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Complete List of Authors:	Alhifany, Abdullah ; Umm Al-Qura University, Pharmacy Almutairi, Abdulaali ; University of Arizona, Pharmacy Almangour, Thamer ; King Saud University, pharmacy Shahbar, Alaa ; Umm Al-Qura University, pharmacy Abraham, Ivo; University of Arizona, pharmacy Alessa, Mohammed; King Saud bin Abdulaziz University for Health Sciences, College of Pharmcy Alnezary, Faris; University of Houston, pharmacy Cheema, Ejaz; University of Birmingham Edgbaston Campus, pharmacy
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Comparing the efficacy and safety of fecal microbiota transplantation with bezlotoxumab as adjunct therapies to standard antibiotics therapy in reducing the risk of recurrent *Clostridium difficile* infections: a systematic review and Bayesian network meta-analysis of randomised controlled trials

Abdullah A Alhifany, PharmD
Department of Clinical Pharmacy, College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia
Email: aahifany@uqu.edu.sa

Abdulaali R Almutairi, PharmD
Department of Pharmacy Practice and Science, University of Arizona College of Pharmacy, Tucson, AZ, USA
Email: almutairi@pharmacy.arizona.edu

Thamer A Almangour, BScPhm, PharmD
Department of Clinical Pharmacy, King Saud University College of Pharmacy, Riyadh, KSA
Email: talmangour@ksu.edu.sa

Alaa N Shahbar, PharmD
Department of Clinical Pharmacy, College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia
Email: anshahbar@uqu.edu.sa

Ivo Abraham, PhD
Department of Pharmacy Practice and Science, University of Arizona College of Pharmacy, Tucson, AZ, USA
Email: abraham@pharmacy.arizona.edu

Mohammed Alessa, BScPhm, PharmD
Department of Pharmacy Practice, College of Pharmacy, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia
Email: essam1@ngha.med.sa

Faris S. Alnezary, PharmD
Department of Pharmacy Practice and Translational Research, University of Houston College of Pharmacy, Houston TX, USA
Email: alnezaryf@gmail.com

Ejaz Cheema, PhD (Corresponding author)
Institute of Clinical Sciences, University of Birmingham, Birmingham, UK
Email: e.cheema@bham.ac.uk

ABSTRACT

Objectives: The risk of recurrent *Clostridium difficile* infections (RCDI) is high when treated with standard antibiotics therapy (SAT) alone. It is suggested that the addition of fecal microbiota transplantation (FMT) or bezlotoxumab to SAT reduces the risk of RCDI. However, there are no head-to-head clinical trials that have compared the efficacy and safety of FMT with bezlotoxumab in reducing the risk of RCDI.

Design: A systematic review and Bayesian network meta-analysis.

Setting: Hospitals.

Inclusion criteria: Randomised controlled trials reporting the resolution of diarrhea associated with RCDI without relapse for at least 60 days after the end of treatments as the primary outcome.

Aim: To compare the efficacy and safety of bezlotoxumab with FMT as adjunct therapies to SAT in reducing the risk of RCDI.

Results: Out of 1003 articles identified, 7 RCTS involving 3,043 patients contributed to the review. The quality of the included RCTs was variable. No difference was reported between the single infusion of FMT and bezlotoxumab in resolving RCDI. However, FMT with two or more infusions showed better resolution than bezlotoxumab in the fixed-effects [Odds Ratio (OR) 2.86, 95% Credible Interval (CrI) 1.29-6.57] but not in the random-effects model [OR 2.58, 95% CrI 0.30-23.53]. Patients treated with SAT alone or bezlotoxumab with SAT showed significantly lower rates of diarrhea than FMT [OR 0, 95% CrI 0-0.09] and [OR 0, 95% CrI 0-0.19], respectively. There was no difference in terms of other adverse events.

Conclusion: Multiple infusions of FMT showed better efficacy than the single infusion of bezlotoxumab as adjunct therapies to SAT. However, FMT was associated with a higher rate of non-serious diarrhea as opposed to SAT used alone or in combination with bezlotoxumab.

Keywords: Recurrent Clostridium difficile Infections, Fecal Microbiota Transplantation, Bezlotoxumab, Standard Antibiotics Therapy, Network meta-analysis

Strengths and limitations

- Safety outcomes were limited due to the early termination of most of the included RCTs and the inconsistent reporting of the adverse events
- The quality of the included RCTs varied with more than half of the studies not reporting blinding of the participants
- The study employed a comprehensive literature search of four databases
- It used Bayesian estimation methods in the indirect comparisons of mABs and FMT to address the absence of head-to-head clinical trial evidence

BACKGROUND

Clostridium difficile is considered to be the most common source of infectious diarrhea in hospitalized patients.[1] *C. difficile*-led infections (CDI) are associated with high mortality particularly in the developed countries including USA, Canada and Europe.[2-4] Around 30% of the *C. difficile* infected patients treated with standard antibiotics therapy (SAT) such as vancomycin, metronidazole or fidaxomicin are reported to develop recurrent *C. difficile* infections (RCDI) that increases up to 60% with subsequent recurrences.[5] This cyclic pattern of recurring CDI-inducing diarrhea is triggered by the use of antibiotics and exotoxins produced by *C. difficile* that contributes to the weakening of the intrinsic fecal microbiota which serves as a natural host defense mechanism against *C. difficile* spores-led colonization.[5-7]The spore-forming ability of *C. difficile* is the main reason behind its nosocomial and community transmission.

Fecal microbiota transplantation (FMT) has been considered a novel intervention to replenish the intrinsic fecal microbiota barrier mechanism that protects against *C. difficile* associated colonization.[8] Evidence from the meta-analyses of randomized controlled trials (RCTs) as well as observational studies have highlighted the benefits of FMT as adjunctive therapy to SAT in resolving CDI over SAT alone.[9-12] Furthermore, the current clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) recommend the use of FMT for the second or subsequent recurrences of CDI.[13] However, the lack of a standardized product, dosage form and method of administration are some of the limitations of FMT.[14]

An alternative approach to FMT is to attenuate the effects of the exotoxins produced by *C. difficile*. Bezlotoxumab, a novel monoclonal antibody (mAB) that has been approved recently by the Food and Drug Administration (FDA) in the USA has been reported to reduce RCDI by attenuating the effect of exotoxin B when used in conjunction with SAT.[15-16] However, there are no head-to-head clinical trials that have compared the efficacy and safety of FMT with bezlotoxumab in reducing the risk of RCDI. In the absence of any head-to-head trials, this systematic review and Bayesian network meta-analysis of randomised controlled trials (RCTs) aims to compare the efficacy and safety of bezlotoxumab with FMT as adjunct therapies to SAT in reducing the risk of RCDI.

METHOD

The systematic review and network meta-analysis were conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension statement for network meta-analyses.[17]

Patient and public involvement

Patients and public were not involved in the design, conducting and reporting of research.

Search strategy

A comprehensive search from inception until 30th February 2019 was conducted in four databases (Medline/Pubmed, Embase, Scopus, Clinicaltrials.gov). Searches were conducted using Patients, Intervention, Comparator, Outcome, and Study design (PICOS) strategy for

clinical evidence of FMT and mAB in CDI (Table 1). Furthermore, manual searches were conducted to identify any additional studies by checking the reference lists of articles retrieved.

INSERT TABLE 1 HERE

Outcome Measure

The primary outcome of interest was the resolution of diarrhea associated to CDI without relapse for at least 60 days after the end of treatments. Furthermore, the adverse events of interest included diarrhea, abdominal pain, leukocytosis, fatigue, nausea, pyrexia, atrial fibrillation, dehydration, sepsis, tachycardia and infusion specific reactions.

Inclusion and Exclusion Criteria

Both published as well as unpublished RCTs that assessed the efficacy and safety of FMT and bezlotoxumab in resolving CDI as adjunct therapies to SAT such as vancomycin, metronidazole or fidaxomicin were eligible for inclusion. Studies were eligible for inclusion if they had included patients 18 years or older diagnosed with RCDI and reporting the resolution rate of CDI as the efficacy outcome.

Data Extraction, risk of bias and quality assessment

Two reviewers (EC and AS) independently reviewed the titles and abstracts. Studies meeting the inclusion criteria were retrieved as full-text to further assess their eligibility for inclusion. The Cochrane Risk of Bias tool was used to assess the quality of included RCTs including randomization, allocation concealment, blinding of participants, reporting of incomplete outcome data, selective reporting and any other bias.[18] Other sources of bias explored included cross-

contamination between study groups, recruitment of participants from a selected population and non-compliance with the study protocol. For each included study, a risk of bias graphs and risk of bias summary were generated.

Statistical Analysis

A Bayesian network meta-analysis was conducted using WinBUGS 1.4.3 (MRC Biostatistics Unit, Cambridge, UK). The outcomes were expressed as Odds Ratio (OR) and 95% Credible Interval (95% Crl) for resolution rate of RCDI and OR with 95% Crl for adverse events. OR for treatment comparisons were estimated based on 20,000 iterations following the discarding of the first 10,000 iterations in the model. Fixed and random-effects models were used for resolution rate of RCDI and fixed-effect for adverse events. Ranking probabilities of treatments were calculated using the surface under the cumulative ranking curve (SUCRA) method. Additionally, a sensitivity analysis was conducted to exclude the studies and/or patients who received non-FDA approved mAB.

RESULTS

The initial search identified 1003 studies (see figure 1). 15 duplicates along with an additional 631 studies that did not meet the inclusion criteria were removed. The abstracts of remaining 357 studies were reviewed, of which a further 297 were excluded. Full texts of the remaining 60 studies were reviewed. Of these, further 53 studies were excluded based on the inclusion and exclusion criteria. The remaining 7 RCTs with 3,043 patients contributed to the review and net-work meta-analysis.

INSERT FIGURE 1 HERE

Characteristics of included studies

The 7 included studies (see table 2 for characteristics of included studies) were published between 2010 and 2017 and involved 3,043 patients.[9 12 15-16 19-21] Four open-labeled RCTs involving 139 patients reported comparisons of FMT as adjunct to vancomycin versus vancomycin alone in patients with an initial episode of CDI or with recurrent CDI and followed for at least 70 days following the end of the treatments.[9 12 19 21] Patients assigned to the FMT arm received an initial course of vancomycin ranging from 3-14 days to assure that patients were covered with an antibiotic therapy at the time of donor screening.

INSERT TABL2 HERE

All studies involving FMT used fresh feces from related donors. The time for infusing the fresh FMT from the time of defecation varied across studies from 3.1 to 48 hours. Three FMT studies were terminated early following an interim analysis; two because of the observed superiority of the FMT[9 12] and one because of inferiority.[19] A fourth study was underpowered and was considered a pilot study.[21] In two studies,[9 12] FMT was reinfused in some patients who experienced a recurrence after the first infusion. In the remaining two studies, FMT was used as a single infusion.[19 21]

Three double-blinded, placebo-controlled RCTs including two multi-center phase two studies and one multi-national, multi-center phase three study investigated the efficacy of mABs.[15-16 20] The two phase two studies investigated the safety and efficacy of newly developed mABs against *C. difficile* exotoxins A and B that corroborated prior evidence of the role of these exotoxins in

the virulence of *C. difficile*.[6] The third RCT confirmed that antagonizing toxin B is the main determinant in suppressing the virulence of *C. difficile*, however it could not rule out the role of toxin A.[7] Three regimens of mABs were tested in these RCTs: anti-toxin A (actoxumab), anti-toxin B (bezlotoxumab) and a combination of both. It is important to highlight that only bezlotoxumab was approved by the FDA for this indication (see figure 2 for network plot of included studies).

INSERT FIGURE 2 HERE

Study quality

The quality of the studies was variable (see figures 3A and 3B). Only three of the seven studies used blinding of participants.[15-16 20]

INSERT FIGURE 3A AND 3B HERE

Comparative efficacy of FMT and mABs in reducing RCDI

The initial analysis comparing the resolution of CDI after receiving one FMT infusion or any mAB regimen found no difference between FMT and bezlotoxumab in the fixed-effects (OR=1.63, 95% CrI=0.77-3.56) and random-effects model (OR=1.53, 95% CrI=0.39-5.16). Yet, FMT showed the best SUCRA probability in the fixed-effects (81.8%) and random-effects models (63.6%) (see figures 4A and 4B). In addition, FMT showed better resolution of CDI than SAT in the fixed-effects (OR=3.07, 95% CrI=1.51-6.44) and random-effects models (OR=2.98, 95% CrI=1.13-7.53). Bezlotoxumab showed better resolution of CDI than SAT in the fixed-effects model (OR=1.89, 95% CrI=1.48-2.41) but not in the random-effects model (OR=1.93, 95% CrI=0.84-4.91) (see table 3).

INSERT FIGURES 4A AND 4B HERE

INSERT TABLE 3 HERE

A secondary comparative analysis that included the resolution outcomes reported for patients who received one or more FMT infusions or any mAB regimen was conducted. FMT showed better resolution of CDI than bezlotoxumab in the fixed-effects (OR=2.86, 95% CrI=1.29-6.57) but not in the random-effects model (OR=2.58, 95% CrI=0.30-23.53). Additionally, FMT showed better resolution of CDI than SAT in the fixed-effects (OR 5.39, 95% CrI 2.54-11.96) and the random-effects models (OR 5.22, 95% CrI 1.26-23.25). Bezlotoxumab showed better resolution of CDI than SAT in the fixed-effects (OR=1.88, 95%CrI=1.48-2.41) but not in the random-effects models (OR=2.01, 95%CrI=0.40-10.51) (see Table 4 here).

INSERT TABLE 4 HERE

Sensitivity analysis

A sensitivity analysis was conducted by excluding the resolution outcomes of patients who received non-FDA approved mABs. There was no difference between one FMT infusion and bezlotoxumab in the fixed-effects (OR=1.67, 95% CrI=0.79-3.64) and random-effects model (OR=1.61, 95%CrI=0.19-12.69). However, one or more FMT infusions showed better resolution of CDI than bezlotoxumab in the fixed-effects (OR=2.93, 95%CrI=1.32-6.78) but not in the random-effects model (OR=2.90, 95%CrI=0.20-45.35). FMT showed the best SUCRA probability in the fixed-effects (99.6%) and the random-effects models (79.7%). The analyses of the safety data for FMT, bezlotoxumab, and SAT revealed a significantly lower rate of non-serious diarrhea in patients receiving bezlotoxumab (OR=0, 95%CrI=0-0.19) and SAT (OR=0, 95% CrI=0-0.09), compared to patients treated with FMT. There were no differences on other adverse events.

DISCUSSION

To the best of authors' knowledge, this is the first network meta-analysis that has compared the recently FDA-approved monoclonal antibody bezlotoxumab with FMT as adjunct therapies to SAT for resolving RCDI. The findings of this study suggested that multiple infusions of FMT have better efficacy than a single infusion of bezlotoxumab as adjunct therapies to SAT. However, FMT was associated with a higher rate of non-serious diarrhea.

Despite the inconsistency in the results of the four RCTs that studied FMT as add-on to SAT versus SAT alone in resolving RCDI,[9 12 19 21] the network analysis showed the superiority of FMT and SAT over SAT alone. This is consistent with the findings of previous meta-analyses of RCTs and observational studies.[10-11] The inconsistency in the results of the four included studies may be attributed to their small sample sizes, lack of blinding and variability between them on the basis of the process of collecting donor feces, preparation of FMT, lag time between feces collection and infusion, method of administration and vancomycin regimen. Furthermore, as evident from the findings of a previous RCT, patients who received FMT monotherapy for an initial episode of CDI without receiving prior antibiotics, retained more bacteroidetes in their gut than patients treated with antibiotics.[22] These findings confirm the effect of antibiotics in attenuating the intrinsic microbiota.[5] It may also explain the inferiority of FMT and SAT over SAT in the study when FMT was preceded by fourteen days of antibiotics.[19] On the contrary, administration of FMT earlier (after the second recurrence of CDI) as opposed to a late administration (after the third or subsequent recurrences) led to shorter length of hospital stay and fewer visits to the emergency department.[23] Thus, the differences in the results of the individual studies included in the current network meta-analysis could have been due to the

variability in starting FMT for initial CDI or RCDI and the inconsistency in the number of previous recurrences among included patients.

Bezlotoxumab showed a favorable efficacy and safety profile in preventing recurrent C. difficile infection in two robust prospective, double-blinded, placebo-controlled RCTs.[16 24] Furthermore, the effect was sustained throughout the three month follow-up.[16] Bezlotoxumab has a novel mechanism of action that reduces the possibility of recurrent C. difficile infection, yet its high cost may limit its utilization.[16 24] Furthermore, even though the network meta-analysis did not report any difference in the resolution rate between bezlotoxumab and FMT after one infusion, the SUCRA probability score favored FMT in the rankogram. Multiple infusions of FMT also showed better resolution rates than a single infusion of bezlotoxumab in the fixed-effects analysis. However, the quality of the included RCTs was variable with more than half of the studies not reporting blinding of the participants. Furthermore, the RCTs studying FMT differed in design, donor selection, FMT preparation, follow-up time, lag time between feces collection and infusion and lag time between SAT discontinuation and FMT; while mABs were infused either during or right away after the discontinuation of SAT. None of the included RCTs reported the number of previous recurrences. Nevertheless, the study addressed a significant issue identified in the 2017 IDSA[13] guidelines by filling the gap in information with regards to the best method in preventing RCDI and the role of FMT and mABs as adjunctive therapies. Further studies are required to assess the efficacy, safety, cost and clinical implications of multiple infusions of bezlotoxumab.

CONCLUSION

Multiple infusions of FMT showed better efficacy than single infusion of bezlotoxumab as adjunct therapies to SAT in resolving RCDI in fixed-effects analyses but with a higher rate of non-serious diarrhea. Further studies are needed to investigate the efficacy and safety of using FMT as monotherapy for CDI, the possible attenuating effect of short-course antibiotics given before FMT and the clinical implications of multiple infusions of bezlotoxumab.

Funding

None

Conflict of interests

None to declare.

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Table 1. PICOS strategy for clinical evidence of FMT and mAB in CDI

PICOS	Clinical Review
Population	Adults with primary or recurrent CDI.
Intervention	Studies that reported the efficacy and safety of fecal microbiota transplantation and/or monoclonal antibodies as add-on therapy to antibiotics at any dosage form and via any route of administration in resolving RCDI.
Comparator	Standard antibiotics therapy, such as vancomycin, metronidazole, or fidaxomicin, at any dosage form and via any route of administration.
Outcome	The resolution of diarrhea associated to CDI without relapse for, at least, 60 days after the end of treatments. Adverse events.
Study design	Published or unpublished randomized controlled trials of any size and duration.

Table 2. Study characteristics and clinical data reporting resolution outcomes of monoclonal antibodies and one FMT infusion from the included studies

Author, year of publication	Study design	Standard Antibiotic Therapy		Actoxumab + Bezlotoxumab		Fecal Microbiota Transplantation		Bezlotoxumab		Actoxumab	
		CDI resolved	Total patients	CDI resolved	Total patients	CDI resolved	Total patients	CDI resolved	Total patients	CDI resolved	Total patients
Nood et al 2013	Open-label RCT	7	26			13	16				
Cammarota et al 2015	Open-label RCT	5	19			13	20				
Hota et al 2017	Open-label RCT	7	14			7	16				

NG et al 2017	Open-label RCT	10	15			11	15				
Leav et al 2010	Phase II double-blinded RCT	14	17							24	29
Lowy et al 2010	Phase III double-blinded RCT	74	99	94	101						
Wilcox et al 2017 (Modify 1)	Phase III double-	286	395	322	383			319	386	172	232

	blinded RCT										
Wilcox et al 2017 (Modify 2)	Phase III double-blinded RCT	281	378	332	390			333	395		

Table 3. Network meta-analysis of the relative efficacy of mABs and one infusion of FMT for CDI resolution

Fecal microbiota transplantation*	1.29 (0.34 – 3.93)	1.53 (0.39 – 5.16)	2.61 (0.64 – 9.74)	**2.98 (1.13 – 7.53)**
1.44 (0.68 – 3.12)	Actoxumab-Bezlotoxumab	1.17 (0.50 – 3.12)	2.01 (0.74 – 6.42)	**2.28 (1.15 – 5.52)**
1.63 (0.77 – 3.56)	1.13 (0.87 – 1.48)	Bezlotoxumab	1.71 (0.57 – 5.49)	1.93 (0.84 – 4.91)
2.74 (1.24 – 6.19)	**1.91 (1.33 – 2.73)**	**1.68 (1.17 – 2.40)**	Actoxumab	1.14 (0.42 – 3.10)
3.07 (1.51 – 6.44)	**2.14 (1.69 – 2.73)**	**1.89 (1.48 – 2.41)**	1.12 (0.80 – 1.58)	Standard Antibiotic Therapy

Treatment Fixed effect model, OR (95% CrI) Random effect model, OR (95% CrI)

*Data are OR (95% CrI) of the row treatment relative to the column treatment (E.g. the effect of 1 infusion of fecal microbiota transplantation relative to Actoxumab-Bezlotoxumab is 1.44 with respect to resolution of recurrent *Clostridium difficile* infection (RCDI) in the fixed effect

model). Bold values indicate comparisons that are statistically significant. ORs above 1 indicate higher efficacy in resolution of RCDI. OR=odds ratio. CrI= credible interval

Table 4. Network meta-analysis of the relative efficacy of mABs and ≥1 FMT infusions for CDI resolution

Fecal microbiota transplantation*	2.10 (0.28 – 15.99)	2.58 (0.30 – 23.53)	4.55 (0.49 – 45.11)	**5.22 (1.26 – 33.25)**
2.52 (1.14 – 5.79)	Actoxumab-Bezlotoxumab	1.22 (0.24 – 6.66)	2.14 (0.33 – 15.50)	2.46 (0.62 – 10.68)
2.86 (1.29 – 6.57)	1.14 (0.87 – 1.49)	Bezlotoxumab	1.75 (0.24 – 13.43)	2.01 (0.40 – 10.51)
4.81 (2.09 – 11.47)	**1.91 (1.33 – 2.73)**	**1.68 (1.17 – 2.40)**	Actoxumab	1.15 (0.20 – 6.39)
5.39 (2.54 – 11.96)	**2.14 (1.68 – 2.73)**	**1.88 (1.48 – 2.41)**	1.12 (0.80 – 1.58)	Standard Antibiotic Therapy

Treatment Fixed effect model, OR (95% CrI) Random effect model, OR (95% CrI)

*Data are OR (95% CrI) of the row treatment relative to the column treatment (E.g. the effect of ≥1 infusions of fecal microbiota transplantation relative to Actoxumab-Bezlotoxumab is 2.52 with respect to resolution of recurrent *Clostridium difficile* infection (RCDI) in the fixed effect model). Bold values indicate comparisons that are statistically significant. ORs above 1 indicate higher efficacy in resolution of RCDI. OR=odds ratio. CrI= credible interval

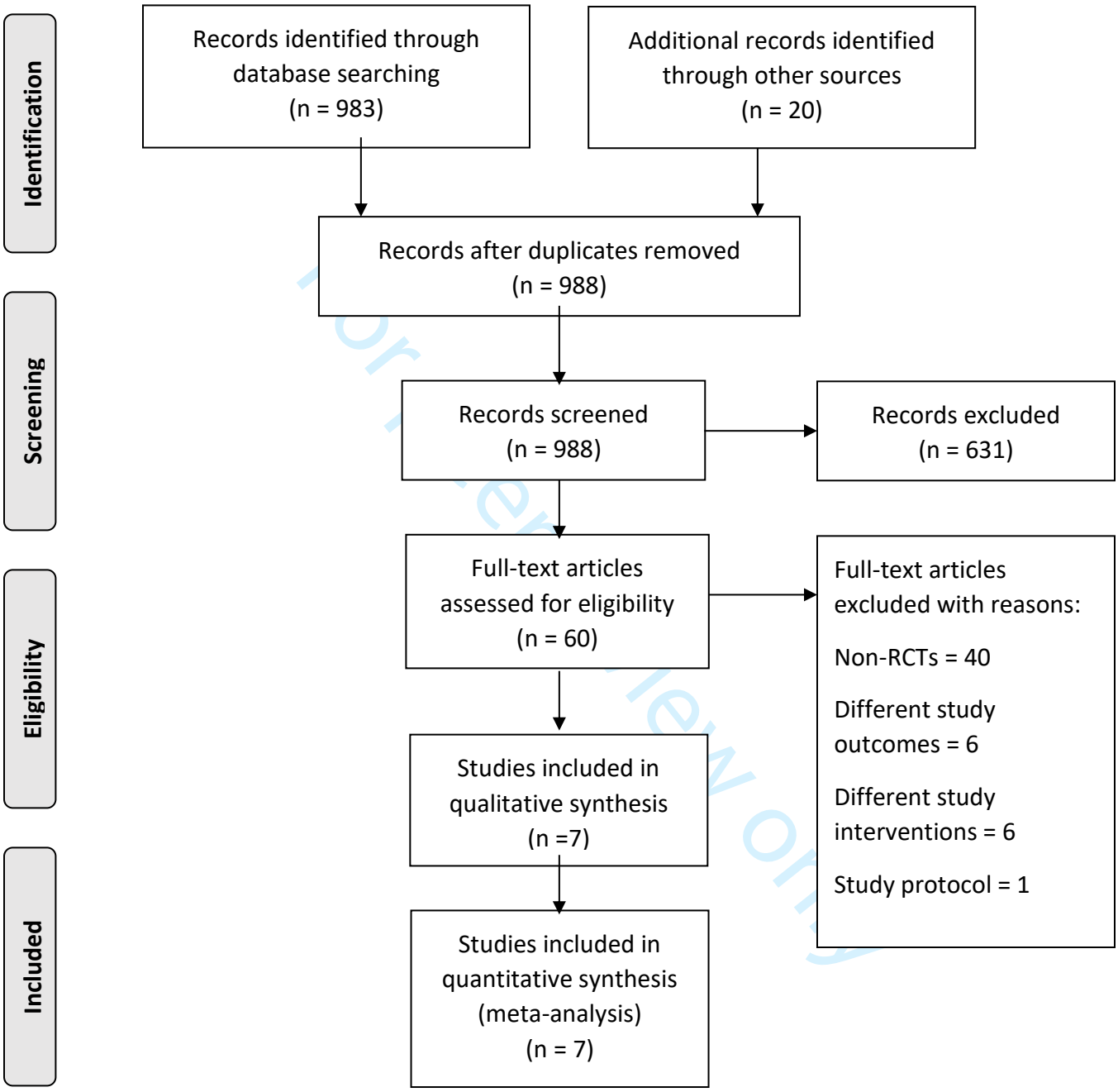


Figure. 1. Study selection process using preferred reporting items for systematic reviews and meta-analyses (PRISMA).

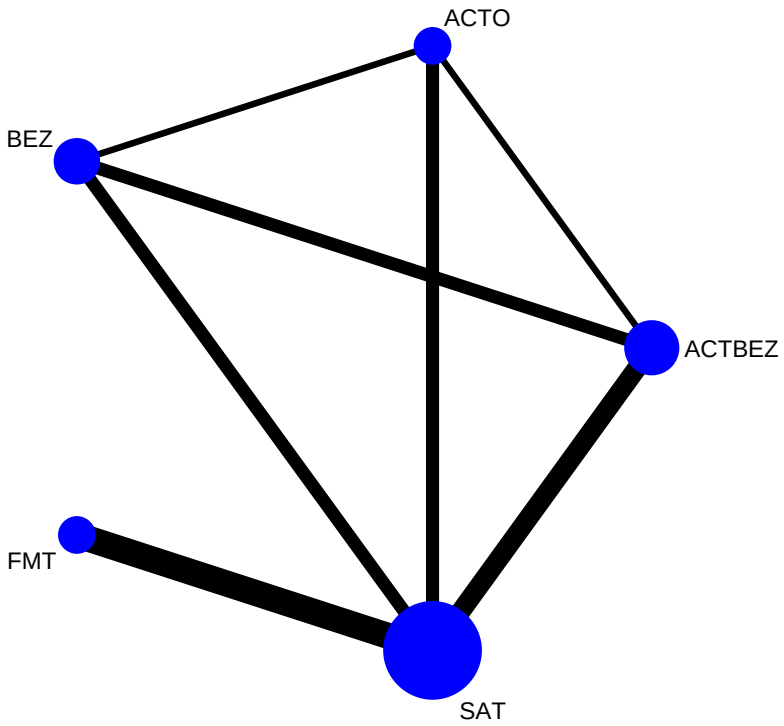


Figure 2. Network plot of included studies. Each circled node represents an intervention, the extent of the circle indicates the number of the included participants, the lines and their thickness represent direct comparisons and the number of studies included in each comparison, respectively. Standard Antibiotic Therapy (SAT), Actoxumab plus Bezlotoxumab (ACTBEZ), Fecal Microbiota Transplantation (FMT), Bezlotoxumab (BEZ), Actoxumab (ACTO).

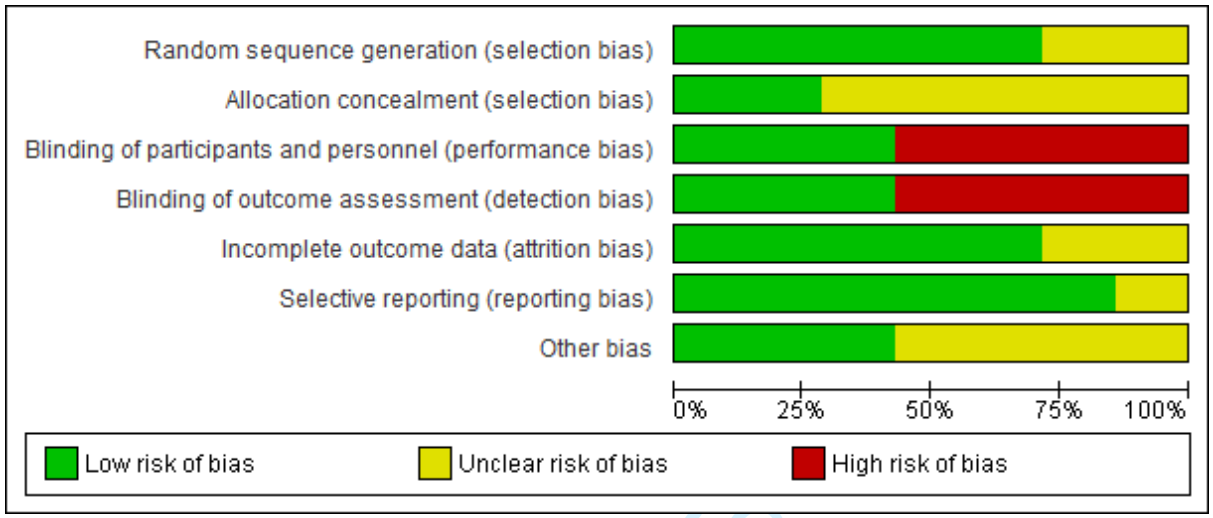


Figure 3 A. Risk of bias graph: review authors' judgments about each risk of bias item presented as percentages across all included studies.

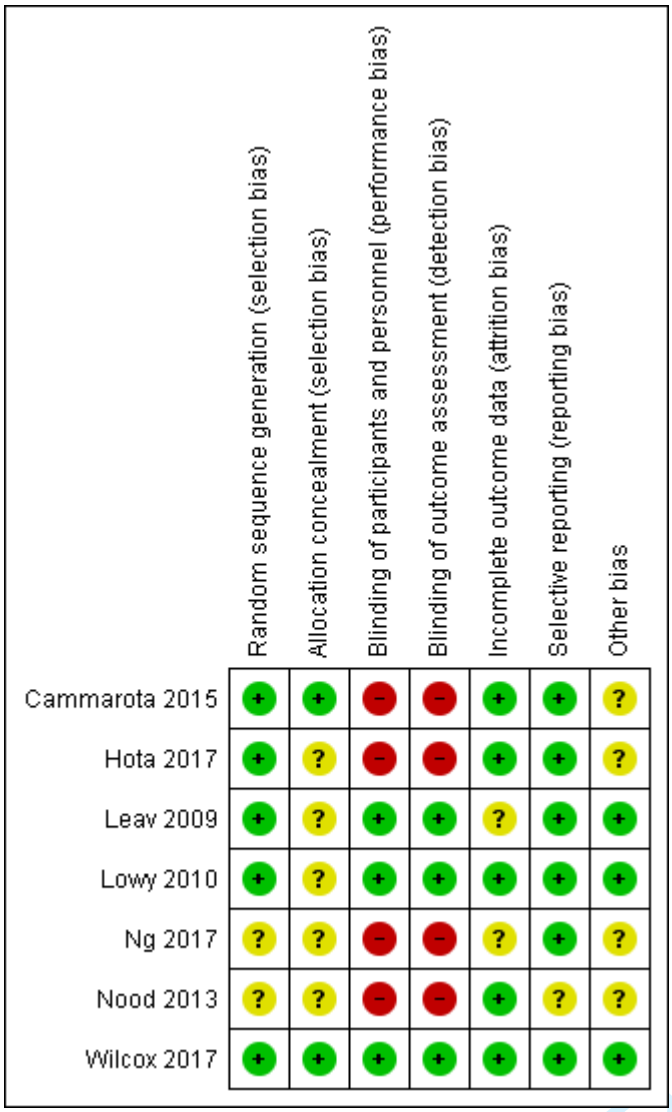
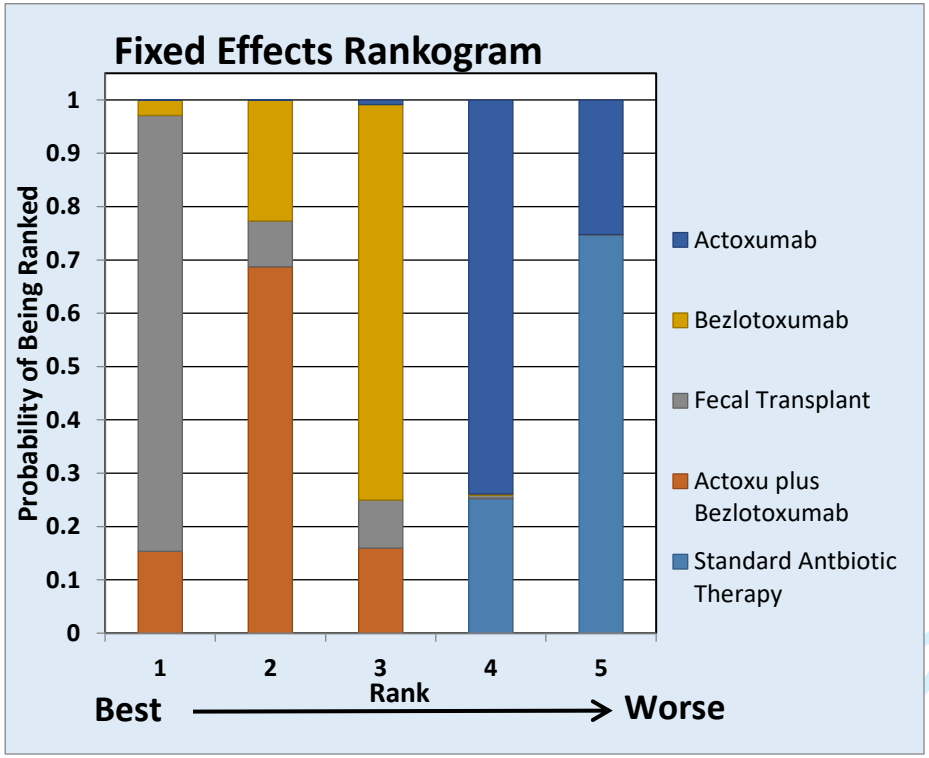
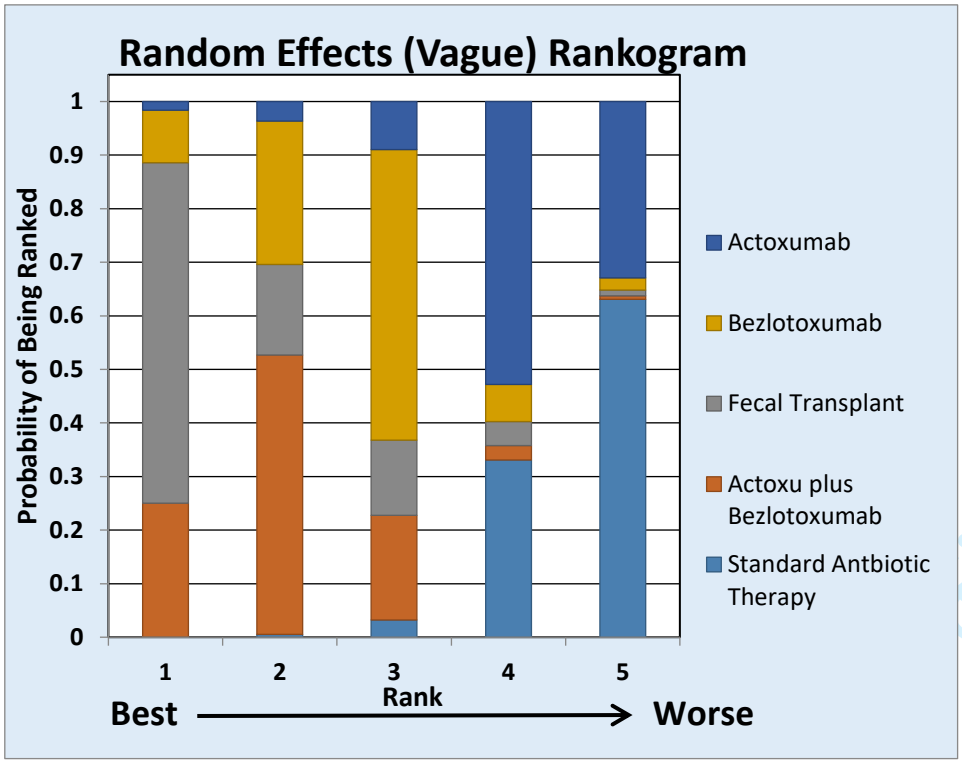


Figure 3 B. Risk of bias summary: review authors' judgments about each risk of bias item for each included study.



Rank	Treatment	SUCRA
1	Fecal Microbiota Transplantation	0.8175
2	Actoxumab plus Bezlotoxumab	0.6869
3	Bezlotoxumab	0.742
4	Actoxumab	0.7388
5	Standard Antibiotics Therapy	0.7468

Figure 4 A. Rankograms shows the ranking of interventions from the best to the worst based on the corresponding surface under the cumulative ranking curve (SUCRA) probability in the fixed effect models.



Rank	Treatment	SUCRA
1	Fecal Microbiota Transplantation	0.6357
2	Actoxumab plus Bezlotoxumab	0.5215
3	Bezlotoxumab	0.5421
4	Actoxumab	0.528
5	Standard Antibiotics Therapy	0.631

Figure 4 B. Rankograms shows the ranking of interventions from the best to the worst based on the corresponding surface under the cumulative ranking curve (SUCRA) probability in the random effect models.

PRISMA 2009 Checklist

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.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
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.	
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.	5-6
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.	5-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5-6
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).	5-6
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.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5-6
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.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
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.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
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).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
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.	7
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.	7-8
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).	8
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.	8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8-9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10
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).	11-12
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).	12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	13
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.	13

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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BMJ Open

Comparing the efficacy and safety of fecal microbiota transplantation with bezlotoxumab in reducing the risk of recurrent Clostridium difficile infections: a systematic review and Bayesian network meta-analysis of randomised controlled trials

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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Infectious diseases
Keywords:	Adult gastroenterology < GASTROENTEROLOGY, Clinical trials < THERAPEUTICS, Adverse events < THERAPEUTICS

Comparing the efficacy and safety of fecal microbiota transplantation with bezlotoxumab in reducing the risk of recurrent *Clostridium difficile* infections: a systematic review and Bayesian network meta-analysis of randomised controlled trials

Abdullah A Alhifany, PharmD
Department of Clinical Pharmacy, College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia
Email: aahifany@uqu.edu.sa

Abdulaali R Almutairi, PharmD
Department of Pharmacy Practice and Science, University of Arizona College of Pharmacy, Tucson, AZ, USA
Email: almutairi@pharmacy.arizona.edu

Thamer A Almangour, BScPhm, PharmD
Department of Clinical Pharmacy, King Saud University College of Pharmacy, Riyadh, KSA
Email: talmangour@ksu.edu.sa

Alaa N Shahbar, PharmD
Department of Clinical Pharmacy, College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia
Email: anshahbar@uqu.edu.sa

Ivo Abraham, PhD
Department of Pharmacy Practice and Science, University of Arizona College of Pharmacy, Tucson, AZ, USA
Email: abraham@pharmacy.arizona.edu

Mohammed Alessa, BScPhm, PharmD
Department of Pharmacy Practice, College of Pharmacy, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia
Email: essam1@ngha.med.sa

Faris S. Alnezary, PharmD
Department of Pharmacy Practice and Translational Research, University of Houston College of Pharmacy, Houston TX, USA
Email: alnezaryf@gmail.com

Ejaz Cheema, PhD (Corresponding author)
Institute of Clinical Sciences, University of Birmingham, Birmingham, UK
Email: e.cheema@bham.ac.uk

ABSTRACT

Objectives: The risk of recurrent *Clostridium difficile* infections (RCDI) is high when treated with standard antibiotics therapy (SAT) alone. It is suggested that the addition of fecal microbiota transplantation (FMT) or bezlotoxumab after SAT reduces the risk of RCDI. In the absence of head-to-head clinical trials, this review attempts to compare the efficacy and safety of bezlotoxumab with FMT in reducing the risk of RCDI in hospitalized patients.

Design: A systematic review and Bayesian network meta-analysis. A comprehensive search from inception until 30th February 2019 was conducted in four databases (Medline/Pubmed, Embase, Scopus, Clinicaltrials.gov). The Cochrane Risk of Bias tool was used to assess the quality of included RCTs.

Setting: Hospitals.

Inclusion criteria: Randomised controlled trials reporting the resolution of diarrhea associated with RCDI without relapse for at least 60 days after the end of treatments as the primary outcome.

Results: Out of 1003 articles identified, seven RCTS involving 3,043 patients contributed to the review. No difference was reported between the single infusion of FMT and bezlotoxumab in resolving RCDI. However, FMT with two or more infusions showed better resolution than bezlotoxumab in the fixed-effects [Odds Ratio (OR) 2.86, 95% Credible Interval (CrI) 1.29-6.57] but not in the random-effects model [OR 2.58, 95% CrI 0.30-23.53]. Patients treated with SAT alone or bezlotoxumab with SAT showed significantly lower rates of diarrhea than FMT [OR 0, 95% CrI 0-0.09] and [OR 0, 95% CrI 0-0.19], respectively. There was no difference in terms of other adverse events.

Discussion: This is the first network meta-analysis that has compared the recently FDA-approved monoclonal antibody bezlotoxumab with FMT for resolving RCDI. The quality of the included RCTs was variable. The findings of this study suggested that multiple infusions of FMT showed better efficacy than the single infusion of bezlotoxumab. However, FMT was associated with a higher rate of non-serious diarrhea as opposed to SAT used alone or in combination with bezlotoxumab.

Keywords: Recurrent Clostridium difficile Infections, Fecal Microbiota Transplantation, Bezlotoxumab, Standard Antibiotics Therapy, Network meta-analysis

Strengths and limitations

- Safety outcomes were limited due to the early termination of most of the included RCTs and the inconsistent reporting of the adverse events
- The quality of the included RCTs varied with more than half of the studies not reporting blinding of the participants
- The study employed a comprehensive literature search of four databases
- It used Bayesian estimation methods in the indirect comparisons of mABs and FMT to address the absence of head-to-head clinical trial evidence

BACKGROUND

Clostridium difficile is considered to be the most common source of infectious diarrhea in hospitalized patients.[1] *C. difficile*-led infections (CDI) are associated with high mortality particularly in the developed countries including USA, Canada and Europe.[2-4] Around 30% of the *C. difficile* infected patients treated with standard antibiotics therapy (SAT) such as vancomycin, metronidazole or fidaxomicin are reported to develop recurrent *C. difficile* infections (RCDI) that increases up to 60% with subsequent recurrences.[5] This cyclic pattern of recurring CDI-inducing diarrhea is triggered by the use of antibiotics and exotoxins produced by *C. difficile* that contributes to the weakening of the intrinsic fecal microbiota which serves as a natural host defense mechanism against *C. difficile* spores-led colonization.[5-7]The spore-forming ability of *C. difficile* is the main reason behind its nosocomial and community transmission.

Fecal microbiota transplantation (FMT) has been considered a novel intervention to replenish the intrinsic fecal microbiota barrier mechanism that protects against *C. difficile* associated colonization.[8] Evidence from the meta-analyses of randomised controlled trials (RCTs) as well as observational studies have highlighted the benefits of FMT in resolving CDI over SAT alone.[9-12] Furthermore, the current clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) recommend the use of FMT for the second or subsequent recurrences of CDI.[13] However, the lack of a standardized product, dosage form and method of administration are some of the limitations of FMT.[14]

An alternative approach to FMT is to attenuate the effects of the exotoxins produced by *C. difficile*. Bezlotoxumab, a novel monoclonal antibody (mAB) that has been approved recently by the Food and Drug Administration (FDA) in the USA has been reported to reduce RCDI by attenuating the effect of exotoxin B when used in conjunction with SAT.[15-16] However, there are no head-to-head clinical trials that have compared the efficacy and safety of FMT with bezlotoxumab in reducing the risk of RCDI. In the absence of any head-to-head trials, this systematic review and Bayesian network meta-analysis of RCTs aims to compare the efficacy and safety of bezlotoxumab with FMT in reducing the risk of RCDI. The review would attempt to determine if FMT when compared to bezlotoxumab has better efficacy and safety in resolving the diarrhea associated with CDI in hospitalized patients without relapse or not.

METHOD

The systematic review and network meta-analysis were conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension statement for network meta-analyses.[17]

Patient and public involvement

Patients and public were not involved in the design, conducting and reporting of research.

Search strategy

A comprehensive search from inception until 30th February 2019 was conducted in four databases (Medline/Pubmed, Embase, Scopus, Clinicaltrials.gov). Searches were conducted using Patients, Intervention, Comparator, Outcome, and Study design (PICOS) strategy for

clinical evidence of FMT and mAB in CDI (Table 1) (see supplementary file for the complete search strategy). Furthermore, manual searches were conducted to identify any additional studies by checking the reference lists of articles retrieved.

INSERT TABLE 1 HERE

Outcome Measure

The primary outcome of interest was the resolution of diarrhea associated to CDI without relapse for at least 60 days after the end of treatments. Furthermore, the adverse events of interest included diarrhea, abdominal pain, leukocytosis, fatigue, nausea, pyrexia, atrial fibrillation, dehydration, sepsis, tachycardia and infusion specific reactions.

Inclusion and Exclusion Criteria

Both published as well as unpublished RCTs that assessed the efficacy and safety of FMT and bezlotoxumab in resolving CDI after a short course of SAT such as vancomycin, metronidazole or fidaxomicin were eligible for inclusion. Studies were eligible for inclusion if they had included patients 18 years or older diagnosed with RCDI and reporting the resolution rate of CDI as the efficacy outcome.

Data Extraction, risk of bias and quality assessment

Two reviewers (EC and AS) independently reviewed the titles and abstracts. Studies meeting the inclusion criteria were retrieved as full-text to further assess their eligibility for inclusion. Reviewer AH independently extracted data from included studies using a data extraction sheet (see table 2 for characteristics of included studies). Reviewer AS checked all data extracted in

the sheets. The data extracted included; author, year of publication, study design and clinical data reporting resolution outcomes of monoclonal antibodies and FMT infusion. The Cochrane Risk of Bias tool was used to assess the quality of included RCTs including randomisation, allocation concealment, blinding of participants, reporting of incomplete outcome data, selective reporting and any other bias.[18] Other sources of bias explored included cross-contamination between study groups, recruitment of participants from a selected population and non-compliance with the study protocol. For each included study, a risk of bias graphs and risk of bias summary were generated.

Statistical Analysis

A Bayesian network meta-analysis was conducted using WinBUGS 1.4.3 (MRC Biostatistics Unit, Cambridge, UK). Binomial data that represented the resolution of diarrhea or adverse events was extracted and analyzed. The binary outcomes were expressed as Odds Ratio (OR) and 95% Credible Interval (95% Crl) for resolution rate of RCDI and OR with 95% Crl for adverse events. OR for treatment comparisons were estimated based on 20,000 iterations following the discarding of the first 10,000 iterations in the model. Both fixed and random-effects models were used for resolution rate of RCDI and fixed-effect for adverse events. This was done to ensure the robustness of the results. Ranking probabilities of treatments were calculated using the surface under the cumulative ranking curve (SUCRA) method. Additionally, a sensitivity analysis was conducted to exclude the studies and/or patients who received non-FDA approved mAB. Furthermore, a pairwise meta-analysis was conducted to check for heterogeneity.

RESULTS

The initial search identified 1003 studies (see figure 1). 15 duplicates along with an additional 631 studies that did not meet the inclusion criteria were removed. The abstracts of remaining 357 studies were reviewed, of which a further 297 were excluded. Full texts of the remaining 60 studies were reviewed. Of these, further 53 studies were excluded based on the inclusion and exclusion criteria. The remaining 7 RCTs with 3,043 patients contributed to the review and network meta-analysis.

INSERT FIGURE 1 HERE

Characteristics of included studies

The 7 included studies (see table 2 for characteristics of included studies) were published between 2010 and 2017 and involved 3,043 patients.[9 12 15-16 19-21] Four open-labeled RCTs involving 139 patients reported comparisons of FMT versus vancomycin alone in patients with an initial episode of CDI or with recurrent CDI and followed for at least 70 days following the end of the treatments.[9 12 19 21] Patients assigned to the FMT arm received an initial course of vancomycin ranging from 3-14 days to assure that patients were covered with an antibiotic therapy at the time of donor screening.

INSERT TABL2 HERE

All studies involving FMT used fresh feces from related donors. The time for infusing the fresh FMT from the time of defecation varied across studies from 3.1 to 48 hours. Three FMT studies were terminated early following an interim analysis; two because of the observed superiority of the FMT[9 12] and one because of inferiority.[19] A fourth study was underpowered and was considered a pilot study.[21] In two studies,[9 12] FMT was reinfused in some patients who

experienced a recurrence after the first infusion. In the remaining two studies, FMT was used as a single infusion.[19 21]

Three double-blinded, placebo-controlled RCTs including two multi-center phase two studies and one multi-national, multi-center phase three study investigated the efficacy of mABs.[15-16 20] The two phase two studies investigated the safety and efficacy of newly developed mABs against *C. difficile* exotoxins A and B that corroborated prior evidence of the role of these exotoxins in the virulence of *C. difficile*.[6] The third RCT confirmed that antagonizing toxin B is the main determinant in suppressing the virulence of *C. difficile*, however it could not rule out the role of toxin A.[7] Three regimens of mABs were tested in these RCTs: anti-toxin A (actoxumab), anti-toxin B (bezlotoxumab) and a combination of both, all of which were infused as a single dose of 10mg/kg either during or right away after a course of SAT. It is important to highlight that only bezlotoxumab was approved by the FDA for this indication (see figure 2 for network plot of included studies).

INSERT FIGURE 2 HERE

Study quality

The quality of the studies was variable (see figures 3A and 3B). Only three of the seven studies used blinding of participants.[15-16 20]

INSERT FIGURE 3A AND 3B HERE

Comparative efficacy of FMT and mABs in reducing RCDI

The initial analysis comparing the resolution of CDI after receiving one FMT infusion or any mAB regimen found no difference between FMT and bezlotoxumab in the fixed-effects (OR=1.63, 95% CrI=0.77-3.56) and random-effects model (OR=1.53, 95% CrI=0.39-5.16). Yet, FMT showed the best SUCRA probability in the fixed-effects (81.8%) and random-effects models (63.6%) (see figures 4A and 4B). In addition, FMT showed better resolution of CDI than SAT in the fixed-effects (OR=3.07, 95% CrI=1.51-6.44) and random-effects models (OR=2.98, 95% CrI=1.13-7.53). Bezlotoxumab showed better resolution of CDI than SAT in the fixed-effects model (OR=1.89, 95% CrI=1.48-2.41) but not in the random-effects model (OR=1.93, 95% CrI=0.84-4.91) (see table 3).

INSERT FIGURES 4A AND 4B HERE

INSERT TABLE 3 HERE

A secondary comparative analysis that included the resolution outcomes reported for patients who received one or more FMT infusions or any mAB regimen was conducted. FMT showed better resolution of CDI than bezlotoxumab in the fixed-effects (OR=2.86, 95% CrI=1.29-6.57) but not in the random-effects model (OR=2.58, 95% CrI=0.30-23.53). Additionally, FMT showed better resolution of CDI than SAT in the fixed-effects (OR 5.39, 95% CrI 2.54-11.96) and the random-effects models (OR 5.22, 95% CrI 1.26-23.25). Bezlotoxumab showed better resolution of CDI than SAT in the fixed-effects (OR=1.88, 95%CrI=1.48-2.41) but not in the random-effects models (OR=2.01, 95%CrI=0.40-10.51) (see Table 4 here).

INSERT TABLE 4 HERE

The analyses of the safety data for FMT, bezlotoxumab, and SAT revealed a significantly lower rate of non-serious diarrhea in patients receiving bezlotoxumab (OR=0, 95%CrI=0-0.19) and SAT (OR=0, 95% CrI=0-0.09), compared to patients treated with FMT. There were no differences on other adverse events.

Sensitivity analysis

A sensitivity analysis was conducted by excluding the resolution outcomes of patients who received non-FDA approved mABs. There was no difference between one FMT infusion and bezlotoxumab in the fixed-effects (OR=1.67, 95% CrI=0.79-3.64) and random-effects model (OR=1.61, 95%CrI=0.19-12.69). However, one or more FMT infusions showed better resolution of CDI than bezlotoxumab in the fixed-effects (OR=2.93, 95%CrI=1.32-6.78) but not in the random-effects model (OR=2.90, 95%CrI=0.20-45.35). FMT showed the best SUCRA probability in the fixed-effects (99.6%) and the random-effects models (79.7%). The pair-wise meta-analysis suggested that heterogeneity I2 for SAT vs FMT and for SAT vs MonoAbs was high which indicated high variability between the studies.

DISCUSSION

To the best of authors' knowledge, this is the first network meta-analysis that has compared the recently FDA-approved monoclonal antibody bezlotoxumab with FMT for resolving RCDI. The findings of this study suggested that multiple infusions of FMT have better efficacy than a single infusion of bezlotoxumab. However, FMT was associated with a higher rate of non-serious diarrhea.

Despite the inconsistency in the results of the four RCTs that studied FMT versus SAT in resolving RCDI,[9 12 19 21] the network analysis showed the superiority of FMT over SAT alone. This is consistent with the findings of previous meta-analyses of RCTs and observational studies.[10-11] The inconsistency in the results of the four included studies may be attributed to their small sample sizes, lack of blinding and variability between them on the basis of the process of collecting donor feces, preparation of FMT, lag time between feces collection and infusion, method of administration and vancomycin regimen. Furthermore, as evident from the findings of a previous RCT, patients who received FMT monotherapy for an initial episode of CDI without receiving prior antibiotics, retained more bacteroidetes in their gut than patients treated with antibiotics.[22] These findings confirm the effect of antibiotics in attenuating the intrinsic microbiota.[5] It may also explain the inferiority of FMT over SAT in the study when FMT was preceded by fourteen days of antibiotics.[19] On the contrary, administration of FMT earlier (after the second recurrence of CDI) as opposed to a late administration (after the third or subsequent recurrences) led to shorter length of hospital stay and fewer visits to the emergency department.[23] Thus, the differences in the results of the individual studies included in the current network meta-analysis could have been due to the variability in starting FMT for initial CDI or RCDI and the inconsistency in the number of previous recurrences among included patients.

Bezlotoxumab showed a favorable efficacy and safety profile in preventing RCDI in two robust prospective, double-blinded, placebo-controlled RCTs.[16 24] Furthermore, the effect was sustained throughout the three month follow-up.[16] Bezlotoxumab has a novel mechanism of action that reduces the possibility of RCDI, yet its high cost may limit its utilization.[16 24] Furthermore, even

though the network meta-analysis did not report any difference in the resolution rate between bezlotoxumab and FMT after one infusion, the SUCRA probability score favored FMT in the rankogram. Multiple infusions of FMT also showed better resolution rates than a single infusion of bezlotoxumab in the fixed-effects analysis.

This review had some limitaions. The quality of the included RCTs was variable with more than half of the studies not reporting blinding of the participants. Furthermore, the RCTs studying FMT differed in design, donor selection, FMT preparation, follow-up time, lag time between feces collection and infusion and lag time between antibiotics discontinuation and FMT infusion; while mABs were infused either during or right away after the discontinuation of antibiotics. None of the included RCTs reported the number of previous recurrences. Furthermore, safety outcomes were limited due to the early termination of most of the included RCTs and the inconsistent reporting of the adverse events. Nevertheless, the review employed a rigorous and comprehensive search strategy to identify the relevant studies. Furthermore, it used the Bayesian estimation methods in the indirect comparisons of mABs and FMT to address the absence of head-to-head clinical trial evidence. By doing so, this review addressed a significant issue identified in the 2017 IDSA[13] guidelines by filling the gap in information concerning the best method in preventing RCDI and the role of FMT and mABs as adjunctive therapies. However, further studies are required to assess the efficacy, safety, cost and clinical implications of multiple infusions of bezlotoxumab.

CONCLUSION

Multiple infusions of FMT showed better efficacy than single infusion of bezlotoxumab in resolving RCDI in fixed-effects analyses but with a higher rate of non-serious diarrhea. Further studies are needed to investigate the efficacy and safety of using FMT as monotherapy for CDI, the possible attenuating effect of short-course antibiotics given before FMT and the clinical implications of multiple infusions of bezlotoxumab.

Funding

None

Conflict of interests

None to declare.

Contributorship statement

Authors AAA designed the research question. Authors ARA, TAA, AAS, MA and FA contributed to the searches, extraction of data and analysis. Authors AAA, IA and EC contributed to the preparation of the manuscript.

Data availability statement

All data relevant to the study are included in the article or uploaded as supplementary information.

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Table 1. PICOS strategy for clinical evidence of FMT and mAB in CDI

PICOS	Clinical Review
Population	Adults with primary or recurrent CDI.
Intervention	Studies that reported the efficacy and safety of fecal microbiota transplantation and/or monoclonal antibodies at any dosage form and via any route of administration in resolving RCDI.
Comparator	Standard antibiotics therapy, such as vancomycin, metronidazole, or fidaxomicin, at any dosage form and via any route of administration.
Outcome	The resolution of diarrhea associated to CDI without relapse for, at least, 60 days after the end of treatments. Adverse events.
Study design	Published or unpublished randomised controlled trials of any size and duration.

Table 2. Study characteristics and clinical data reporting resolution outcomes of monoclonal antibodies and one FMT infusion from the included studies

Author, year of publication	Study design	Standard Antibiotic Therapy	Actoxumab + Bezlotoxumab	Fecal Microbiota Transplantation	Bezlotoxumab	Actoxumab
		Method of administration (CDI resolved/ Total patients)	CDI resolved/ Total patients	CDI resolved /Total patients	CDI resolved /Total patients	CDI resolved /Total patients
Nood et al 2013	Open-label RCT	Vanocomycin 500mg orally four times daily for 14 days (7/26)		Vancomycin 500mg orally four times daily for 4 days followed by FMT (13/16)		
Cammarota et al 2015	Open-label RCT	Vancomycin 125mg orally four times daily for 10 days followed by 125-500mg/day every 2-3 days for 3 weeks (5/19)		Vacomycin 125mg orallt four times daily for 3 days followed by FMT (13/20)		
Hota et al 2017	Open-label RCT	Vancomycin 125mg orally four times daily for 14days, then 125mg orally two times daily for 7 days, then 125mg orally daily for 7 days, then 125mg		Vancomycin 125mg orally four times daily for 14 days followed by FMT after 48 hours (7/16)		

		orally every second day for 7 days, then 125mg orally every third day for 7days. (7/12)				
NG et al 2017 (Abstract)	Open-label RCT	Vancomycin 500mg orally four times daily for 10 days (10/15)		Vancomycin 500mg orally four times daily followed by FMT (11/15)		
Leav et al 2010	Phase II double-blinded RCT	Standard treatment course of Vancomycin or Metronidazole (14/17)				Standard treatment course of Vancomycin or Metronidazole followed by a single dose of 10mg/kg IV of Atoxumab (24/29)
Lowy et al 2010	Phase III double-blinded RCT	Standard treatment course of Vancomycin or Metronidazole (74/99)	Standard treatment course of Vancomycin or Metronidazole followed by a single dose 10mg/kg IV of each Atoxumab and Bezlotoxumab (94/101)			
Wilcox et al 2017 (Modify 1)	Phase III double-blinded RCT	Standard treatment course of Vancomycin, Metronidazole or	Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin followed by a single dose 10mg/kg IV of		Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin followed	Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin

		Fidaxomicin for 10-14 days (286/395)	each Atoxumab and Bezlotoxumab (322/383)		by a single dose 10mg/kg IV of Bezlotoxumab (319/386)	followed by a single dose 10mg/kg IV of Atoxumab (172/232)
Wilcox et al 2017 (Modify 2)	Phase III double-blinded RCT	Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin for 10-14 days (281/378)	Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin followed by a single dose 10mg/kg IV of each Atoxumab and Bezlotoxumab (332/390)		Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin followed by a single dose 10mg/kg IV of Bezlotoxumab (333/395)	

Table 3. Network meta-analysis of the relative efficacy of mABs and one infusion of FMT for CDI resolution

Fecal microbiota transplantation*	1.29 (0.34 – 3.93)	1.53 (0.39 – 5.16)	2.61 (0.64 – 9.74)	**2.98 (1.13 – 7.53)**
1.44 (0.68 – 3.12)	Actoxumab-Bezlotoxumab	1.17 (0.50 – 3.12)	2.01 (0.74 – 6.42)	**2.28 (1.15 – 5.52)**
1.63 (0.77 – 3.56)	1.13 (0.87 – 1.48)	Bezlotoxumab	1.71 (0.57 – 5.49)	1.93 (0.84 – 4.91)
2.74 (1.24 – 6.19)	**1.91 (1.33 – 2.73)**	**1.68 (1.17 – 2.40)**	Actoxumab	1.14 (0.42 – 3.10)
3.07 (1.51 – 6.44)	**2.14 (1.69 – 2.73)**	**1.89 (1.48 – 2.41)**	1.12 (0.80 – 1.58)	Standard Antibiotic Therapy

Treatment Fixed effect model, OR (95% CrI) Random effect model, OR (95% CrI)

*Data are OR (95% CrI) of the row treatment relative to the column treatment (E.g. the effect of 1 infusion of fecal microbiota transplantation relative to Actoxumab-Bezlotoxumab is 1.44 with respect to resolution of recurrent *Clostridium difficile* infection (RCDI) in the fixed effect

model). Bold values indicate comparisons that are statistically significant. ORs above 1 indicate higher efficacy in resolution of RCDI. OR=odds ratio. CrI= credible interval

Table 4. Network meta-analysis of the relative efficacy of mABs and ≥1 FMT infusions for CDI resolution

Fecal microbiota transplantation*	2.10 (0.28 – 15.99)	2.58 (0.30 – 23.53)	4.55 (0.49 – 45.11)	**5.22 (1.26 – 33.25)**
2.52 (1.14 – 5.79)	Actoxumab-Bezlotoxumab	1.22 (0.24 – 6.66)	2.14 (0.33 – 15.50)	2.46 (0.62 – 10.68)
2.86 (1.29 – 6.57)	1.14 (0.87 – 1.49)	Bezlotoxumab	1.75 (0.24 – 13.43)	2.01 (0.40 – 10.51)
4.81 (2.09 – 11.47)	**1.91 (1.33 – 2.73)**	**1.68 (1.17 – 2.40)**	Actoxumab	1.15 (0.20 – 6.39)
5.39 (2.54 – 11.96)	**2.14 (1.68 – 2.73)**	**1.88 (1.48 – 2.41)**	1.12 (0.80 – 1.58)	Standard Antibiotic Therapy

Treatment Fixed effect model, OR (95% CrI) Random effect model, OR (95% CrI)

*Data are OR (95% CrI) of the row treatment relative to the column treatment (E.g. the effect of ≥1 infusions of fecal microbiota transplantation relative to Actoxumab-Bezlotoxumab is 2.52 with respect to resolution of recurrent *Clostridium difficile* infection (RCDI) in the fixed effect model). Bold values indicate comparisons that are statistically significant. ORs above 1 indicate higher efficacy in resolution of RCDI. OR=odds ratio. CrI= credible interval

Figure. 1. Search strategy: Study selection process using preferred reporting items for systematic reviews and meta-analyses (PRISMA).

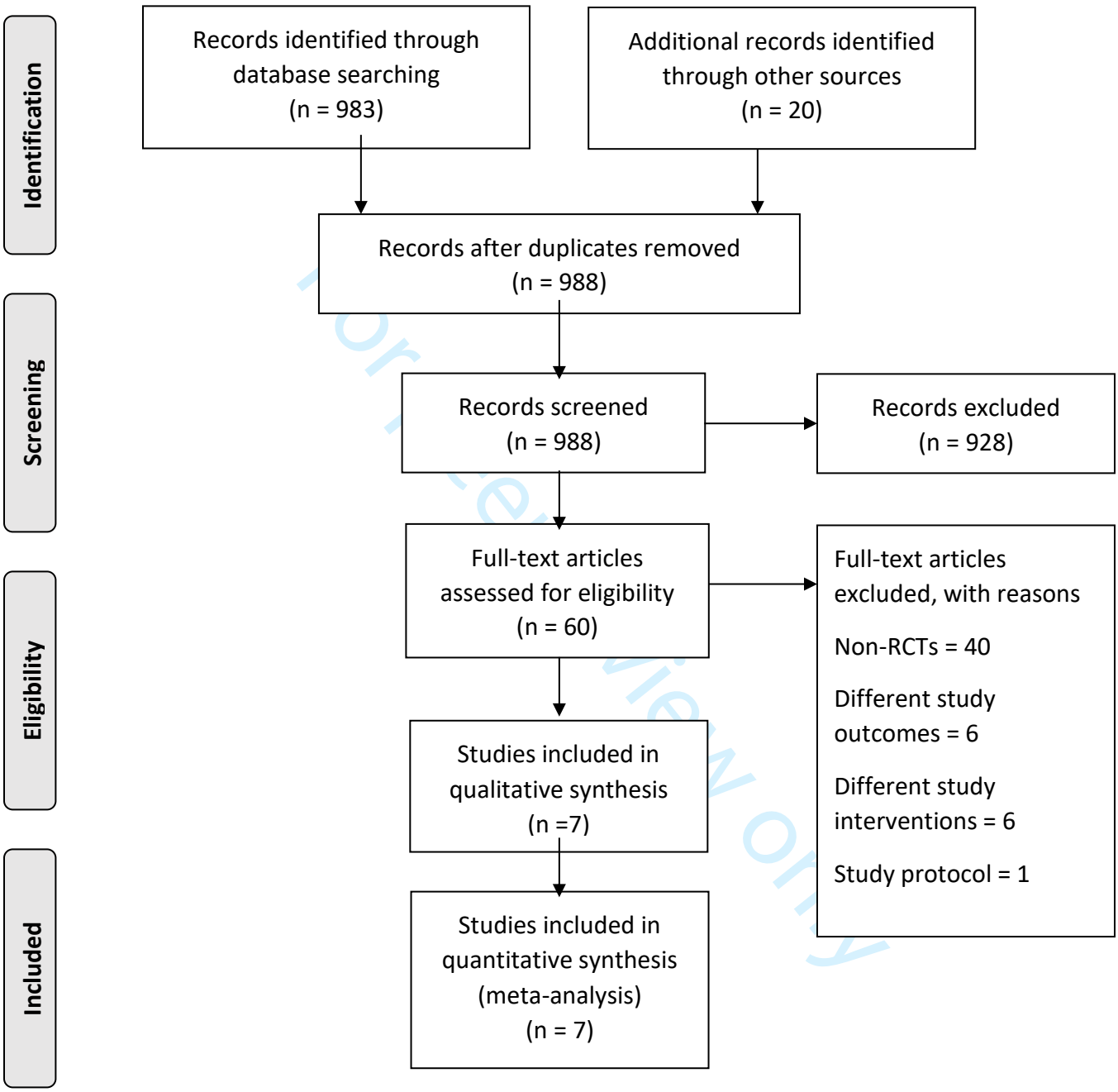
Figure 2. Network plot of included studies. Each circled node represents an intervention, the extent of the circle indicates the number of the included participants, the lines and their thickness represent direct comparisons and the number of studies included in each comparison, respectively. Standard Antibiotic Therapy (SAT), Actoxumab plus Bezlotoxumab (ACTBEZ), Fecal Microbiota Transplantation (FMT), Bezlotoxumab (BEZ), Actoxumab (ACTO).

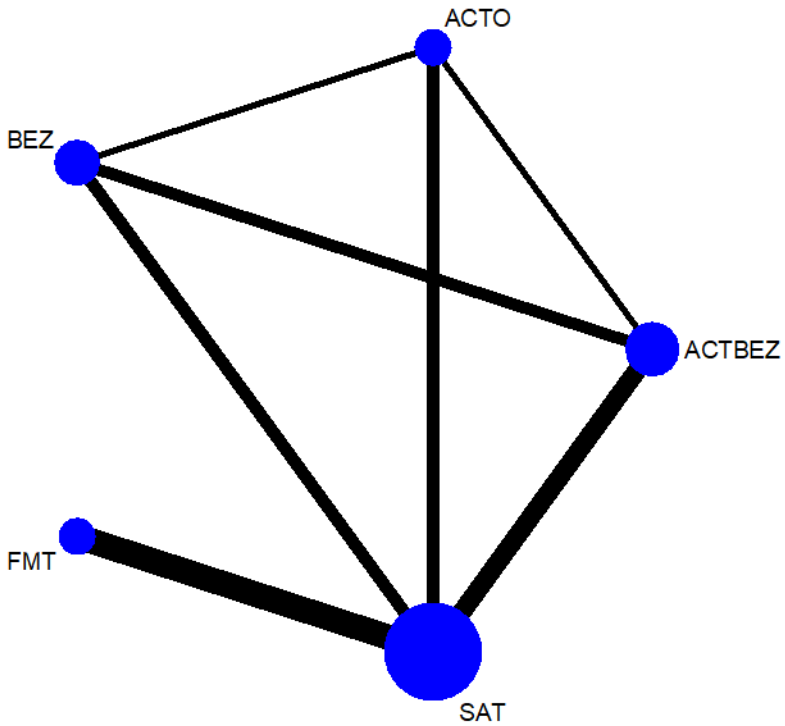
Figure 3A. Risk of bias graph: review authors' judgments about each risk of bias item presented as percentages across all included studies.

Figure 3B. Risk of bias summary: review authors' judgments about each risk of bias item for each included study.

Figure 4A. Rankograms shows the ranking of interventions from the best to the worst based on the corresponding surface under the cumulative ranking curve (SUCRA) probability in the fixed effect models.

Figure 4B. Rankograms shows the ranking of interventions from the best to the worst based on the corresponding surface under the cumulative ranking curve (SUCRA) probability in the random effect models.





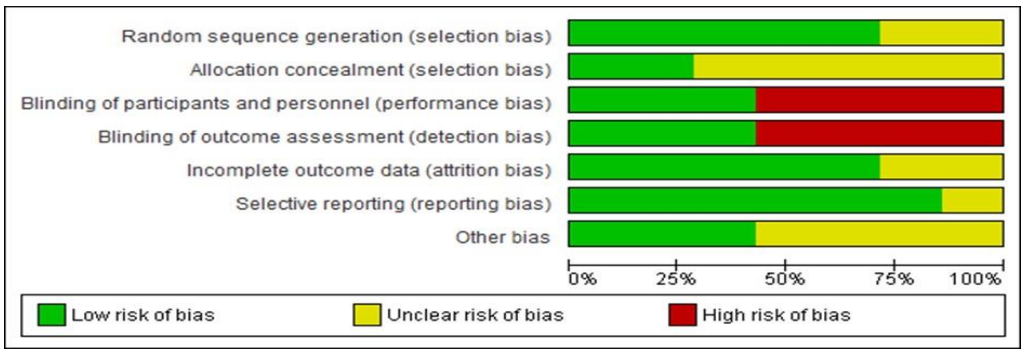


FIGURE 3A

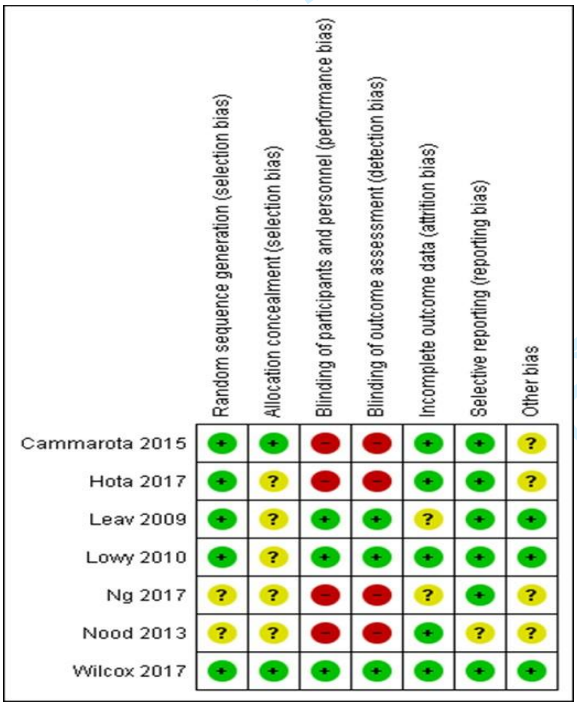
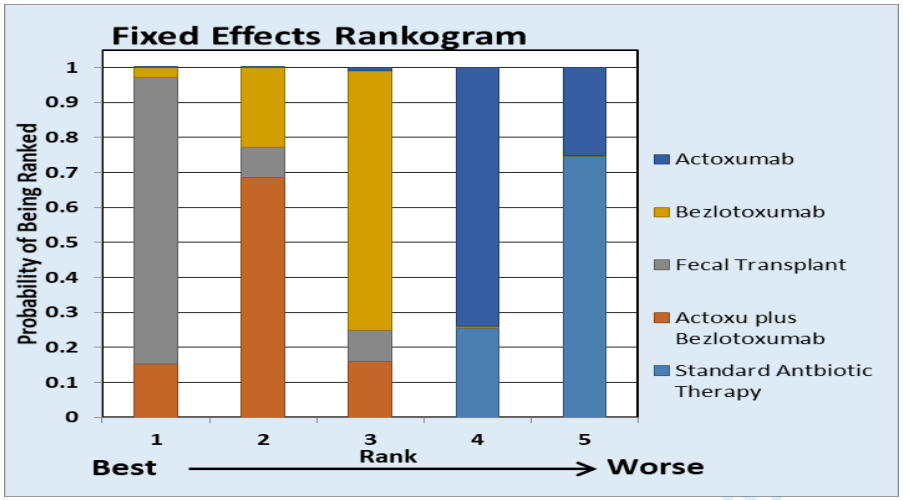


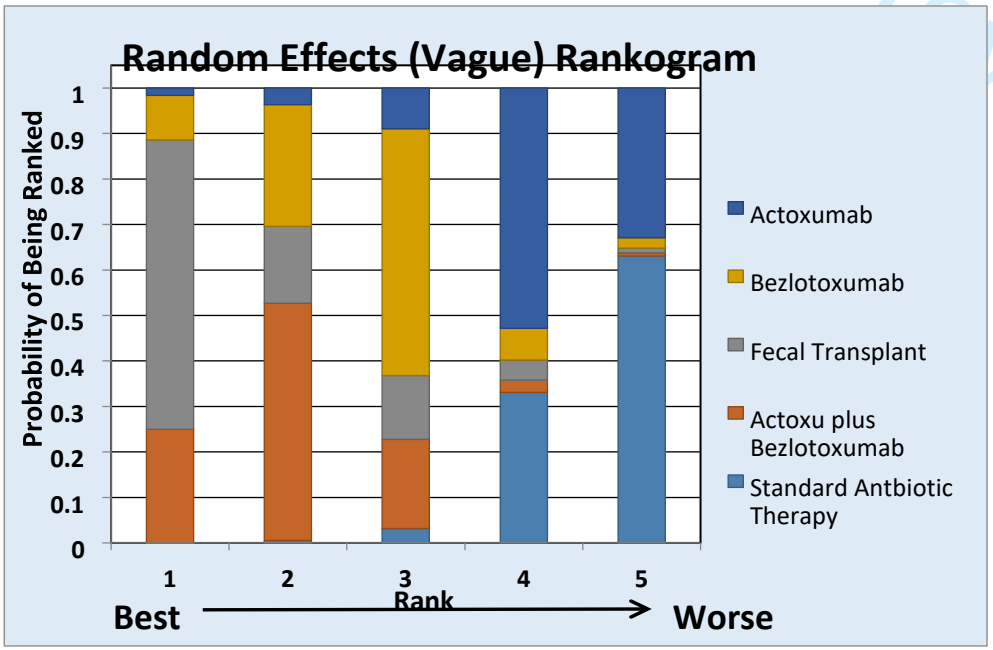
FIGURE 3B

Figure 4A



Rank	Treatment	SUCRA
1	Fecal Microbiota Transplantation	0.8175
2	Actoxumab plus Bezlotoxumab	0.6869
3	Bezlotoxumab	0.742
4	Actoxumab	0.7388
5	Standard Antibiotics Therapy	0.7468

Figure 4B



Rank	Treatment	SUCRA
1	Fecal Microbiota Transplantation	0.6357
2	Actoxumab plus Bezlotoxumab	0.5215
3	Bezlotoxumab	0.5421
4	Actoxumab	0.528
5	Standard Antibiotics Therapy	0.631

Example of search strategy

Medline-PubMed
((((((((((("Fecal Microbiota Transplantation"[Mesh]) OR Fecal Microbiota Transplantation)) OR ((monoclonal antibodies[MeSH Terms]) OR monoclonal antibodies)) OR ((vancomycin[MeSH Terms]) OR vancomycin)) OR ((metronidazole[MeSH Terms]) OR metronidazole)) OR ((fidaxomicin[MeSH Terms]) OR fidaxomicin)) OR ((resolution of diarrhea[MeSH Terms]) OR resolution of diarrhea)) AND ((clostridium difficile[MeSH Terms]) OR clostridium difficile)) OR ((clostridium difficile infection[MeSH Terms]) OR clostridium difficile infection)) AND ((randomized controlled trial[MeSH Terms]) OR randomized controlled trial))) = 619 citations
Embase
('fecal microbiota transplantation'/exp OR 'monoclonal antibody'/exp OR 'vancomycin'/exp OR 'metronidazole'/exp OR 'fidaxomicin'/exp) AND 'clostridium difficile infection'/exp AND [randomized controlled trial]/lim =170 citations

PRISMA 2009 Checklist

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.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
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.	
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.	5-6
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.	5-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5-6
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).	5-6
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.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5-6
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.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
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.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
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).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
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.	7
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.	7-8
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).	8
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.	8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8-9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10
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).	11-12
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).	12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	13
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.	13

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

BMJ Open

Comparing the efficacy and safety of fecal microbiota transplantation with bezlotoxumab in reducing the risk of recurrent Clostridium difficile infections: a systematic review and Bayesian network meta-analysis of randomised controlled trials

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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Infectious diseases
Keywords:	Adult gastroenterology < GASTROENTEROLOGY, Clinical trials < THERAPEUTICS, Adverse events < THERAPEUTICS

Comparing the efficacy and safety of fecal microbiota transplantation with bezlotoxumab in reducing the risk of recurrent *Clostridium difficile* infections: a systematic review and Bayesian network meta-analysis of randomized controlled trials

Abdullah A Alhifany, PharmD
Department of Clinical Pharmacy, College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia
Email: aahifany@uqu.edu.sa

Abdulaali R Almutairi, PharmD
Department of Pharmacy Practice and Science, University of Arizona College of Pharmacy, Tucson, AZ, USA
Email: almutairi@pharmacy.arizona.edu

Thamer A Almangour, BScPhm, PharmD, BCPS, BCIDP
Department of Clinical Pharmacy, College of Pharmacy, King Saud University Riyadh, Saudi Arabia, P.O.Box 2457, Riyadh 11451
Email: talmangour@ksu.edu.sa

Alaa N Shahbar, PharmD
Department of Clinical Pharmacy, College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia
Email: anshahbar@uqu.edu.sa

Ivo Abraham, PhD
Department of Pharmacy Practice and Science, University of Arizona College of Pharmacy, Tucson, AZ, USA
Email: abraham@pharmacy.arizona.edu

Mohammed Alessa, BScPhm, PharmD
Department of Pharmacy Practice, College of Pharmacy, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia
Email: essam1@ngha.med.sa

Faris S. Alnezary, PharmD
Department of Pharmacy Practice and Translational Research, University of Houston College of Pharmacy, Houston TX, USA
Email: alnezaryf@gmail.com

Ejaz Cheema, PhD (Corresponding author)
Institute of Clinical Sciences, University of Birmingham, Birmingham, UK
Email: e.cheema@bham.ac.uk

ABSTRACT

Objectives: The risk of recurrent *Clostridium difficile* infections (RCDI) is high when treated with standard antibiotics therapy (SAT) alone. It is suggested that the addition of fecal microbiota transplantation (FMT) or bezlotoxumab after SAT reduces the risk of RCDI. In the absence of head-to-head randomized clinical trials (RCTs), this review attempts to compare the efficacy and safety of bezlotoxumab with FMT in reducing the risk of RCDI in hospitalized patients.

Design: A systematic review and Bayesian network meta-analysis.

Data Source: A comprehensive search from inception until 30th February 2019 was conducted in four databases (Medline/Pubmed, Embase, Scopus, Clinicaltrials.gov).

Eligibility criteria: RCTs reporting the resolution of diarrhea associated with RCDI without relapse for at least 60 days after the end of treatments as the primary outcome.

Data extraction and synthesis: We extracted author, year of publication, study design and binomial data that represented the resolution of diarrhea or adverse events of monoclonal antibodies and FMT infusion. Random-effects models were used for resolution rate of RCDI and and adverse events. The Cochrane Risk of Bias tool was used to assess the quality of included RCTs.

Results: Out of 1003 articles identified, seven RCTs involving 3,043 patients contributed to the review. No difference was reported between single or multiple infusions of FMT and bezlotoxumab in resolving RCDI, [Odds Ratio (OR) 1.53, 95% Credible Interval (CrI) 0.39-5.16] and [OR 2.86, 95% CrI=1.29-6.57], respectively. Patients treated with SAT alone or

bezlotoxumab with SAT showed significantly lower rates of diarrhea than FMT [OR 0, 95% CrI 0-0.09] and [OR 0, 95% CrI 0-0.19], respectively. There was no difference in terms of other adverse events.

Conclusions: This is the first network meta-analysis that has compared the recently FDA-approved monoclonal antibody bezlotoxumab with FMT for resolving RCDI. The quality of the included RCTs was variable. The findings of this study suggested no difference between single or multiple infusions of FMT and bezlotoxumab. However, FMT was associated with a higher rate of non-serious diarrhea as opposed to SAT used alone or in combination with bezlotoxumab.

Keywords: Recurrent *Clostridium difficile* Infections, Fecal Microbiota Transplantation, Bezlotoxumab, Standard Antibiotics Therapy, Network meta-analysis

Strengths and limitations

- Safety outcomes were limited due to the early termination of most of the included RCTs and the inconsistent reporting of the adverse events
- The quality of the included RCTs varied with more than half of the studies not reporting blinding of the participants
- The study employed a comprehensive literature search of four databases
- It used Bayesian estimation methods in the indirect comparisons of mABs and FMT to address the absence of head-to-head clinical trial evidence

BACKGROUND

Clostridium difficile is considered to be the most common source of infectious diarrhea in hospitalized patients.[1] *C. difficile*-led infections (CDI) are associated with high mortality particularly in the developed countries including USA, Canada and Europe.[2-4] Around 30% of the *C. difficile* infected patients treated with standard antibiotics therapy (SAT) such as vancomycin, metronidazole or fidaxomicin are reported to develop recurrent *C. difficile* infections (RCDI) that increase up to 60% with subsequent recurrences.[5] This cyclic pattern of recurring CDI-inducing diarrhea is triggered by the use of antibiotics and exotoxins produced by *C. difficile* that contributes to the weakening of the intrinsic fecal microbiota which serves as a natural host defense mechanism against *C. difficile* spores-led colonization.[5-7]The spore-forming ability of *C. difficile* is the main reason behind its nosocomial and community transmission.

Fecal microbiota transplantation (FMT) has been considered a novel intervention to replenish the intrinsic fecal microbiota barrier mechanism that protects against *C. difficile* associated colonization.[8] Evidence from the meta-analyses of randomized controlled trials (RCTs) as well as observational studies have highlighted the benefits of FMT in resolving CDI over SAT alone.[9-12] Furthermore, the current clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) recommend the use of FMT for the second or subsequent recurrences of CDI.[13] However, the lack of a standardized product, dosage form and method of administration are some of the limitations of FMT.[14]

An alternative approach to FMT is to attenuate the effects of the exotoxins produced by *C. difficile*. Bezlotoxumab, a novel monoclonal antibody (mAB) that has been approved recently by the Food and Drug Administration (FDA) in the USA has been reported to reduce RCDI by attenuating the effect of exotoxin B when used in conjunction with SAT.[15-16] However, there are no head-to-head clinical trials that have compared the efficacy and safety of FMT with bezlotoxumab in reducing the risk of RCDI. In the absence of any head-to-head trials, this systematic review and Bayesian network meta-analysis of RCTs aims to compare the efficacy and safety of bezlotoxumab with FMT in reducing the risk of RCDI. The review would attempt to determine if FMT when compared to bezlotoxumab has better efficacy and safety in resolving the diarrhea associated with CDI in hospitalized patients without relapse or not.

METHOD

The systematic review and network meta-analysis were conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension statement for network meta-analyses.[17]

Patient and public involvement

Patients and public were not involved in the design, conducting and reporting of research.

Search strategy

A comprehensive search from inception until 30th February 2019 was conducted in four databases (Medline/Pubmed, Embase, Scopus, Clinicaltrials.gov). Searches were conducted using Patients, Intervention, Comparator, Outcome, and Study design (PICOS) strategy for

clinical evidence of FMT and mAB in CDI (Table 1) (see supplementary file for the complete search strategy). Furthermore, manual searches were conducted to identify any additional studies by checking the reference lists of articles retrieved.

INSERT TABLE 1 HERE

Outcome Measure

The primary outcome of interest was the resolution of diarrhea associated with CDI without relapse for at least 60 days after the end of treatments. Furthermore, the adverse events of interest included diarrhea, abdominal pain, leukocytosis, fatigue, nausea, pyrexia, atrial fibrillation, dehydration, sepsis, tachycardia and infusion specific reactions.

Inclusion and Exclusion Criteria

Both published as well as unpublished RCTs that assessed the efficacy and safety of FMT and bezlotoxumab in resolving CDI after a short course of SAT such as vancomycin, metronidazole or fidaxomicin were eligible for inclusion. Studies were eligible for inclusion if they had included patients 18 years or older diagnosed with RCDI and reported the resolution rate of CDI as the efficacy outcome.

Data Extraction, risk of bias and quality assessment

Two reviewers (EC and AS) independently reviewed the titles and abstracts. Studies meeting the inclusion criteria were retrieved as full-text to further assess their eligibility for inclusion. Reviewer AAA independently extracted data from included studies using a data extraction sheet (see table 2 for characteristics of included studies). Reviewer AS checked all data extracted in

the sheets. The data extracted included; author, year of publication, study design and clinical data reporting resolution outcomes of monoclonal antibodies and FMT infusion. The Cochrane Risk of Bias tool was used to assess the quality of included RCTs including randomization, allocation concealment, blinding of participants, reporting of incomplete outcome data, selective reporting and any other bias.[18] Other sources of bias explored included cross-contamination between study groups, recruitment of participants from a selected population and non-compliance with the study protocol. For each included study, a risk of bias graphs and risk of bias summary were generated.

Statistical Analysis

A Bayesian network meta-analysis was conducted using WinBUGS 1.4.3 (MRC Biostatistics Unit, Cambridge, UK). Binomial data that represented the resolution of diarrhea or adverse events were extracted and analyzed. The binary outcomes were expressed as Odds Ratio (OR) and 95% Credible Interval (95% Crl) for resolution rate of RCDI and OR with 95% Crl for adverse events. OR for treatment comparisons were estimated based on 20,000 iterations following the discarding of the first 10,000 iterations in the model. Random-effects model was used for the resolution rate of RCDI and adverse events due to the assumed variability between the included studies. A binomial likelihood with a logit link was used in the model. Furthermore, noninformative priors were used for all parameters.Ranking probabilities of treatments were calculated using the surface under the cumulative ranking curve (SUCRA) method. Additionally, a sensitivity analysis was conducted to exclude the studies and/or patients who received non-FDA approved mAB. Furthermore, a pairwise meta-analysis was conducted to check for heterogeneity.

RESULTS

The initial search identified 1003 studies (see figure 1). 15 duplicates along with an additional 631 studies were removed at title level due to not meeting the inclusion criteria. The abstracts of remaining 357 studies were reviewed, of which a further 297 were excluded. Full texts of the remaining 60 studies were reviewed. Of these, further 53 studies were excluded based on the inclusion and exclusion criteria. The remaining 7 RCTs with 3,043 patients contributed to the review and network meta-analysis.

INSERT FIGURE 1 HERE

Characteristics of included studies

The 7 included studies (see table 2 for characteristics of included studies) were published between 2010 and 2017 and involved 3,043 patients.[9 12 15-16 19-21] Four open-labeled RCTs involving 139 patients reported comparisons of FMT versus vancomycin alone in patients with an initial episode of CDI or with recurrent CDI and followed for at least 70 days following the end of the treatments.[9 12 19 21] Patients assigned to the FMT arm received an initial course of vancomycin ranging from 3-14 days to assure that patients were covered with an antibiotic therapy at the time of donor screening.

INSERT TABL2 HERE

All studies involving FMT used fresh feces from related donors. The time for infusing the fresh FMT from the time of defecation varied across studies from 3.1 to 48 hours. Three FMT studies were terminated early following an interim analysis; two because of the observed superiority of

the FMT[9 12] and one because of inferiority.[19] A fourth study was underpowered and was considered a pilot study.[21] In two studies,[9 12] FMT was reinfused in some patients who experienced a recurrence after the first infusion. In the remaining two studies, FMT was used as a single infusion.[19 21]

Three double-blinded, placebo-controlled RCTs including two multi-center phase two studies and one multi-national, multi-center phase three study investigated the efficacy of mABs.[15-16 20] The two phase two studies investigated the safety and efficacy of newly developed mABs against *C. difficile* exotoxins A and B that corroborated prior evidence of the role of these exotoxins in the virulence of *C. difficile*.[6] The third RCT confirmed that antagonizing toxin B is the main determinant in suppressing the virulence of *C. difficile*, however it could not rule out the role of toxin A.[7] Three regimens of mABs were tested in these RCTs: anti-toxin A (actoxumab), anti-toxin B (bezlotoxumab) and a combination of both, all of which were infused as a single dose of 10mg/kg either during or right away after a course of SAT. It is important to highlight that only bezlotoxumab was approved by the FDA for this indication (see figure 2 for network plot of included studies).

INSERT FIGURE 2 HERE

Study quality

The quality of the studies was variable (see figures 3A and 3B). Only three of the seven studies used blinding of participants.[15-16 20]

INSERT FIGURE 3A AND 3B HERE

Comparative efficacy of FMT and mABs in reducing RCDI

The initial analysis comparing the resolution of CDI after receiving one FMT infusion or any mAB regimen found no statistical difference between FMT and bezlotoxumab (OR=1.53, 95% CrI=0.39-5.16). Yet, FMT showed the best SUCRA probability (63.6%) (see figure 4). In addition, FMT showed better resolution of CDI than SAT (OR=2.98, 95% CrI=1.13-7.53). Whereas, bezlotoxumab showed no statistical difference in resolution of CDI than SAT (OR=1.93, 95% CrI=0.84-4.91) (see table 3).

INSERT FIGURE 4 HERE

A secondary comparative analysis that included the resolution outcomes reported for patients who received two or more FMT infusions or any mAB regimen was conducted. FMT did not show statistical difference in resolution of CDI than bezlotoxumab (OR=2.58, 95% CrI=0.30-23.53). In addition, bezlotoxumab showed no statistical difference in resolution of CDI than SAT (OR=2.01, 95%CrI=0.40-10.51) However, FMT showed better resolution of CDI than SAT (OR 5.22, 95% CrI 1.26-23.25). (see Table 3).

INSERT TABLE 3 HERE

The analyses of the safety data for FMT, bezlotoxumab, and SAT revealed a significantly lower rate of non-serious diarrhea in patients receiving bezlotoxumab (OR 0, 95%CrI 0-0.19) and SAT (OR 0, 95% CrI 0-0.09), compared to patients treated with FMT. There were no differences on other adverse events.

Sensitivity analysis

A sensitivity analysis was conducted by excluding the resolution outcomes of patients who received non-FDA approved mABs. There was no difference between single or multiple FMT infusion and bezlotoxumab (OR 1.61, 95%CrI 0.19-12.69), (OR=2.90, 95%CrI=0.20-45.35), respectively. However, FMT showed the best SUCRA probability (79.7%). The pair-wise meta-analysis suggested that heterogeneity I2 for SAT vs FMT and for SAT vs mABs was high which indicated high variability between the studies.

DISCUSSION

To the best of authors' knowledge, this is the first network meta-analysis that has compared the recently FDA-approved monoclonal antibody bezlotoxumab with FMT for resolving RCDI. The findings of this study suggested that single or multiple infusions of FMT showed no difference in efficacy than a single infusion of bezlotoxumab. However, FMT was associated with a higher rate of non-serious diarrhea.

Despite the inconsistency in the results of the four RCTs that studied FMT versus SAT in resolving RCDI, [9 12 19 21] the network analysis showed the superiority of FMT over SAT alone. This is consistent with the findings of previous meta-analyses of RCTs and observational studies.[10-11] The inconsistency in the results of the four included studies may be attributed to their small sample sizes, lack of blinding and variability between them on the basis of the process of collecting donor feces, preparation of FMT, lag time between feces collection and infusion, method of administration and SAT regimen. Furthermore, as evident from the findings of a previous RCT, patients who received FMT monotherapy for an initial episode of CDI without

receiving prior antibiotics, retained more bacteroidetes in their gut than patients treated with antibiotics.[22] These findings confirm the effect of antibiotics in attenuating intrinsic microbiota.[5] It may also explain the inferiority of FMT over SAT in the study when FMT was preceded by fourteen days of antibiotics.[19] On the contrary, administration of FMT earlier (after the second recurrence of CDI) as opposed to a late administration (after the third or subsequent recurrences) led to shorter length of hospital stay and fewer visits to the emergency department.[23] Thus, the differences in the results of the individual studies included in the current network meta-analysis could have been due to the variability in starting FMT for initial CDI or RCDI and the inconsistency in the number of previous recurrences among included patients.

Bezlotoxumab showed a favorable efficacy and safety profile in preventing RCDI in two robust prospective, double-blinded, placebo-controlled RCTs.[16 24] Furthermore, the effect was sustained throughout the three month follow-up.[16] Bezlotoxumab has a novel mechanism of action that reduces the possibility of RCDI, yet its high cost may limit it's utilization.[16 24] Furthermore, even though the network meta-analysis did not report any difference in the resolution rate between bezlotoxumab and FMT, the SUCRA probability score favored FMT in the rankogram.

This review had some limitaions. The quality of the included RCTs was variable with more than half of the studies not reporting blinding of the participants. Furthermore, the RCTs studying FMT differed in design, donor selection, FMT preparation, follow-up time, lag time between feces collection and infusion and lag time between antibiotics discontinuation and FMT infusion; while mABs were infused either during or right away after the discontinuation of antibiotics. None of the included RCTs reported the number of previous recurrences. Furthermore, safety outcomes were limited due to the early termination of most of the included RCTs and the

inconsistent reporting of the adverse events. Nevertheless, the review employed a rigorous and comprehensive search strategy to identify relevant studies. Furthermore, it used the Bayesian estimation methods in the indirect comparisons of mABs and FMT to address the absence of head-to-head clinical trial evidence. By doing so, this review addressed a significant issue identified in the 2017 IDSA[13] guidelines by filling the gap in information concerning the best method in preventing RCDI and the role of FMT and mABs therapies. However, further studies are required to assess the efficacy, safety, cost and clinical implications of multiple infusions of bezlotoxumab.

CONCLUSION

Single of multiple infusions of FMT showed no difference in efficacy than single infusion of bezlotoxumab in resolving RCDI but with a higher rate of non-serious diarrhea. Further studies are needed to investigate the efficacy and safety of using FMT as monotherapy for CDI, the possible attenuating effect of short-course antibiotics given before FMT and the clinical implications of multiple infusions of bezlotoxumab.

Funding

None

Conflict of interests

None to declare.

Data availability statement

No additional data available

Contributorship statement

Authors AAA designed the research question. Authors ARA, TAA, AAS, MA and FA contributed to the searches, extraction of data and analysis. Authors AAA, IA and EC contributed to the preparation of the manuscript.

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Table 1. PICOS strategy for clinical evidence of FMT and mAB in CDI

PICOS	Clinical Review
Population	Adults with primary or recurrent CDI.
Intervention	Studies that reported the efficacy and safety of fecal microbiota transplantation and/or monoclonal antibodies at any dosage form and via any route of administration in resolving RCDI.
Comparator	Standard antibiotics therapy, such as vancomycin, metronidazole, or fidaxomicin, at any dosage form and via any route of administration.
Outcome	The resolution of diarrhea associated to CDI without relapse for, at least, 60 days after the end of treatments. Adverse events.
Study design	Published or unpublished randomised controlled trials of any size and duration.

Table 2. Study characteristics and clinical data reporting resolution outcomes of monoclonal antibodies and one FMT infusion from the included studies

Author, year of publication	Study design	Standard Antibiotic Therapy	Actoxumab + Bezlotoxumab	Fecal Microbiota Transplantation	Bezlotoxumab	Actoxumab
		Method of administration (CDI resolved/ Total patients)	CDI resolved/ Total patients	CDI resolved /Total patients	CDI resolved /Total patients	CDI resolved /Total patients
Nood et al 2013	Open-label RCT	Vanocomycin 500mg orally four times daily for 14 days (7/26)		Vancomycin 500mg orally four times daily for 4 days followed by FMT (13/16)		
Cammarota et al 2015	Open-label RCT	Vancomycin 125mg orally four times daily for 10 days followed by 125-500mg/day every 2-3 days for 3 weeks (5/19)		Vacomycin 125mg orallt four times daily for 3 days followed by FMT (13/20)		
Hota et al 2017	Open-label RCT	Vancomycin 125mg orally four times daily for 14days, then 125mg orally two times daily for 7 days, then 125mg orally daily for 7 days, then 125mg		Vancomycin 125mg orally four times daily for 14 days followed by FMT after 48 hours (7/16)		

		orally every second day for 7 days, then 125mg orally every third day for 7days. (7/12)				
NG et al 2017 (Abstract)	Open-label RCT	Vancomycin 500mg orally four times daily for 10 days (10/15)		Vancomycin 500mg orally four times daily followed by FMT (11/15)		
Leav et al 2010	Phase II double-blinded RCT	Standard treatment course of Vancomycin or Metronidazole (14/17)				Standard treatment course of Vancomycin or Metronidazole followed by a single dose of 10mg/kg IV of Atoxumab (24/29)
Lowy et al 2010	Phase III double-blinded RCT	Standard treatment course of Vancomycin or Metronidazole (74/99)	Standard treatment course of Vancomycin or Metronidazole followed by a single dose 10mg/kg IV of each Atoxumab and Bezlotoxumab (94/101)			
Wilcox et al 2017 (Modify 1)	Phase III double-blinded RCT	Standard treatment course of Vancomycin, Metronidazole or	Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin followed by a single dose 10mg/kg IV of		Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin followed	Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin

		Fidaxomicin for 10-14 days (286/395)	each Atoxumab and Bezlotoxumab (322/383)		by a single dose 10mg/kg IV of Bezlotoxumab (319/386)	followed by a single dose 10mg/kg IV of Atoxumab (172/232)
Wilcox et al 2017 (Modify 2)	Phase III double-blinded RCT	Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin for 10-14 days (281/378)	Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin followed by a single dose 10mg/kg IV of each Atoxumab and Bezlotoxumab (332/390)		Standard treatment course of Vancomycin, Metronidazole or Fidaxomicin followed by a single dose 10mg/kg IV of Bezlotoxumab (333/395)	

Table 3. Network meta-analysis of the relative efficacy of mABs and single or multiple infusions of FMT for CDI resolution

Fecal microbiota transplantation*	1.29 (0.34 – 3.93)	1.53 (0.39 – 5.16)	2.61 (0.64 – 9.74)	**2.98 (1.13 – 7.53)**
2.10 (0.28 – 15.99)	Actoxumab-Bezlotoxumab	1.17 (0.50 – 3.12)	2.01 (0.74 – 6.42)	**2.28 (1.15 – 5.52)**
2.58 (0.30 – 23.53)	1.22 (0.24 – 6.66)	Bezlotoxumab	1.71 (0.57 – 5.49)	1.93 (0.84 – 4.91)
4.55 (0.49 – 45.11)	2.14 (0.33 – 15.50)	1.75 (0.24 – 13.43)	Actoxumab	1.14 (0.42 – 3.10)
5.22 (1.26 – 23.25)	2.46 (0.62 – 10.68)	2.01 (0.40 – 10.51)	1.16 (0.20 – 6.39)	Standard Antibiotic Therapy

Treatment Multiple infusions of FMT, OR (95% CrI) Single infusion of FMT, OR (95% CrI)

*Data are OR (95% CrI) of the row treatment relative to the column treatment (E.g. the effect of multiple infusions of fecal microbiota transplantation relative to Actoxumab-Bezlotoxumab is 2.10 with respect to resolution of recurrent *Clostridium difficile* infection (RCDI). Bold

values indicate comparisons that are statistically significant. ORs above 1 indicate higher efficacy in resolution of RCDI. OR=odds ratio. CrI= credible interval.

Figure Legends

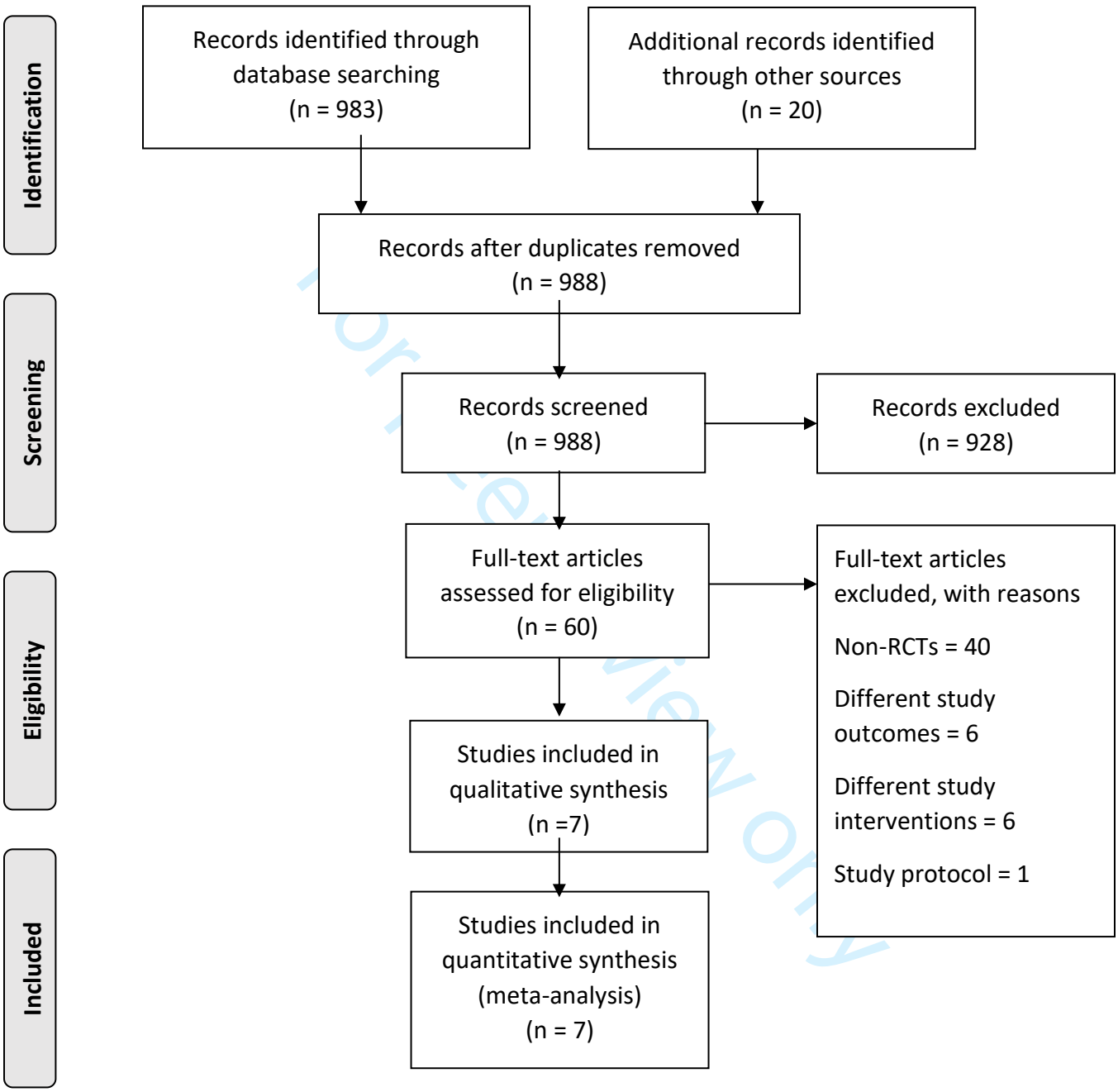
Figure 1. Study selection process using preferred reporting items for systematic reviews and meta-analyses (PRISMA).

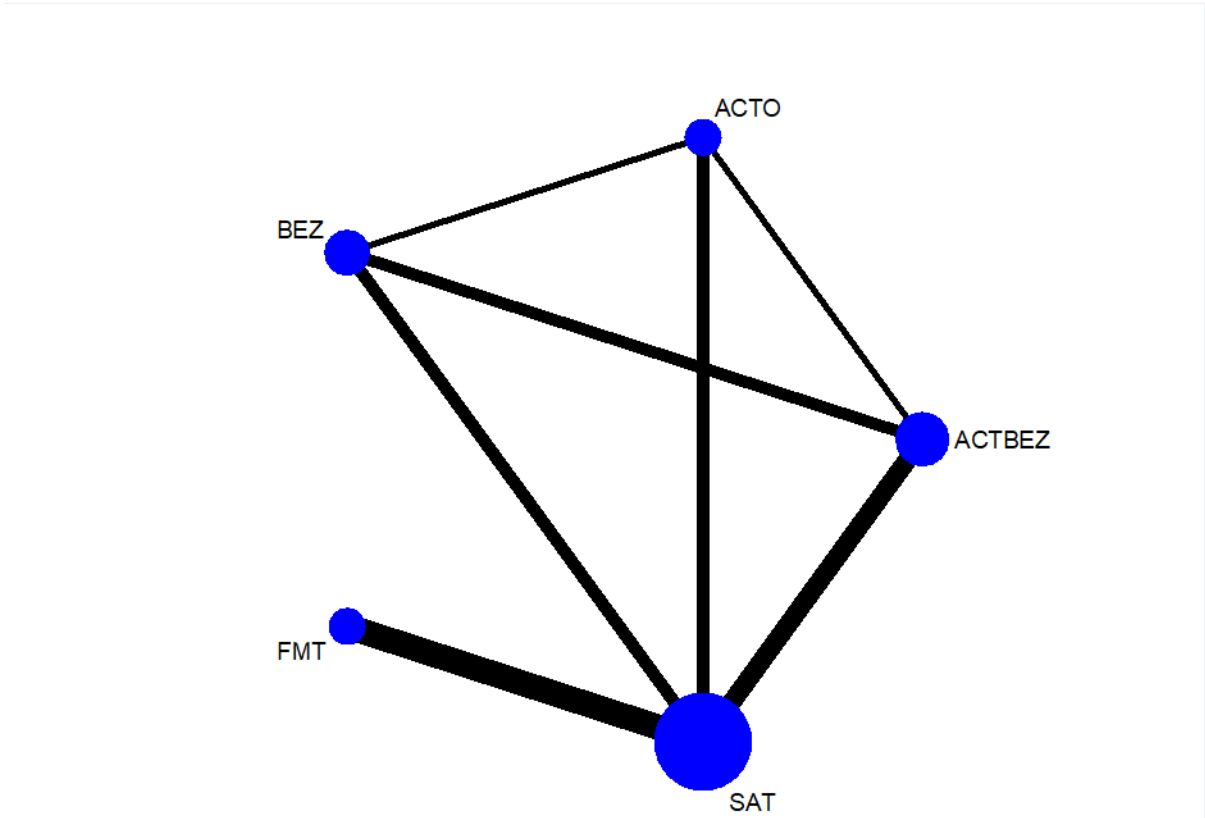
Figure 2. Network plot of included studies. Each circled node represents an intervention, the extent of the circle indicates the number of the included participants, the lines and their thickness represent direct comparisons and the number of studies included in each comparison, respectively. Standard Antibiotic Therapy (SAT), Actoxumab plus Bezlotoxumab (ACTBEZ), Fecal Microbiota Transplantation (FMT), Bezlotoxumab (BEZ), Actoxumab (ACTO).

Figure 3 A. Risk of bias graph: review authors' judgments about each risk of bias item presented as percentages across all included studies.

Figure 3 B. Risk of bias summary: review authors' judgments about each risk of bias item for each included study.

Figure 4. Rankograms shows the ranking of interventions from the best to the worst based on the corresponding surface under the cumulative ranking curve (SUCRA) probability in the random effect models.





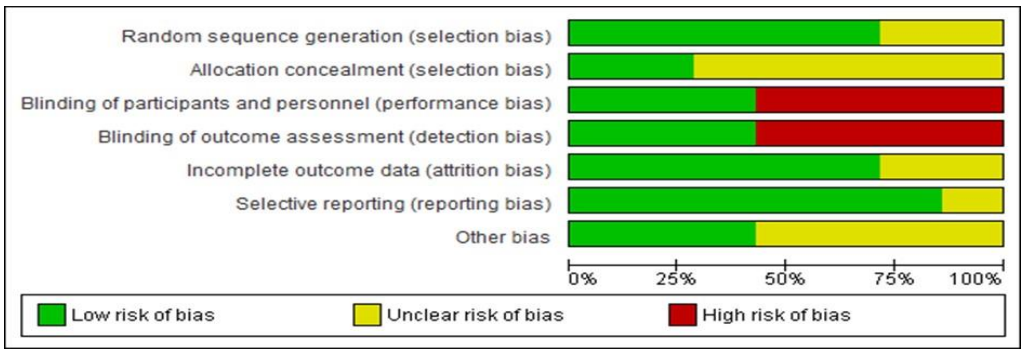


FIGURE 3A

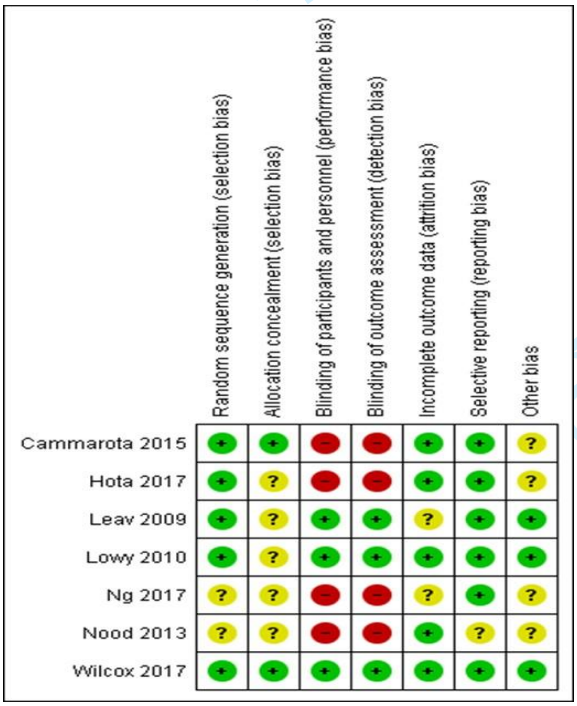
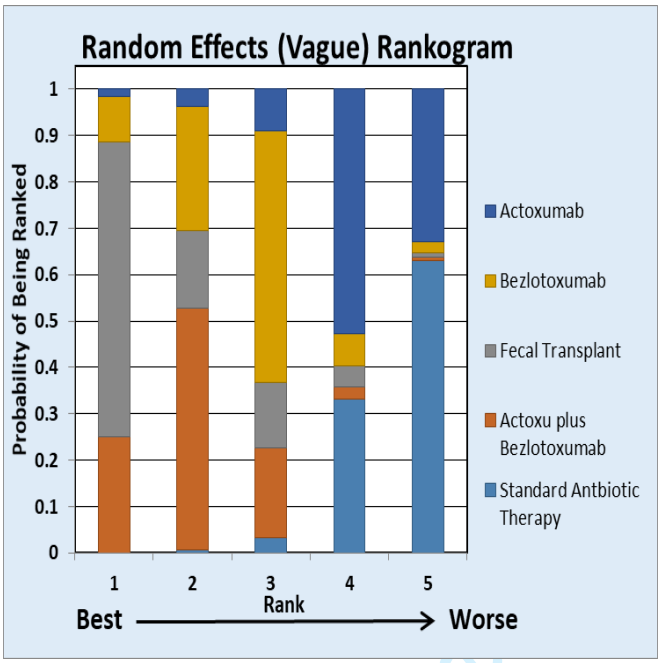


FIGURE 3B



Rank	Treatment	SUCRA
1	Fecal Microbiota Transplantation	0.6357
2	Actoxumab plus Bezlotoxumab	0.5215
3	Bezlotoxumab	0.5421
4	Actoxumab	0.528
5	Standard Antibiotics Therapy	0.631

Figure 4. Rankograms shows the ranking of interventions from the best to the worst based on the corresponding surface under the cumulative ranking curve (SUCRA) probability.

Example of search strategy

Medline-PubMed
((((((((((("Fecal Microbiota Transplantation"[Mesh]) OR Fecal Microbiota Transplantation)) OR ((monoclonal antibodies[MeSH Terms]) OR monoclonal antibodies)) OR ((vancomycin[MeSH Terms]) OR vancomycin)) OR ((metronidazole[MeSH Terms]) OR metronidazole)) OR ((fidaxomicin[MeSH Terms]) OR fidaxomicin)) OR ((resolution of diarrhea[MeSH Terms]) OR resolution of diarrhea)) AND ((clostridium difficile[MeSH Terms]) OR clostridium difficile)) OR ((clostridium difficile infection[MeSH Terms]) OR clostridium difficile infection)) AND ((randomized controlled trial[MeSH Terms]) OR randomized controlled trial))) = 619 citations
Embase
('fecal microbiota transplantation'/exp OR 'monoclonal antibody'/exp OR 'vancomycin'/exp OR 'metronidazole'/exp OR 'fidaxomicin'/exp) AND 'clostridium difficile infection'/exp AND [randomized controlled trial]/lim =170 citations

PRISMA 2009 Checklist

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.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
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.	
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.	5-6
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.	5-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5-6
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).	5-6
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.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5-6
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.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
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.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
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).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
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.	7
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.	7-8
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).	8
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.	8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8-9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10
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).	11-12
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).	12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	13
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.	13

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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