REVIEW

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Escherichia coli Nissle 1917 in Ulcerative Colitis Treatment: Systematic Review and Meta-analysis

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ABSTRACT

Background & Aims: *Escherichia coli Nissle* 1917 (EcN) has been recommended as a therapeutic tool for ulcerative colitis (UC) treatment. However, to date, no meta-analysis has been performed on this topic.
Methods. We performed a literature search on PubMed, MEDLINE, Science Direct and EMBASE. We evaluated success rates for induction of remission, relapse rates and side effects, expressed as Intention-To-Treat. Odd ratios (OR), pooled OR and 95% confidence intervals (CI) were calculated, based on the Mantel-Haenszel method. Heterogeneity was assessed by using the χ^2 and I^2 statistics and, if present, a random-effects model was adopted.
Results. We selected six eligible trials, with 719 patients, 390 assigned to the study group and 329 to the control group. EcN induced remission in 61.6% of cases, while in the control group (mesalazine) the remission was achieved in 69.5% of cases, with a mean difference of 7.9%. The pooled OR was 0.92 (95% CI 0.15-9.66, p=0.93). A single study showed a better performance of EcN than the placebo. A relapse of the disease occurred in 36.8% in the EcN group and in 36.1% in the control group (mesalazine), with a mean difference of 0.8%, OR=1.07, with a 95% CI of 0.70-1.64 (p=0.74). Side effects were comparable (OR=1.44, 95% CI 0.80-2.59, p=0.22).
Conclusions. EcN is equivalent to mesalazine in preventing disease relapse, thus confirming current guideline recommendations. EcN seems to be as effective as controls in inducing remission and therefore, its use cannot be recommended as in one study the comparison was performed against placebo. Further studies may be helpful for this subject.

Key words: ulcerative colitis – inflammatory bowel disease – probiotics – Escherichia coli Nissle – microbiota.

Abbreviations: EcN: Escherichia coli Nissle; UC: ulcerative colitis; IBD: inflammatory bowel disease; CD: Crohn's disease; ITT: intention-to-treat.

INTRODUCTION

Escherichia coli Nissle 1917 (EcN) is a non pathogenic Gram-negative bacterium belonging to the *Enterobacteriaceae* family [1]. It is a well-known probiotic strain with multiple beneficial effects on intestinal homeostasis. Firstly, in contrast to other *E. coli* strains, it does not produce virulence factors, so that it is unable to induce damage to the surface of the intestinal epithelium [2]. Conversely, EcN can stimulate the production of human beta-defensin 2, a molecule that has proven to be crucial in the protection of mucosal barrier against the adhesion and invasion of pathogenic bacterial species [3]. Due to this peculiarity, several *in vitro* and *in vivo* studies have demonstrated a protective function of EcN against Salmonella, Shigella, Candida and other invasive bacteria [4-6]. Furthermore, EcN may restore a damaged epithelium through the modulation of tight junction and zonula occludens proteins [7]. Additionally, this singular bacterial strain may secrete some factors (microcins, adhesins, proteases) that enhance the production of adenosine triphosphate (ATP), thus improving the energy availability [8]. Finally, EcN may modulate the mucosal inflammatory response by a direct action on activated T-lymphocytes. As a consequence, reduced levels of proinflammatory cytokines, such as interleukin (IL)-2, interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) have been observed in experimental models, as well as an increase of regulatory cytokines (IL-10, IL-1, IL-8) [9, 10].

For these reasons, EcN has been employed in several clinical trials for the treatment of gastrointestinal disorders [11], including inflammatory bowel diseases (IBD) [1], a heterogeneous group of chronic recurrent inflammatory disorders of the gastrointestinal tract [12]. Inflammatory bowel diseases are commonly divided into ulcerative colitis (UC) and Crohn's disease (CD). Two pathogenetic hypotheses are predominant in the current view: a predisposing genetic background and an immune response against the human microbiota [13-15]. Intestinal microbiota is constituted by a wide variety of bacterial species, and it has been considered as an "organ within an organ" for its extreme variety of strains and ability to control the local immunity and response to antigens [16]. On this basis, the possibility that a pharmacological modulation of intestinal microbiota could mirror the outcome of IBD and other gastrointestinal disorders has been investigated with increasing success [17-20].

The aim of the present systematic review with meta-analysis was to investigate the role of EcN administration in patients suffering from UC, focusing on the effectiveness in inducing and maintaining the remission phase and the safety profile.

METHOD

Eligibility criteria and study selection

Methods of analysis and inclusion criteria were based on "Preferred Reporting Items for Systematic reviews and Meta-Analyses" (PRISMA) recommendations [21], and a PICOS checklist has been enclosed (see Supplementary material). Only randomized clinical trials were included, while review articles, experimental *in vivo* or *in vitro* studies and non randomized trials were excluded. Abstracts were excluded.

Data collection process

A literature search was performed in June 2015. Relevant publications were identified by a research in PubMed, MEDLINE, Science Direct and EMBASE. The search terms were *Escherichia coli*, Nissle, treatment, inflammatory bowel disease and ulcerative colitis. We used the following string: [(ulcerative colitis) AND (*Escherichia coli* OR E coli OR EcN OR Nissle)]. Titles and abstracts of papers were screened by two reviewers (G.L. and E.I.). Studies were independently prescreened in blind for relevance by the two reviewers using full reports. Discussion put an end to any disagreements. Successively, data were extracted from the relevant studies by one reviewer and checked by a second reviewer, and thus inserted into dedicated tables. A third reviewer (M.P.) came to a decision about any disagreements.

Reviewers independently abstracted from each paper the following data: (i) year of publication, (ii) country where the study was performed, (iii) single- or multicentre study, (iv) study design, (v) number of patients included, (vi) methods of randomization, (vii) success rate expressed as intention to treat (ITT) for induction and maintenance of remission, (viii) side effects.

Risk of bias

To ascertain the validity of eligible randomized trials, pairs of reviewers working independently and with adequate reliability determined the adequacy of randomization and concealment of allocation, blinding of patients, health care providers, data collectors, and outcome assessors. Additionally, we provided funnel plots to determine the risk of publication bias: absence of significant publication bias occurred when symmetry in the graph appeared.

Summary measures and aim of the meta-analysis

The end-point was to compare the administration of EcN strain in patients with UC, in comparison to a control group, represented either by placebo or mesalazine administration. The outcomes extracted for the meta-analysis were the success rates for induction of remission, relapse rates and side effects. These data have been expressed as percentages.

Planned methods of analysis

Data on remission achievement or maintenance, expressed as ITT, and side effects were extracted from the studies. Odd ratios (OR), pooled OR and 95% confidence intervals (CI) were calculated, based on the Mantel-Haenszel method. Data were entered into the RevMan 5.3 software (The Nordic Cochrane Centre, Copenhagen, Denmark) (Cochrane library). A p value <0.05 was considered statistically significant. Heterogeneity was assessed by using the χ^2 and I^2 statistics. In particular, heterogeneity was considered to be present if the χ^2 test delivered a $p < 0.05$ and, therefore, I^2 statistic was used to quantify the proportion of heterogeneity between the studies. In the presence of heterogeneity, a revision of included studies was carried out to assess the main reason explaining the phenomenon and, therefore, the subgroup analysis was performed. Only if this attempt failed, a random effects model was employed, otherwise a fixed effects model was adopted [22]. Finally, the Jadad scale was selected in order to evaluate the methodological quality of eligible trials [23]. A score ≥ 3 points indicated an adequate quality of the trial.

RESULTS

Study selection

The literature search identified 299 articles, of which only 6 met the inclusion criteria after the selection by the reviewers. The flow diagram of this systematic review is displayed in Fig. 1. The main characteristics of the six studies [24-29] are summarized in Table I. All studies but one [29] were performed on the adult population. The dose of EcN was the same in all studies. In four studies [24, 25, 28, 29], EcN was compared to mesalazine, while in the remaining two [26, 27] it was compared to a placebo. In one study [26], EcN was given via enema for proctitis or proctosigmoiditis. Overall, 719 patients were recruited for the meta-analysis; 390 were assigned to the study group and 329 to the control group.

The quality of eligible trials according to the Jadad scale is reported in Table II. All studies had a score equal or greater than 3, resulting in a good quality of the trial.

Induction of remission

Only three studies evaluated the ability of EcN to induce remission [26-28]. EcN was able to induce remission in 61.6% of cases (106 out of 172), while in the control group the remission was achieved in 69.5% of cases (66 out of 95),

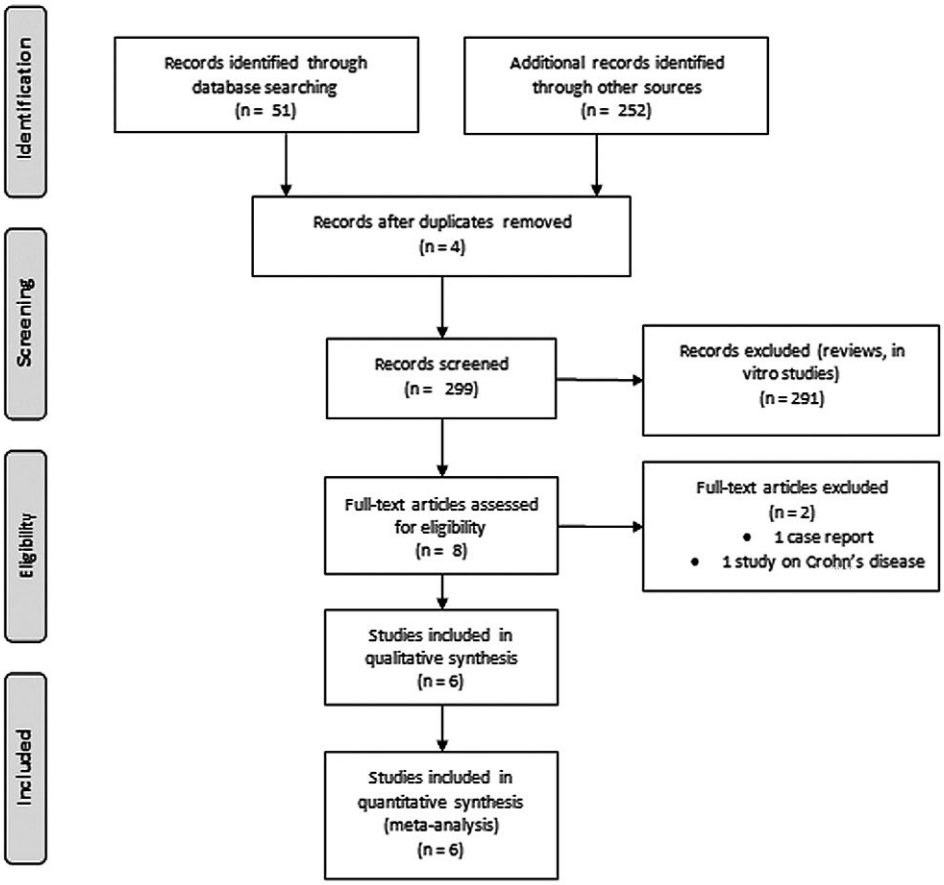

Fig. 1. Diagram of the study selection, according to the PRISMA statements.

with a mean difference of 7.9%. The pooled OR was 0.92 (95% CI 0.15-9.66), not statistically significant (p=0.93). In conclusion, EcN was as effective as the control group. The heterogeneity test provided a high level of heterogeneity (χ^2=13.85, p=0.001 and I2=86%), thus prompting the use of a random effect model.

However, in the three studies analyzing the induction of remission, one study compared EcN to a placebo, while in

Table I. Main characteristics of studies included in the meta-analysis

Study [reference]	Study design and duration	Treatment groups		Scale used for activity index	Age	Extent of the disease
		EcN group	Control group			
Kruis 1997 [24]	Double blind, randomized, placebo controlled – 12 weeks	200 mg/day + placebo	Mesalazine 500 mg t.i.d + placebo	CAI	Adults	Proctitis 27% Proctosigmoiditis 39% Left colon 18% Pancolitis 16%
Kruis 2004 [25]	Double blind, randomized, placebo controlled – 9 months	200 mg/day + placebo	Mesalazine 500 mg t.i.d + placebo	CAI	Adults	Proctitis 58% Left colon 19% Pancolitis 23%
Matthes 2010 [26]	Double blind, randomized, placebo controlled – 8 weeks	Enema 40, 20 or 10 mL	Placebo 40, 20 or 10 mL	CAI	Adults	Proctitis or proctosigmoiditis
Petersen 2014 [27]	Double blind, randomized, placebo controlled – 12 weeks	200 mg/day	Placebo	CAI	Adults	Proctitis 14% Left colon 62% Pancolitis 24%
Rembacken 1999 [28]	Double blind, randomized, placebo controlled – 12 months	200 mg/day	Mesalazine 800 mg t.i.d	CAI	Adults	Proctitis 29% Left colon 31% Subtotal colitis 5% Pancolitis 35 %
Henker 2008 [29]	Open-labelled pilot study – 12 months	200 mg/day	Mesalazine 500 mg t.i.d	CAI	Pediatric population	N/A

CAI: clinical activity index; EcN: Escherichia coli Nissle; N/A not available.

Table II. Assessment of the quality of eligible trials according to the Jadad scale.

Study [reference]	Randomization	Blinding	Withdrawal and drop-outs	Total points
Kruis 1997 [24]	2	1	1	4
Kruis 2004 [25]	2	2	1	5
Matthes 2010 [26]	2	2	1	5
Petersen 2014 [27]	2	2	1	5
Rembacken 1999 [28]	1	1	1	3
Henker 2008 [29]	1	1	1	3

the other two a standard of care (mesalazine or ciprofloxacin) regimen was used. For this reason, we performed a subgroup analysis. EcN was superior to a placebo (OR = 7.77, 95% CI 1.58-38.15; p = 0.01), but this comparison comprised only one study. On the other hand, no statistically significant difference was found between EcN and standard of care (OR = 0.38, 95% CI 0.09-1.63; p = 0.19).

Finally, we undertook a further analysis by excluding the only study that investigated EcN via enema [26]: in this case we confirmed that either EcN was comparable to the control group (OR = 0.38, 95% CI 0.09-1.63; p = 0.19), and the heterogeneity remained high (χ^2=3.78, p=0.05 and I2=74%).

The forest plot of this analysis is reported in Fig. 2.

Maintenance of remission

The maintenance of remission was evaluated in four studies [24, 25, 28, 29]. In all cases, the control groups received the standard of care (mesalazine). In the overall analysis, a relapse of the disease occurred in 36.8% (82 out of 223) in the EcN group and in 36.1% (79 out of 219) in the control group, with a mean difference of 0.8%, not statistically significant (p=0.74), meaning that EcN was as successful as mesalazine in the maintenance of remission. In detail the pooled OR was 1.07, with a 95% CI of 0.70-1.64. No heterogeneity was found (χ^2=0.91, p=0.82 and I2=0%), therefore a fixed effects model was adopted. The forest plot of the present analysis is shown in Fig. 3.

Side effects

All the six studies investigated side effects, which were observed in 35.9% of the EcN group and in 26.1% in the control group. Pooled OR was 1.44, 95% CI = 0.80-2.59, p=0.22, as displayed in Fig. 4. Therefore, the rates of side effects were comparable between the two groups. We adopted a random effects model, since a moderate heterogeneity was detected (χ^2=7.7, p=0.1 and I^2=48%). The most common adverse reactions were diarrhea and bloating, more common in the EcN group.

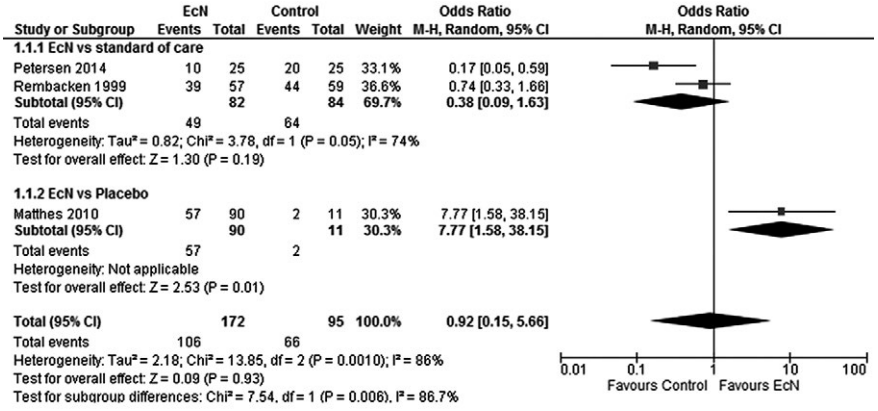

Fig. 2. Forest plot of the efficacy of EcN in inducing remission for ulcerative colitis.

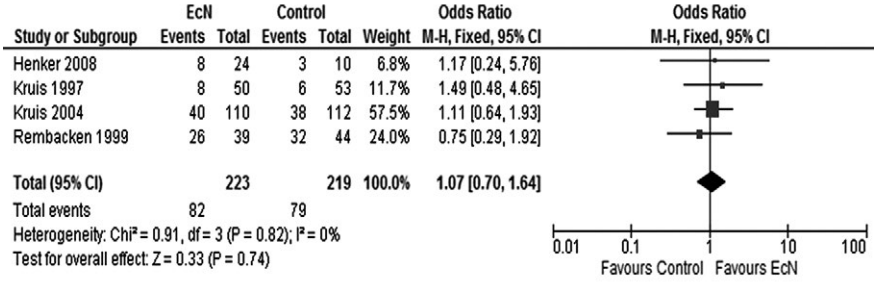

Fig. 3. Forest plot of the efficacy of EcN in maintaining remission for ulcerative colitis.

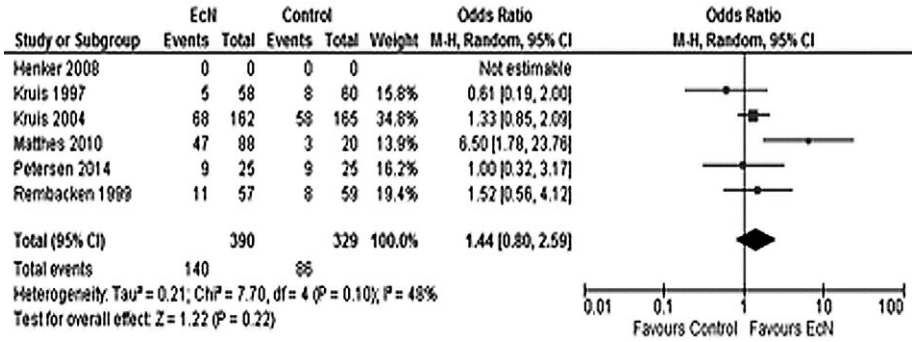

Fig. 4. Forest plot of the side effects observed in EcN treatment.

Risk of bias in interventional studies and heterogeneity

Figure 5 shows the funnel plots of the three cited comparisons (induction of remission, maintenance of remission and side effects, respectively), demonstrating the symmetry of the study distribution, thus excluding the possibility of publication bias.

Heterogeneity. A high grade of heterogeneity was found between the studies. Several factors may contribute to this phenomenon, and they may be summarized as follows: i) a discrepancy in the composition of the control group, since in two studies a placebo [26, 27] and in the other four mesalazine were assumed, ii) the different dose of mesalazine, iii) one study was performed on a pediatric population [29], iv) in one study EcN was given by enema [26] and v) the different duration of the follow up, ranging from 12 weeks to 12 months. All these factors could represent limitations for the meta-analysis, despite we suppose that the exclusion of the study on pediatric population could be a source of publication bias.

DISCUSSION

The use of probiotics in the treatment of UC has been postulated and investigated in several trials with different results [30]. Current guidelines [31] report that single probiotic strains are not effective in inducing the remission, according to various clinical trials [32, 33]. Their use in IBD is widespread for the safety profile although not all strains are equally effective. A probiotic mixture called VSL#3TM has been proven to be successful for induction and maintenance of remission in children with active UC, in a 1-year placebo-controlled, double-blind study [34]. The same mixture is considered as a gold standard for the treatment of pouchitis, and therefore it is advised by guidelines [31, 35, 36]. Additionally, EcN is recommended by the guidelines as an alternative to mesalazine for the maintenance of remission in UC. This recommendation was based on the results of four trials, which were available at the time of guideline elaboration. The present review encloses two more recently published studies which permitted a meta-analysis on this topic, which represents therefore the first one in the literature.

A first relevant finding of our analysis is that EcN is as effective as the regimen used in controls for induction of remission (OR: 0.92; p=0.93). Indeed, in two of the three analyzed studies, the control groups received a placebo. On this basis of the verification of the null hypothesis, it may be argued that EcN is not superior to a placebo in the remission phase induction. The only study [28] that compared EcN to

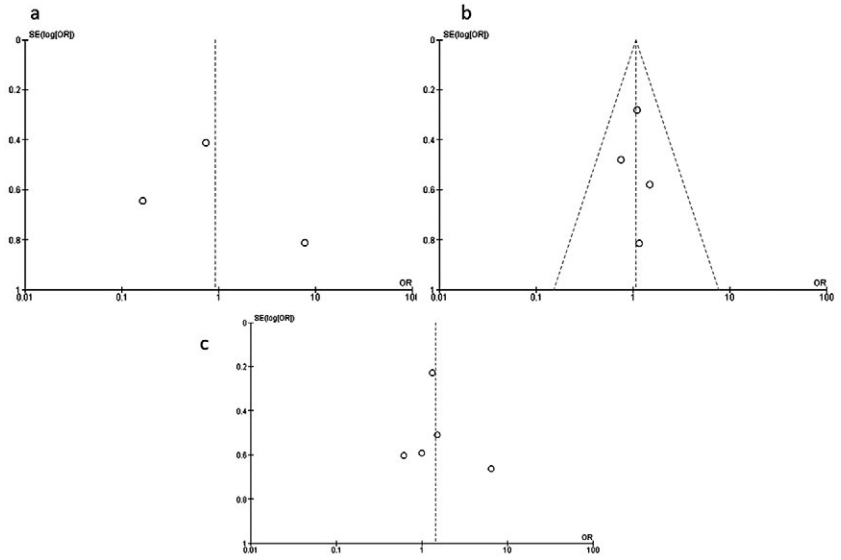

Fig. 5. Funnel plots of the efficacy of EcN in inducing remission in ulcerative colitis (5a), in maintaining remission (5b), and Funnel plot of the side effects observed in EcN treatment (5c).

mesalazine, showed an OR of 0.74 (95% CI: 0.33-1.66) in favor of EcN, but not statistically significant.

On the other hand, the evaluation of the maintenance of remission was performed in comparison to mesalazine in all studies. This detail allows to draw more solid conclusions, due to the absence of heterogeneity between the studies (χ^2=0.91, p=0.82 and I^2=0%). In this comparison EcN was as successful as mesalazine in preventing the relapse, with an OR=1.07, p=0.74. This finding represents the statistical validation of guideline statements regarding this topic.

A peculiar observation concerns the extension of the disease. In two studies [24, 25], with a large proportion of patients with proctitis and proctosigmoiditis enrolled, EcN demonstrated a high effectiveness (Fig. 3). Moreover, in the only study in which EcN was administered by enema for proctosigmoiditis, its efficacy was better than a placebo [26]. For these reasons, we may hypothesize that EcN could be more effective for the treatment of distal UC.

A first limitation of our meta-analysis is related to the small number of eligible studies. This detail may be a limit, since a solid conclusion could not be drawn, and further high-quality trials are needed. The second limitation is related to the high heterogeneity of the included trials, which is another drawback in order to perform a solid comparison between studies. We have underlined that this finding may be explained by a discrepancy in the composition of the control group, by the different dose of mesalazine, the age of enrolled patients, the route of EcN administration and the different duration of the follow up, ranging from 12 weeks to 12 months. Moreover, in the three studies analyzing the induction of remission, one study compared EcN to placebo, one to mesalazine and one study to placebo plus standard of care (which for some patients also included mesalazine), thus contributing to the high heterogeneity.

The present meta-analysis could provide some key points regarding the treatment of UC with EcN. First, EcN is equivalent to mesalazine in preventing disease relapse. Second, its use in inducing the remission cannot be recommended. However, other studies may be helpful to support this assertion, since the level of evidence for probiotic use in inducing remission is low (level 5 according to guidelines) [31]. In particular, the use of EcN as an add-on treatment to traditional therapy could represent an interesting field of investigation. Finally, EcN could be advised especially for proctitis and proctosigmoiditis, but this proposition needs to be investigated more in depth.

CONCLUSION

E. coli Nissle 1917 is a valid probiotic for UC treatment and its use could represent an effective option even if indications need to be better detailed through new trials able to provide a more reliable support for a further meta-analysis.

Conflicts of interest: None to declare.

Authors' contributions: G.L., M.P. and A.D.L. planned the study. G.L., A.C. and A.I. performed the literature search. G.L. performed the statistical analysis. G.L., E.I. and M.P. wrote the manuscript. All authors read and approved the final version.

Supplementary material: To access the supplementary material visit the online version of the *J Gastrointestin Liver Dis* at http://www.jgld.ro/wp/archive/y2015/n4/a15 and http://dx.doi.org/10.15403/jgld.2014.1121.244.ecn

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Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3, last paragraph
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5

Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	4
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	4
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5, 6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5, 6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	6, 7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	7
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	7,8
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	8
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	8, 9
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	none