzaki A, Rodi M, Tsianos EV. Rifaximin improves thrombocytopenia in patients with alcoholic cirrhosis in association with reduction of endotoxaemia. Liver Int 2012;32(3):467–75. https://doi.org/10.1111/j.1478-3231.2011.02650.x.

[8] Jiménez C, Ventura-Cots M, Sala M, Calafat M, Garcia-Retortillo M, Cirera I, et al. Effect of rifaximin on infections, acute-on-chronic liver failure and mortality in alcoholic hepatitis: a pilot study (RIFA-AH). Liver Int 2022;42(5):1109–20. https://doi.org/10.1111/liv.15207.

[9] Louvet A, Labreuche J, Dao T, Thévenot T, Oberti F, Bureau C, et al. Effect of prophylactic antibiotics on mortality in severe alcohol-related hepatitis: a randomized clinical trial. JAMA 2023;329(18):1558–66. https://doi.org/10.1001/jama.2023.4902.

[10] Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol 2014;14:135. https://doi.org/10.1186/1471-2288-14-135.

[11] Luo D, Wan X, Liu J, Tong T. Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. Stat Methods Med Res 2018;27(6):1785–805. https://doi.org/10.1177/0962280216669183.

[12] Cumpston M, Li T, Page MJ, Chandler J, Welch VA, Higgins JP, et al. Updated guidance for trusted systematic reviews: a new edition of the cochrane handbook for systematic reviews of interventions. Cochrane Database Syst Rev 2019;10(10): Ed000142. https://doi.org/10.1002/14651858.Ed000142.

[13] Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. OHRI 2011;2 (1):1–12.

[14] Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21(11):1539–58. https://doi.org/10.1002/sim.1186.

[15] Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315(7109):629–34. https://doi.org/10.1136/bmj.315.7109.629.

[16] Støy S, Laursen TL, Eriksen LL, Grønbæk H, Vilstrup H, Sandahl TD. No effect in alcoholic hepatitis of gut-selective, broad-spectrum antibiotics on bacterial translocation or hepatic and systemic inflammation. Clin Transl Gastroenterol 2021;12 (2):e00306. https://doi.org/10.14309/ctg.0000000000000306.

[17] Parker R, Im G, Jones F, Hernández OP, Nahas J, Kumar A, et al. Clinical and microbiological features of infection in alcoholic hepatitis: an international cohort study. J Gastroenterol 2017;52(11):1192–200. https://doi.org/10.1007/s00535-017-1336-z.

[18] Vlachogiannakos J, Viazis N, Vasianopoulou P, Vafiadis I, Karamanolis DG, Ladas SD. Long-term administration of rifaximin improves the prognosis of patients with decompensated alcoholic cirrhosis. J Gastroenterol Hepatol 2013;28 (3):450–5. https://doi.org/10.1111/jgh.12070.

[19] Kimer N, Meldgaard M, Hamberg O, Kronborg TM, Lund AM, Møller HJ, et al. The impact of rifaximin on inflammation and metabolism in alcoholic hepatitis: a randomized clinical trial. PLoS One 2022;17(3):e0264278. https://doi.org/10.1371/journal.pone.0264278.

[20] Bode C, Schäfer C, Fukui H, Bode JC. Effect of treatment with paromomycin on endotoxemia in patients with alcoholic liver disease—a double-blind, placebo-controlled trial. Alcohol Clin Exp Res 1997;21(8):1367–73.

[21] Kulkarni AV, Tirumalle S, Premkumar M, Kumar K, Fatima S, Rapole B, et al. Primary norfloxacin prophylaxis for apasl-defined acute-on-chronic liver failure: a placebo-controlled double-blind randomized trial. Am J Gastroenterol 2022;117 (4):607–16. https://doi.org/10.14309/ajg.0000000000001611.

[22] Moreau R, Elkrief L, Bureau C, Perarnau JM, Thévenot T, Saliba F, et al. Effects of long-term norfloxacin therapy in patients with advanced cirrhosis. Gastroenterology 2018;155(6):1816–27 e9. https://doi.org/10.1053/j.gastro.2018.08.026.

[23] Song S, Yang Y, Geng C, Tang Z, Wang C, Li X. Norfloxacin versus alternative antibiotics for prophylaxis of spontaneous bacteria peritonitis in cirrhosis: a systematic review and meta-analysis. BMC Infect Dis 2023;23(1):557. https://doi.org/10.1186/s12879-023-08557-6.

[24] Grangé JD, Roulot D, Pelletier G, Pariente EA, Denis J, Ink O, et al. Norfloxacin primary prophylaxis of bacterial infections in cirrhotic patients with ascites: a double-blind randomized trial. J Hepatol 1998;29(3):430–6. https://doi.org/10.1016/s0168-8278(98)80061-5.

[25] Goel A, Rahim U, Nguyen LH, Stave C, Nguyen MH. Systematic review with meta-analysis: rifaximin for the prophylaxis of spontaneous bacterial peritonitis.

[26] Moon AM, Dominitz JA, Ioannou GN, Lowy E, Beste LA. Use of antibiotics among patients with cirrhosis and upper gastrointestinal bleeding is associated with reduced mortality. Clin Gastroenterol Hepatol 2016;14(11):1629–37 e1. https://doi.org/10.1016/j.cgh.2016.05.040.

[27] Vlachogiannakos J, Saveriadis AS, Viazis N, Theodoropoulos I, Foudoulis K, Manolakopoulos S, et al. Intestinal decontamination improves liver haemodynamics in patients with alcohol-related decompensated cirrhosis. Aliment Pharmacol Ther 2009;29(9):992–9. https://doi.org/10.1111/j.1365-2036.2009.03958.x.

[28] Ripoll C, Groszmann R, Garcia-Tsao G, Grace N, Burroughs A, Planas R, et al. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. Gastroenterology 2007;133(2):481–8. https://doi.org/10.1053/j.gastro.2007.05.024.

[29] Runyon BA. Management of adult patients with ascites due to cirrhosis: an update. Hepatology 2009;49(6):2087–107. https://doi.org/10.1002/hep.22853.

[30] Wu D, Wu SM, Lu J, Zhou YQ, Xu L, Guo CY. Rifaximin versus nonabsorbable disaccharides for the treatment of hepatic encephalopathy: a meta-analysis. Gastroenterol Res Pract 2013;2013:236963. https://doi.org/10.1155/2013/236963.

[31] Bhide A, Shah PS, Acharya G. A simplified guide to randomized controlled trials. Acta Obstet Gynecol Scand 2018;97(4):380–7. https://doi.org/10.1111/aogs.13309.

[32] Vergis N, Atkinson SR, Knapp S, Maurice J, Allison M, Austin A, et al. In patients with severe alcoholic hepatitis, prednisolone increases susceptibility to infection and infection-related mortality, and is associated with high circulating levels of bacterial DNA. Gastroenterology 2017;152(5):1068–77 e4. https://doi.org/10.1053/j.gastro.2016.12.019.

[33] Mendez-Sanchez N, Coronel-Castillo CE, Cordova-Gallardo J, Qi X. Antibiotics in chronic liver disease and their effects on gut microbiota. Antibiotics (Basel) 2023;12(10). https://doi.org/10.3390/antibiotics12101475.

[34] Wang R, Tang R, Li B, Ma X, Schnabl B, Tilg H. Gut microbiome, liver immunology, and liver diseases. Cell Mol Immunol 2021;18(1):4–17. https://doi.org/10.1038/s41423-020-00592-6.

[35] Zoratti C, Moretti R, Rebuzzi L, Albergati IV, Di Somma A, Decorti G, et al. Antibiotics and liver cirrhosis: what the physicians need to know. Antibiotics (Basel) 2021;11(1). https://doi.org/10.3390/antibiotics11010031.

[36] Yu X, Jin Y, Zhou W, Xiao T, Wu Z, Su J, et al. Rifaximin modulates the gut microbiota to prevent hepatic encephalopathy in liver cirrhosis without impacting the resistome. Front Cell Infect Microbiol 2021;11:761192. https://doi.org/10.3389/fcimb.2021.761192.

[37] Elfert A, Abo Ali L, Soliman S, Ibrahim S, Abd-Elsalam S. Randomized-controlled trial of rifaximin versus norfloxacin for secondary prophylaxis of spontaneous bacterial peritonitis. Eur J Gastroenterol Hepatol 2016;28(12):1450–4. https://doi.org/10.1097/meg.0000000000000724.

[38] Lee SW, Choi Y, Jang K, Yoon SH, Chung JY. Multiple-dose pharmacokinetics and safety of amoxicillin/clavulanate in healthy elderly subjects. Int J Clin Pharmacol Ther 2020;58(11):634–41. https://doi.org/10.5414/cp203773.

[39] Fernández J, Acevedo J, Castro M, Garcia O, de Lope CR, Roca D, et al. Prevalence and risk factors of infections by multiresistant bacteria in cirrhosis: a prospective study. Hepatology 2012;55(5):1551–61. https://doi.org/10.1002/hep.25532.

[40] von Hippel PT. The heterogeneity statistic I(2) can be biased in small meta-analyses. BMC Med Res Methodol 2015;15:35. https://doi.org/10.1186/s12874-015-0024-z.