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Interventions for preventing diarrhoea-associated haemolytic uraemic syndrome (Review)

Imdad A, Mackoff SP, Urciuoli DM, Syed T, Tanner-Smith EE, Huang D, Gomez-Duarte OG

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Interventions for preventing diarrhoea-associated haemolytic uraemic syndrome.
Cochrane Database of Systematic Reviews 2021, Issue 7. Art. No.: CD012997.
DOI: 10.1002/14651858.CD012997.pub2.

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[Intervention Review]

Interventions for preventing diarrhoea-associated haemolytic uraemic syndrome

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Editorial group: Cochrane Kidney and Transplant Group.
Publication status and date: Edited (no change to conclusions), published in Issue 7, 2021.

Citation: Imdad A, Mackoff SP, Urciuoli DM, Syed T, Tanner-Smith EE, Huang D, Gomez-Duarte OG. Interventions for preventing diarrhoea-associated haemolytic uraemic syndrome. *Cochrane Database of Systematic Reviews* 2021, Issue 7. Art. No.: CD012997. DOI: 10.1002/14651858.CD012997.pub2.

ABSTRACT

Background

Haemolytic uraemic syndrome (HUS) is a common cause of acquired kidney failure in children and rarely in adults. The most important risk factor for development of HUS is a gastrointestinal infection by Shiga toxin-producing *Escherichia coli* (STEC). This review addressed the interventions aimed at secondary prevention of HUS in patients with diarrhoea who were infected with a bacteria that increase the risk of HUS.

Objectives

Our objective was to evaluate evidence regarding secondary preventative strategies for HUS associated with STEC infections. In doing so, we sought to assess the effectiveness and safety of interventions as well as their potential to impact the morbidity and death associated with this condition.

Search methods

We searched the Cochrane Kidney and Transplant Register of Studies up to 12 November 2020 through contact with the Information Specialist using search terms relevant to this review. Studies in the Register are identified through searches of CENTRAL, MEDLINE, and EMBASE, conference proceedings, the International Clinical Trials Register (ICTRP) Search Portal and ClinicalTrials.gov.

Selection criteria

Studies were considered based on the methods, participants, and research goals. Only randomised controlled trials were considered eligible for inclusion. The participants of the studies were paediatric and adult patients with diarrhoeal illnesses due to STEC. The primary outcome of interest was incidence of HUS.

Data collection and analysis

We used standard methodological procedures as recommended by Cochrane. Summary estimates of effect were obtained using a random-effects model, and results were expressed as risk ratios (RR) and their 95% confidence intervals (CI) for dichotomous outcomes. Confidence in the evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.

Main results

We identified four studies (536 participants) for inclusion that investigated four different interventions including antibiotics (trimethoprim-sulfamethoxazole), anti-Shiga toxin antibody-containing bovine colostrum, Shiga toxin binding agent (Synsorb Pk: a silicon dioxide-based agent), and a monoclonal antibody against Shiga toxin (urtoxazumab). The overall risk of bias was unclear for selection, performance and detection bias and low for attrition, reporting and other sources of bias.

It was uncertain if trimethoprim-sulfamethoxazole reduced the incidence of HUS compared to no treatment (47 participants: RR 0.57, 95% CI 0.11-2.81, very low certainty evidence). Adverse events relative to this review, need for acute dialysis, neurological complication and death were not reported.

There were no incidences of HUS in either the bovine colostrum group or the placebo group. It was uncertain if bovine colostrum caused more adverse events (27 participants: RR 0.92, 95% CI 0.42 to 2.03; very low certainty evidence). The need for acute dialysis, neurological complications or death were not reported.

It is uncertain whether Synsorb Pk reduces the incidence of HUS compared to placebo (353 participants: RR 0.93, 95% CI 0.39 to 2.22; very low certainty evidence). Adverse events relevant to this review, need for acute dialysis, neurological complications or death were not reported.

One study compared two doses of urtoxazumab (3.0 mg/kg and 1.0 mg/kg) to placebo. It is uncertain if either 3.0 mg/kg urtoxazumab (71 participants: RR 0.34, 95% CI 0.01 to 8.14) or 1.0 mg/kg urtoxazumab (74 participants: RR 0.95, 95% CI 0.79 to 1.13) reduced the incidence of HUS compared to placebo (very low certainty evidence). Low certainty evidence showed there may be little or no difference in the number of treatment-emergent adverse events with either 3.0 mg/kg urtoxazumab (71 participants: RR 1.00, 95% CI 0.84 to 1.18) or 1.0 mg/kg urtoxazumab (74 participants: RR 0.95, 95% CI 0.79 to 1.13) compared to placebo. There were 25 serious adverse events reported in 18 patients: 10 in the placebo group, and 9 and 6 serious adverse events in the 1.0 mg/kg and 3.0 mg/kg urtoxazumab groups, respectively. It is unclear how many patients experienced these adverse events in each group, and how many patients experienced more than one event. It is uncertain if either dose of urtoxazumab increased the risk of neurological complications or death (very low certainty evidence). Need for acute dialysis was not reported.

Authors' conclusions

The included studies assessed antibiotics, bovine milk, and Shiga toxin inhibitor (Synsorb Pk) and monoclonal antibodies (Urtoxazumab) against Shiga toxin for secondary prevention of HUS in patients with diarrhoea due to STEC. However, no firm conclusions about the efficacy of these interventions can be drawn given the small number of included studies and the small sample sizes of those included studies. Additional studies, including larger multicentre studies, are needed to assess the efficacy of interventions to prevent development of HUS in patients with diarrhoea due to STEC infection.

PLAIN LANGUAGE SUMMARY

Interventions for prevention of haemolytic uraemic syndrome in patients

What is the issue?

Haemolytic uraemic syndrome (HUS) is a serious illness that primarily affects children and can have severe side effects such as anaemia (low red blood cell counts), kidney damage, brain damage, and death in some cases. HUS most commonly occurs as a complication of diarrhoeal illness caused by a particular form of *Escherichia coli* (*E. coli*) bacteria called Shiga toxin-producing *E. coli* (STEC). Despite the severity of this illness, there are currently no standard practices for treating these patients.

What did we do?

We summarized the available evidence that was collected to date on methods for preventing HUS in patients diagnosed with STEC-associated diarrhoea (loose bowel movements). We searched the literature for past studies looking at different treatments aimed at preventing HUS in children with diarrhoeal illness and summarized the findings in our review,

What did we find?

Four studies randomising 536 patients were included. These studies looked at four different preventative treatments including antibiotic therapy, anti-Shiga toxin antibody-containing bovine colostrum, Shiga toxin binding agent (Synsorb Pk: a silicon dioxide-based agent) and a monoclonal antibody against Shiga toxin (urtoxazumab). The included studies had small number of participants and the results did not favour any one intervention to reduce the progression of the disease to HUS in patients who were infected with STEC.

Conclusions

No conclusion on the best method for preventing HUS in patients with STEC-associated diarrhoea can be drawn from this data; more studies with a larger group of patients is required before any recommendation can be made.

Summary of findings 1. Antibiotics versus no treatment for secondary prevention of HUS

Trimethoprim-sulfamethoxazole versus no treatment for secondary prevention of HUS in patients with diarrhoea due to Shiga toxin-producing *E. coli*

Patient or population: patients with diarrhoea due to Shiga toxin-producing *E. coli*
Intervention: trimethoprim-sulfamethoxazole
Comparison: no treatment

Outcomes	No. of participants (studies)	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects* (95% CI)	
				Risk with no treatment	Risk with antibiotics
Incidence of HUS Follow up: 10 days	47 (1)	⊕⊖⊖⊖ VERY LOW [1,2]	RR 0.57 (0.11 to 2.81)	160 per 1,000	69 fewer per 1,000 (142 fewer to 290 more)
Adverse events	Not reported	--	--	--	--
Need for acute dialysis	Not reported	--	--	--	--
Neurological complications	Not reported	--	--	--	--
Death (any cause)	Not reported	--	--	--	--

***The risk in the intervention group** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
HUS: haemolytic uraemic syndrome; **CI:** Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Downgraded once for risk of bias: The risk of bias was high for blinding of participants and personnel
[2] Downgraded twice for imprecision: very wide confidence intervals crossing the line of no effect and few events

Summary of findings 2. Bovine colostrum versus placebo for secondary prevention of HUS

Bovine colostrum versus placebo for secondary prevention of HUS in patients with diarrhoea due to Shiga toxin-producing *E. coli*

Patient or population: patients with diarrhoea due to Shiga toxin-producing *E. coli*
Intervention: bovine colostrum
Comparison: placebo

Outcomes	No. of participants (studies)	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects* (95% CI)	
				Risk with placebo	Risk with bovine colostrum
Incidence of HUS Follow up: 21 days	27 (1)	⊕⊖⊖⊖ VERY LOW [1,2]	not estimable	No events	No events
Adverse events Follow up: 21 days	27 (1)	⊕⊖⊖⊖ VERY LOW [1,3]	RR 0.92 (0.42 to 2.03)	500 per 1,000	40 fewer per 1,000 (290 fewer to 515 more)
Need for acute dialysis	Not reported	--	--	--	--
Neurological complications	Not reported	--	--	--	--
Death (any cause)	Not reported	--	--	--	--

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
HUS: haemolytic uraemic syndrome; **CI:** Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Downgraded once for risk of bias: Selection and detection bias were unclear
[2] Downgraded twice for imprecision: No events in the intervention and control group
[3] Downgraded twice for imprecision: very wide confidence intervals crossing the line of no effect and few events

Summary of findings 3. Synsorb Pk versus placebo for secondary prevention of HUS

Synsorb Pk versus placebo for secondary prevention of HUS in patients with diarrhoea due to Shiga toxin-producing *E. coli*

Patient or population: patients with diarrhoea due to Shiga toxin-producing *E. coli*
Intervention: Synsorb Pk
Comparison: placebo

Outcomes	No. of participants (studies)	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects* (95% CI)	
				Risk with placebo	Risk with Synsorb Pk
Incidence of HUS Follow up: 7 days	353 (1)	⊕⊖⊖⊖ VERY LOW [1,2]	RR 0.93 (0.39 to 2.22)	56 per 1,000	4 fewer per 1,000 (34 fewer to 68 more)
Adverse events	Not reported	--	--	--	--
Need for acute dialysis	Not reported	--	--	--	--
Neurological complications	Not reported	--	--	--	--
Death (any cause)	Not reported	--	--	--	--

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
HUS: haemolytic uraemic syndrome; **CI:** Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Downgraded once for risk of bias: high risk of performance bias and unclear risk of selection and detection bias
[2] Downgraded twice for imprecision: The number of events was small in both groups and the CI of the summary estimate was large

Summary of findings 4. Urtoxazumab (3.0 mg/kg) versus placebo for secondary prevention of HUS

Urtoxazumab (3.0 mg/kg) versus placebo for secondary prevention of HUS

Patient or population: paediatric patients with diarrhoea due to Shiga toxin-producing *E. coli*
Intervention: urtoxazumab (3.0 mg/kg)
Comparison: placebo

Outcomes	No. of partici-pants	Certainty of the evidence	Relative effect (95% CI)	Anticipated absolute effects* (95% CI)

	(studies)	(GRADE)		Risk with placebo	Risk with 3.0 mg/kg urtoxazumab
Incidence of HUS Follow up: 7 days	71 (1)	⊕⊖⊖⊖ VERY LOW [1,2]	RR 0.34 (0.01 to 8.14)	28 per 1,000	18 fewer per 1,000 (27 fewer to 198 more)
Adverse events - treatment emergent Follow up: to 56 days	71 (1)	⊕⊕⊖⊖ LOW [1,3]	RR 1.00 (0.84 to 1.18)	889 per 1,000	0 fewer per 1,000 (142 fewer to 160 more)
Adverse events - serious Follow-up: to 56 days	71 (1)	⊕⊕⊖⊖ LOW [1,4]	risk ratio could not be calculated[4]	-	-
Need for acute dialysis	Not reported	--	--	--	--
Neurological complications Follow up: to 56 days	71 (1)	⊕⊖⊖⊖ VERY LOW [1,2]	RR 2.06 (0.20 to 21.68)	28 per 1,000	29 more per 1,000 (22 fewer to 574 more)
Death (any cause) Follow up: to 56 days	71 (1)	⊕⊖⊖⊖ VERY LOW [1,2]	RR 0.34 (0.01 to 8.14)	1/36**	No events

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

** Event rate derived from the raw data. A 'per thousand' rate is non-informative in view of the scarcity of evidence and zero events in the treatment group

HUS: haemolytic uraemic syndrome; **CI:** Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Downgraded once for risk of bias: The risk of selection, performance and detection bias were all unclear
[2] Downgraded twice for imprecision: The number of events were small in the intervention and control group and the summary estimate had wide confidence intervals
[3] Downgraded once for imprecision because the CI included 1
[4] Downgraded once for imprecision: The study reported 25 serious adverse events in 18 patients: 10 in the placebo group, and 9 and 6 serious adverse events in the 1.0 mg/kg and 3.0 mg/kg urtoxazumab groups, respectively. It is unclear how many patients experienced these adverse events in each group, and how many patients experienced more than one event. So risk ratio could not be calculated in this scenario

Summary of findings 5. Urtoxazumab (1.0 mg/kg) versus placebo for secondary prevention of HUS

Urtoxazumab (1.0 mg/kg) versus placebo for secondary prevention of HUS

Patient or population: paediatric patients with diarrhoea due to Shiga toxin-producing *E. coli*
Intervention: urtoxazumab 1.0 mg/kg
Comparison: placebo

Outcomes	No. of participants (studies)	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects* (95% CI)	
				Risk with placebo	Risk with 1.0 mg/kg urtoxazumab
Incidence of HUS Follow up: 56 days	74 (1)	⊕⊖⊖⊖ VERY LOW [1,2]	RR 0.95 (0.06 to 14.59)	28 per 1,000	1 fewer per 1,000 (26 fewer to 378 more)
Adverse events - treatment emergent Follow up: 56 days	74 (1)	⊕⊕⊖⊖ LOW [1,3]	RR 0.95 (0.79 to 1.13)	889 per 1,000	44 fewer per 1,000 (187 fewer to 116 more)
Adverse events - serious Follow up: 56 days	74 (1)	⊕⊕⊖⊖ LOW [1,4]	--	--	--
Need for acute dialysis	Not reported	--	--	--	--
Neurological complications Follow up: 56 days	74 (1)	⊕⊖⊖⊖ VERY LOW [1,2]	RR 2.84 (0.31 to 26.08)	28 per 1,000	51 more per 1,000 (19 fewer to 697 more)
Death (any cause) Follow up: 56 days	74 (1)	⊕⊖⊖⊖ VERY LOW [1,2]	RR 0.95 (0.06 to 14.59)	28 per 1,000	1 fewer per 1,000 (26 fewer to 378 more)

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

HUS: haemolytic uraemic syndrome; **CI:** Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect

Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

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[2] Downgraded twice for imprecision: The number of events were small in the intervention and control group and the summary estimate had wide confidence intervals
[3] Downgraded once for imprecision because the CI included 1.
[4] Downgraded once for imprecision: The study reported 25 serious adverse events in 18 patients: 10 in the placebo group, and 9 and 6 serious adverse events in the 1.0 mg/kg and 3.0 mg/kg urtoxazumab groups, respectively. It is unclear how many patients experienced these adverse events in each group, and how many patients experienced more than one event. So risk ratio could not be calculated in this scenario.

BACKGROUND

Description of the condition

Haemolytic uraemic syndrome (HUS) is a serious condition caused by abnormal destruction of red blood cells and kidney damage and diagnosed clinically as a triad of microangiopathic haemolytic anaemia, thrombocytopenia and acute kidney injury (Fakhouri 2017; Mele 2014). HUS most commonly occurs secondary to infections with about 90% cases developing after diarrhoeal disease due to Shiga toxin-producing *Escherichia coli* (STEC) (Jokiranta 2017). *Shigella dysenteriae* is another common cause of HUS. Children are most commonly affected (Mody 2015; Talarico 2016), however cases of adults with HUS have been reported (Gould 2011; Mele 2014). Available evidence suggest endothelial cell damage as a primary event in the pathogenesis of HUS, mediated by Shiga-toxin in case of STEC infection and complement activation in atypical and secondary causes of HUS (Corrigan 2001; Fakhouri 2017). Additionally, Shiga toxin-producing organisms infect the gastrointestinal tract, and induce diarrhoea that may progress to haemorrhagic colitis (Melton-Celsa 2014).

This review focuses on HUS associated with diarrhoeal disease due to STEC. The annual incidence of STEC HUS in the U.S. and Europe is estimated to be between 1.9 and 2.9 cases per 100,000 children, three to five years of age (Majowicz 2014; Ylinen 2020). The incidence in Latin American is estimated to be 10 times higher than other continents with an incidence between 10 and 17 cases per 100,000 children less than five years of age (Rivas 2014). Most infections are due to STECs that belong to serogroup O157, although other serotypes are also implicated in HUS. Incubation periods last anywhere from one to 12 days and symptoms can include nausea, vomiting, cramping, abdominal pain, and watery diarrhoea that then turns bloody within two to three days (Bell 1994; Keir 2015; Riley 1983). Progression to HUS typically occurs 7 to 10 days after the onset of symptoms. It is estimated that 10% to 15% of cases of diarrhoeal illness due to STEC infection will progress to HUS. Of these patients affected by HUS, 30% will go on to develop serious complications such as end-stage kidney disease and neurological sequelae and death (Garg 2003; Keir 2015; Rowe 1998; Siegler 1994).

Description of the intervention

Prevention of diarrhoea-associated HUS can be in the form of primary or secondary prevention. Primary prevention relies on identifying and modifying predisposing risk factors for STEC infection, such as food safety, handwashing, and waste disposal. Secondary prevention relies on taking actions to reduce the risk of developing HUS once the predisposing disease, in this case, infectious diarrhoea, has been diagnosed. Some examples of intervention for secondary prevention of HUS include aggressive hydration, antibiotics, monoclonal antibodies against Shiga toxin and Shiga toxin binding proteins (i.e. Synsorb Pk) (Grisaru 2017; Thomas 2013).

How the intervention might work

Use of antibiotics to treat STEC infection to prevent HUS is debatable (Fakhouri 2017). The Centers for Disease Control and Prevention and American Gastroenterology Association both recommend against the use of antibiotics to treat STEC to prevent HUS, due to concerns that antibiotic can potentially increase risk of HUS after STEC infection (CDC 2018a; Riddle 2016). These recommendations are mostly based on findings from observational studies and one randomised controlled trial (RCT) that did not show any increased or protective effect of antibiotics with relation to HUS (Freedman 2016;Proulx 1992; Thomas 2013; Wong 2000). There is some evidence in favour of the use of antibiotics. During the 2011 outbreak of HUS in Germany, an observational study assessed the use of azithromycin in children with STEC infections found that treatment with azithromycin was associated with a lower frequency of long-term STEC carriage (Nitschke 2012). Monoclonal antibodies against Shiga toxin are another potential novel approach for clinical detection of the toxin (Skinner 2016). Anti-Shiga toxin monoclonal antibodies have been investigated as potential treatments in animal models and in healthy volunteers, yet the evidence for their efficacy is still inconclusive (Bitzan 2009; Dowling 2005; Lopez 2010a; Mejias 2016; Melton-Celsa 2014). Monoclonal antibodies against Shiga toxins 1 and 2 may be used as a preventative strategy in preventing the onset of HUS (Melton-Celsa 2014; Thomas 2013). Synsorb Pk is a silicon dioxide-based compound containing the trisaccharide part of Gb3 that serves as binding protein to prevent the absorption of Shiga toxin from the gastrointestinal system (Armstrong 1991; Trachtman 2003). In addition to Synsorb Pk, several other Shiga toxin receptor analogues have been developed including STARFISH, Daisy, SUPER TWIG, Gb3 polymers, Ac-PPPtet, and probiotic with a Shiga toxin binder (Melton-Celsa 2014). These developments can potentially prevent HUS by neutralizing the action of Shiga-toxins (Melton-Celsa 2014).

Why it is important to do this review

Treatment strategies for HUS have been discussed in a prior Cochrane review (Michael 2009); however, no Cochrane review has focused on secondary prevention of diarrhoea-associated HUS. Other non-Cochrane reviews have focused on selective interventions (Freedman 2016; Grisaru 2017; Thomas 2013) and need an update. We therefore aim to synthesize up to date evidence regarding secondary preventative strategies for diarrhoea-associated HUS.

OBJECTIVES

To assess the effectiveness and safety of intervention for secondary prevention of morbidity and death from diarrhoea-associated HUS in children and adults, compared to placebo or no secondary intervention use.

METHODS

Criteria for considering studies for this review

Types of studies

All RCTs and quasi-RCTs (RCTs in which allocation to treatment was obtained by alternation, use of alternate medical records, date of birth or other predictable methods) examining secondary prevention strategies of diarrhoea-associated HUS were included. We included randomised studies if the randomisation was conducted at the individual or the cluster level. We also considered cross-over RCTs eligible for inclusion. We excluded all observational studies such as cohort, case-control, case series, and case reports.

Types of participants

We included evidence from studies that focused on paediatric and adult patients with diarrhoea who are at risk of developing HUS, such as those infected with STEC including both O157 and non-O157 serogroups. We included studies with participants at risk of developing diarrhoea-associated HUS regardless of a particular setting, educational status, gender, race, geographic location, or socioeconomic status of the participants.

We excluded studies with patients that are at risk of non-diarrhoea-associated HUS such as those associated with *Streptococcus pneumoniae* infections, disorders of complement regulation, ADAMTS13 deficiency, cancer, organ transplant and pregnancy. This is because pathophysiology of non-diarrhoea-associated HUS is thought to be different than diarrhoea-associated HUS and it is hard to predict occurrence of HUS in non-diarrhoea-associated cases (Fakhouri 2017).

Types of interventions

We included evidence from studies that evaluated the following interventions used to prevent diarrhoea-associated HUS:

- Antibiotics
- Anti-Shiga toxin monoclonal antibodies
- Shiga toxin binding protein (i.e. Synsorb Pk)
- Aggressive hydration.

We included studies regardless of the type of antibiotics used, mode of delivery of intervention (oral versus intravascular/intramuscular), or frequency of intervention.

Eligible comparison groups included placebo and standard of care conditions. We included studies with multiple treatment arms such as factorial design trials, as long as the study reports contrasted in a way whereby the only difference between two groups was the intervention.

We excluded studies that evaluated interventions delivered after the diagnosis of HUS, given that these interventions were outside the scope of this review. We also excluded studies in which the intervention was provided as a primary form of prevention for diarrhoea itself, as these interventions were also outside the scope of the review.

Types of outcome measures

Primary outcomes

- Incidence of HUS in patients with diarrhoea
  - HUS was defined as a triad of microangiopathic haemolytic anaemia, thrombocytopenia, and acute kidney injury that happened within three weeks of the diarrhoeal episode. The laboratory evidence included anaemia with microangiopathic changes such as presence of schistocytes, burr cells, or helmet cells on peripheral blood smear and kidney injury evidenced by haematuria, proteinuria or elevated creatinine or blood urea nitrogen (CDC 1996). We also included cases of thrombotic thrombocytopenic purpura (TTP) after a diarrhoeal episode. The definition of TTP includes the triad of HUS plus central nervous system involvement and fever. If the definition of the primary outcomes was not provided explicitly in the study, we contacted the authors for further information. If no information was available on how the HUS was defined, we still included the data from that study but planned a sensitivity analysis to assess if the inclusion/exclusion of the study altered our findings and conclusions.

Secondary outcomes

1. Oligoanuric kidney failure defined as urine output < 0.5 mL/kg/hour
2. Need for acute kidney replacement therapy
3. Need for prolonged dialysis for one to three months post HUS acute phase, or develop dialysis-dependent kidney failure needing kidney transplant
4. Death (any cause)
5. Adverse events or any serious acute phase complications such as bowel perforation or obstruction, peritonitis, sepsis, cardiac injury, or pancreatitis
6. Need for blood transfusion and platelet transfusions
7. Incidence of neurological complications (e.g. stroke, seizures).

Search methods for identification of studies

Electronic searches

We searched the Cochrane Kidney and Transplant Register of Studies up to 12 November 2020 through contact with the Information Specialist using search terms relevant to this review. The Register contains studies identified from the following sources.

1. Monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL)
2. Weekly searches of MEDLINE OVID SP
3. Handsearching of kidney-related journals and the proceedings of major kidney conferences
4. Searching of the current year of EMBASE OVID SP
5. Weekly current awareness alerts for selected kidney and transplant journals
6. Searches of the International Clinical Trials Register (ICTRP) Search Portal and ClinicalTrials.gov.

Studies contained in the Register are identified through searches of CENTRAL, MEDLINE, and EMBASE based on the scope of Cochrane Kidney and Transplant. Details of search strategies, as well as a list of handsearched journals, conference proceedings and current awareness alerts, are available on the Cochrane Kidney and Transplant website under CKT Register of Studies.

See Appendix 1 for search terms used in strategies for this review.

Searching other resources

1. Reference lists of review articles, relevant studies and clinical practice guidelines.
2. Letters seeking information about unpublished or incomplete RCTs to investigators known to be involved in previous studies.
3. Grey literature from the Conference Proceeding Citation Index database (hosted on Web of Science).
4. Manually searched reference lists of potentially included studies and previous reviews on topic.

Data collection and analysis

Selection of studies

The search strategy was used to identify titles and abstracts of studies that were potentially relevant to the review. The titles and abstracts identified in the search were screened independently by two authors who selected potentially eligible titles to progress to the next stage of full text review. Any conflict between the two authors was resolved by discussion or contacting a senior author. Two authors independently assessed retrieved full text articles and made a determination about the study's eligibility for inclusion in the review. If there was no consensus on inclusion/exclusion of a study between two authors, a third author was consulted for a final decision.

Data extraction and management

Data extraction was carried out independently by two authors using standard data extraction forms. Studies reported in non-English language journals were to be translated before assessment. Where more than one publication of one study existed, reports were grouped together and the publication with the most complete data was used for the purposes of effect size estimation. Where relevant outcomes were only published in earlier versions, these earlier versions were used for the purposes of effect size estimation. Any discrepancy between published versions was highlighted in the text of the review.

For eligible studies, at least two authors extracted the data and any discrepancies in extracted data were resolved based on discussion with a third author.

A codebook was used to define and describe all the variables abstracted from included studies. We abstracted the information on the following variables: study type, study site, baseline mortality and morbidity, inclusion/exclusion criteria, details of the intervention (e.g. type, route, frequency) risk of bias, attrition, coverage of intervention, characteristics of participants (e.g. age, race, gender, socioeconomic status), place of living (home versus facility), and outcome data.

When information regarding any of the above was unclear, we attempted to contact the authors of the original reports to provide further details. If authors had not performed an analysis for a particular variable, we asked authors to perform that analysis or provide the original dataset so that we could perform the analysis for that outcome.

Assessment of risk of bias in included studies

The following items were independently assessed by two authors using the Cochrane risk of bias assessment tool for randomised trials (Higgins 2011) (see Appendix 2). All risk of bias items were coded as high, low, or unclear risk of bias, with additional textual support for each item.

- Was there adequate sequence generation (selection bias)?
- Was allocation adequately concealed (selection bias)?
- Was knowledge of the allocated interventions adequately prevented during the study?
  - Participants and personnel (performance bias)
  - Outcome assessors (detection bias)
- Were incomplete outcome data adequately addressed (attrition bias)?
- Are reports of the study free of suggestion of selective outcome reporting (reporting bias)?
- Was the study apparently free of other problems that could put it at a risk of bias?

Two review authors independently assessed the risk of bias for each study using the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011). We resolved any disagreement by discussion or by involving a third author.

Measures of treatment effect

For dichotomous outcomes (incidence of HUS, need for acute dialysis, dialysis-dependent kidney failure) results were expressed as risk ratio (RR) with 95% confidence intervals (CI). Where continuous scales of measurement were used to assess the effects of treatment, the mean difference (MD) was used, or the standardised mean difference (SMD) if different scales were used.

Unit of analysis issues

We planned to consider the data from individual and cluster RCTs in the same meta-analysis. However, none of the included studies used a cluster randomised trial design. We had planned to use cluster-adjusted values when reported by authors. If authors did not appropriately adjust for their cluster designs, we would have conducted our own adjustments by inflating the standard error (SE) of the effect size estimate by multiplying it by the square root of design effect as described by the Cochrane handbook (Higgins 2011). If the design effect could not have been estimated for a primary study (e.g. if the cluster sizes, intra-class correlation coefficients, or both were not reported), a design effect from similar study would have been considered.

For studies using cross-over designs, we planned to only include data from the first phase of the study prior to the first cross-over. However, no eligible studies using a cross-over design were ultimately included.

Dealing with missing data

Attrition is an important factor in RCTs and differential loss to follow-up may lead to biased results. We therefore extracted information on attrition and reported missing data, including dropouts and reasons for dropout as reported by authors. We contacted authors if data were missing, there were no reasons provided for missing data, or both. When authors reported data for completers as well as controlling for dropout (e.g. imputed using regression methods), we extracted the latter. We also contacted authors to obtain data if a study did not report data for a primary or secondary outcome of this review.

Data were included based on an intention-to-treat analysis, i.e., all participants randomised to each group in the analyses were analysed based on initial allocation, regardless of whether or not they received the allocated intervention.

Assessment of heterogeneity

We first assessed heterogeneity by visual inspection of the forest plot. We quantified statistical heterogeneity using the I^2 statistic, which describes the percentage of total variation across studies

that is due to heterogeneity rather than sampling error (Higgins 2003). A guide to the interpretation of I^2 values was as follows.

- 0% to 40%: might not be important
- 30% to 60%: may represent moderate heterogeneity
- 50% to 90%: may represent substantial heterogeneity
- 75% to 100%: considerable heterogeneity.

The importance of the observed value of I^2 depends on the magnitude and direction of treatment effects, the total amount of observed heterogeneity, and the strength of evidence for heterogeneity (e.g. P-value from the Chi^2 test, value of τ, confidence interval for I^2) (Higgins 2011).

Clinical heterogeneity was described in terms of the different types, durations, and frequencies of the included interventions. Methodological heterogeneity was described in terms of the prevalence of individual versus cluster-randomised trials. No meta-analysis was performed in this review, so inferences were made about the statistical heterogeneity.

Assessment of reporting biases

If 10 or more studies were included in the meta-analysis, we planned to investigate reporting bias such as publication bias using funnel plots. We intended to assess funnel plot asymmetry visually. If asymmetry was suggested by a visual assessment, we then planned to perform additional analyses to investigate it by using an Egger regression test to quantify the magnitude of asymmetry, and a trim and fill analysis to assess the potential effect of funnel plot asymmetry on the estimated mean effect size. There were not enough included studies to conduct these assessments of reporting bias.

Data synthesis

We planned to combine data from individual trials for meta-analysis when the interventions, patient groups, and outcomes were sufficiently similar (as determined by consensus). We planned to synthesize effect sizes in the meta-analysis using a random effects model, using the restricted maximum likelihood estimator for the random-effects variance component. Because none of the included studies were conceptually similar in terms of interventions, patient groups, and outcomes, no meta-analyses were performed.

Subgroup analysis and investigation of heterogeneity

We planned the following subgroup analyses. Again, however, we were unable to conduct any subgroup analyses given that no meta-analyses were performed.

- STEC versus other causes of diarrhoea-associated HUS
- Children (< 18 years) versus adults (≥ 18 years)
- Outbreak settings versus non-outbreak settings
- Hospital setting versus community-based studies versus mixed/undefined settings
- Low and middle-income countries versus high-income countries.

Sensitivity analysis

We planned the following sensitivity analyses to explore the influence of the following factors on the estimated mean effect sizes.

- Repeating the analysis excluding unpublished studies
- Repeating the analysis excluding studies with high risk of bias on sequence generation
- Repeating the analysis excluding any small sample size studies.

We also planned to conduct sensitivity analyses to investigate the effect of missing data.

- 5% to 10% missing data
- 10% to 20% missing data
- 20% or more missing data.

Again, however, no sensitivity analyses were conducted given that no meta-analyses were performed.

Summary of findings and assessment of the certainty of the evidence

We present the main results of the review in 'Summary of findings' tables. These tables present key information concerning the quality of the evidence, the magnitude of the effects of the interventions examined, and the sum of the available data for the main outcomes (Schunemann 2011a). The 'Summary of findings' tables also include an overall grading of the evidence related to each of the main outcomes using the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) approach (GRADE 2008; GRADE 2011). The GRADE approach defines the quality of a body of evidence as the extent to which one can be confident that an estimate of effect or association is close to the true quantity of specific interest. The quality of a body of evidence involves consideration of within-trial risk of bias (methodological quality), directness of evidence, heterogeneity, precision of effect estimates and risk of publication bias (Schunemann 2011b). We presented the following outcomes in the 'Summary of findings' tables.

- Incidence of HUS in patients with diarrhoea
- Adverse events
- Need for acute dialysis
- Incidence of neurological complications
- Death (any cause)

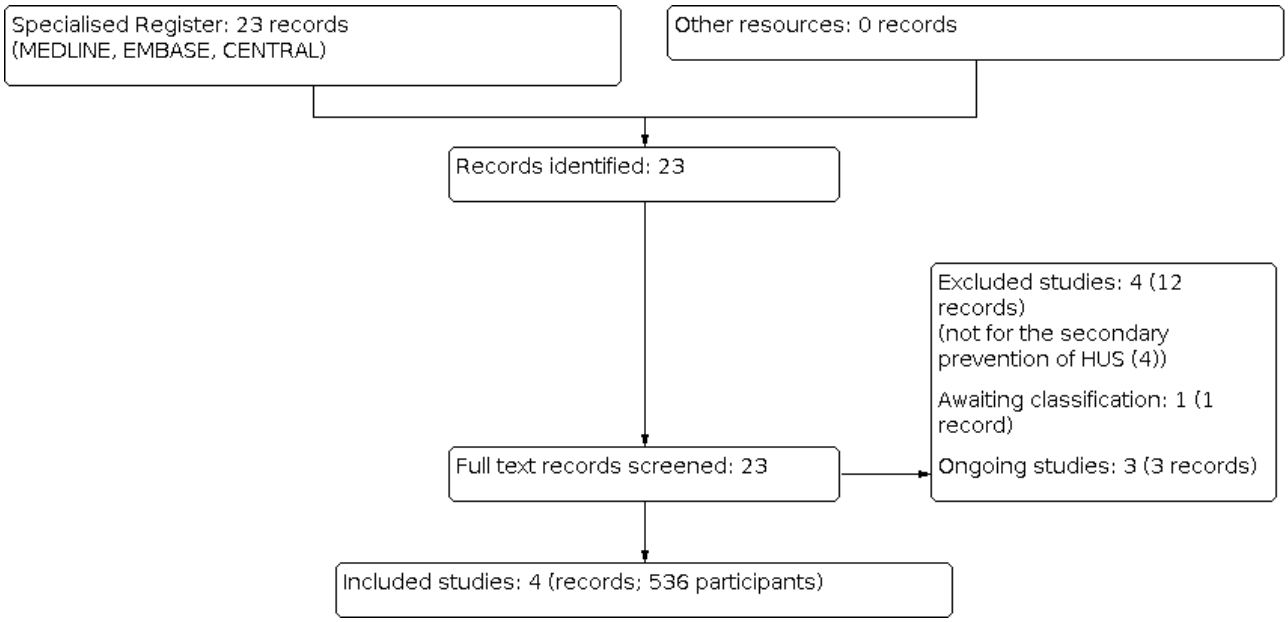
RESULTS

Description of studies

Results of the search

We searched Cochrane Kidney and Transplant's Specialised Register and identified 23 reports. Figure 1 provides the PRISMA flow diagram for selection of studies. Four studies were included (Huppertz 1999; Lopez 2010; Proulx 1992; Rowe 1995) and four studies were excluded (Caletti 2011; HUS-SYNSORB Pk 1998; NCT02205541; Pape 2009). There are three ongoing studies (NCT03275792; NCT04132375; SHIGATEC 2011) and one study is awaiting classification (McLaine 1995). These four studies will be evaluated in a future update of this review. See Figure 1.

Figure 1. Study flow diagram.



Included studies

See Characteristics of included studies.

Four studies (Huppertz 1999; Proulx 1992; Rowe 1995, Lopez 2010) randomising 536 patients were included. These four studies assessed four different interventions. These studies were then categorized according to the intervention assessed for the secondary prevention of HUS:

- Antibiotic therapy (trimethoprim/sulfamethoxazole)
- Anti-Shiga toxin antibody containing bovine colostrum
- Oral Shiga toxin binding agent (Synsorb Pk)
- Humanized monoclonal antibody (urtoxazumab).

Antibiotic therapy (trimethoprim/sulfamethoxazole)

Proulx 1992 was a parallel RCT that assessed the use of antibiotic therapy for *E. coli* O157:H7 enteritis. The study included 47 children with proven *E. coli* O157:H7 enteritis. Participants were randomised to one of the two treatment groups. The intervention group received 4/20 mg/kg of oral trimethoprim-sulfamethoxazole twice daily for five days. The control group received no treatment. The primary outcomes assessed were incidence of HUS, duration of diarrhoeal symptoms, and faecal excretion of pathogen. No funding source was specified by the authors.

Anti-Shiga toxin antibody containing bovine colostrum

Huppertz 1999 was a parallel RCT that assessed the use of anti-Shiga toxin containing antibodies bovine colostrum for treatment of diarrhoea-associated with diarrheogenic Shiga toxin-producing *E. coli* expressing intimin and haemolysin. The study included 30 children with diarrhoea whose stool cultures yielded *E. coli* containing gene *eae* which encodes intimin, in addition to Stx1, Stx2, or both or enterohaemorrhagic *E. coli* (EHEC)-haemolysin. The included children were randomised to one of the two treatment groups. The intervention group received 7 grams of bovine colostrum preparation given orally before meals three times/day for 14 days. The comparison group received placebo preparation that was similar in chemical composition and identical in appearance to the bovine colostrum treatment. The primary outcomes assessed were bovine colostrum oral tolerance, diarrhoeal stool frequency reduction, and faecal excretion of *E. coli* and occurrence of HUS. The study also included two patients who already had HUS. No funding source was specified by the authors.

Oral Shiga toxin binding agent (Synsorb Pk)

Rowe 1995 was a parallel RCT that assessed the use of Synsorb Pk for the prevention of HUS in children with STEC. Synsorb Pk is silicon-based oral Shiga toxin-binding agent that competitively inhibits the absorption of Shiga toxin from the gut. The study included 364 children diagnosed with *E. coli* O157, other verotoxin-producing *E. coli* (VTEC) infection, close contact with individuals diagnosed with HUS or VTEC infection, and symptoms consistent with VTEC infection. Participants were randomised to one of the two treatment groups. The intervention group received 500 mg/kg of Synsorb Pk mixed in baby food given orally divided into 2 doses/day for 7 days. The placebo group received equal volumes of corn meal. The primary outcome assessed was development of HUS at day 7 of treatment. No funding source was specified by the authors.

Humanised monoclonal antibody (urtoxazumab)

Lopez 2010 evaluated the pharmacokinetics and safety of urtoxazumab in adults and children. Urtoxazumab is a humanised monoclonal IgG subclass IgG1 against the B subunit of against Shiga toxin (Stx) 2. The first phase of the study included otherwise healthy adults aged 19 to 65 years. The adults were given a single IV 100 mL dose of urtoxazumab at four different dosage levels (0.1, 0.3, 1.0, 3.0 mg/kg/body weight). The control group received placebo. Safety, tolerability and pharmacokinetic were measured after the administration of the medication until day 7. The paediatric part of the study was a parallel RCT and included 109 children, aged from one to 15 years who had diarrhoea for less than 3 days and tested positive for STEC infection. The study excluded children with

chronic diseases and the children who had already developed the HUS. The paediatric study was conducted in two parts. In the first part of the study, children were divided into two cohorts. In the first cohort, participants were randomised to receive IV infusion of 1.0 mg/kg urtoxazumab and the control group received placebo. The second cohort received IV infusion of 3.0 mg/kg urtoxazumab and the control group received placebo. Safety and pharmacokinetic outcomes were measured. Once the safety was established, further 85 children were recruited into cohorts of 1.0 mg/kg and 3.0 mg/kg of urtoxazumab. The primary outcomes assessed were adverse events and efficacy outcomes. The authors identified the Program of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research of Japan as a funding source.

Excluded studies

See Characteristics of excluded studies

None of the four excluded studies gave the interventions for the secondary prevention of HUS. Two gave the intervention during episodes of HUS (HUS-SYNSORB Pk 1998; NCT02205541); one gave the intervention after HUS developed (Caletti 2011); and the intervention was given as a treatment for HUS (Pape 2009).

Studies awaiting classification

McLaine 1995 was only available as an abstract and no full text publication has been identified. This study compared Synsorb Pk to placebo, however no details on population, dose or outcomes were available.

See Characteristics of studies awaiting classification.

Ongoing studies

Three ongoing studies were identified and will be assessed in a future update of this review (NCT03275792; NCT04132375; SHIGATEC 2011). These studies plan to compare the following.

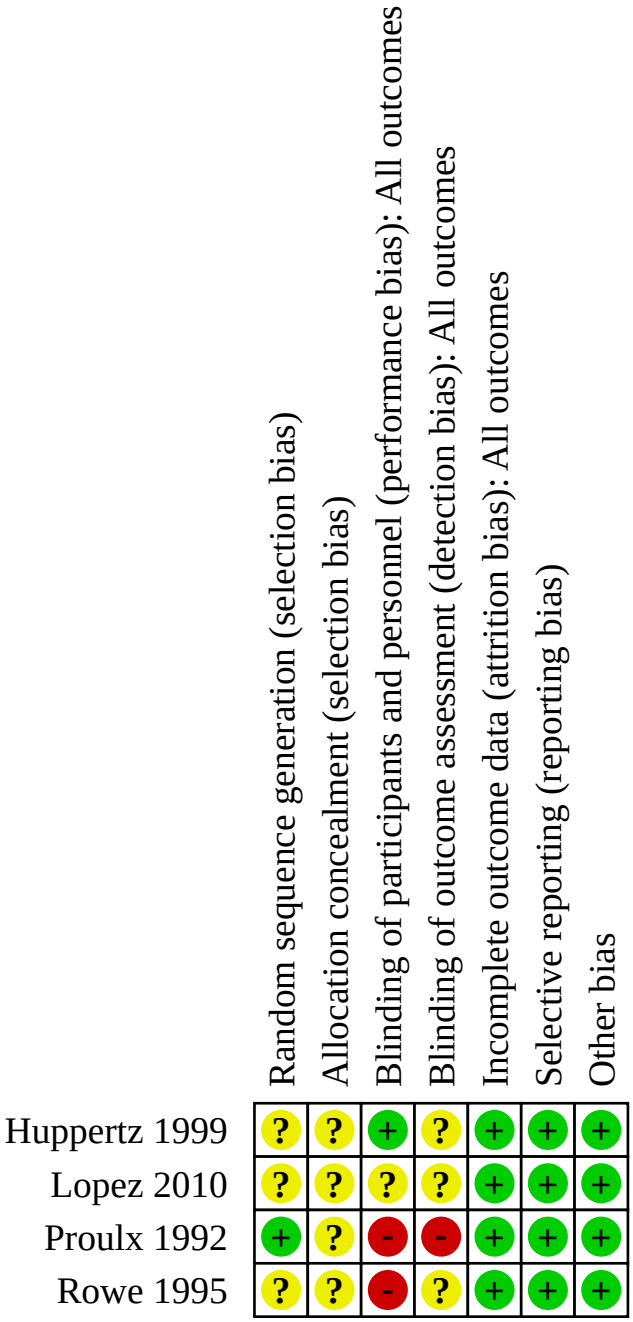
- Infusion of 40 mL/kg of 0.9% normal saline IV over 60 minutes, 0.9% normal saline with 5% dextrose at 150% of standard maintenance volume compared to standard emergency department care (NCT03275792)
- IV dose of 4 mg/kg INM004 (anti-Stx hyperimmune equine immunoglobulin F[ab']2 fragments) and a 2nd IV dose of 4 mg/kg of INM004 compared to placebo (NCT04132375)
- Shigamabs infused over one hour as a single infusion, Shigamabs. Two doses (1 and 3 mg/kg) are being tested in 2 sequential cohorts of children. Shigamabs is infused over one hour as a single infusion (SHIGATEC 2011)

See Characteristics of ongoing studies.

Risk of bias in included studies

The overall risk of bias in regard to allocation and selection across all four studies was generally unclear due to lack of discussion regarding allocation methods. The overall risk of bias in regard to blinding, performance and detection was generally high and unclear. We determined that the overall risk of bias in terms of incomplete outcome data and attrition was low across all four studies. Finally, we found that the overall risk of bias in regard to selective reporting and other potential biases was low for all four studies. Figure 2 show the risk of bias in the included studies.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

Random sequence generation

Proulx 1992 was judged to be at low risk of bias for random sequence generation, while Huppertz 1999, Lopez 2010 and Rowe 1995 were judged to have unclear risk of bias.

Allocation concealment

The risk of allocation bias was judged to be unclear for all four studies due to a lack of discussion of efforts to conceal allocation.

Blinding

Performance bias

Huppertz 1999 was judged to be a low risk of performance bias, Proulx 1992 and Rowe 1995 were judged to be at high risk of performance bias, and Lopez 2010 was determined to be at unclear risk of performance bias.

Detection bias

Huppertz 1999; Lopez 2010; Rowe 1995 were judged to be at unclear risk of detection bias while Proulx 1992 was judged to be at high risk of detection bias/

Incomplete outcome data

All four studies were judged to be at low risk of attrition bias.

Selective reporting

All four studies were judged to be a low risk of reporting bias.

Other potential sources of bias

There were no other potential sources of bias in any of the four studies.

Effects of interventions

See: **Summary of findings 1** Antibiotics versus no treatment for secondary prevention of HUS; **Summary of findings 2** Bovine colostrum versus placebo for secondary prevention of HUS; **Summary of findings 3** Synsorb Pk versus placebo for secondary prevention of HUS; **Summary of findings 4** Urtoxazumab (3.0 mg/kg) versus placebo for secondary prevention of HUS; **Summary of findings 5** Urtoxazumab (1.0 mg/kg) versus placebo for secondary prevention of HUS

Trimethoprim-sulfamethoxazole versus no treatment

Proulx 1992 compared trimethoprim-sulfamethoxazole with no treatment and randomised a total of 47 participants. There were 2/22 (9%) patients with HUS in the trimethoprim-sulfamethoxazole group compared to 4/25 (16%) in the control group. See Summary of findings 1.

Incidence of HUS

It is uncertain whether trimethoprim-sulfamethoxazole reduces the incidence of HUS compared to no treatment (Analysis 1.1: RR 0.57; 95 % CI 0.11 to 2.81; very low certainty evidence) and was downgraded due to imprecision and risk of bias.

Other outcomes

This study did not report adverse events relevant to this review, need for acute dialysis, neurological complications or death.

The authors reported the data on effect of antibiotics on duration of symptoms compared to control. The data showed inconclusive evidence on whether trimethoprim-sulfamethoxazole had any influence on duration of symptoms for bloody stools, diarrhoea, abdominal pain, vomiting and fever.

Bovine colostrum versus placebo

Huppertz 1999 compared bovine colostrum to placebo and randomised a total of 27 participants. See Summary of findings 2.

Incidence of HUS

There were no incidences of HUS in either the treatment or control groups. The evidence was graded very low and was downgraded due to risk of bias and imprecision.

Adverse events

It is uncertain whether bovine colostrum causes more adverse events compared to placebo (Analysis 2.2: RR 0.92, 95% CI 0.42 to 2.03; very low certainty evidence) and was downgraded based on risk of bias and imprecision. Symptoms considered adverse were determined to be poor appetite, abdominal colic, and occasional vomiting.

Other outcomes

This study did not report need for acute dialysis, neurological complications or death.

The authors did, however, report on the effect of bovine colostrum on median stool frequency. The median stool frequency decreased from three stools/day to one stool/day in the intervention group whereas there was no change in stool frequency (three stools/day) in the control group.

Synsorb Pk versus placebo

Rowe 1995 compared Synsorb Pk with placebo and randomised a total of 353 participants. See Summary of findings 3.

Incidence of HUS

It is uncertain whether Synsorb Pk reduces the incidence of HUS compared to placebo (Analysis 3.1: RR 0.93, 95% CI 0.39 to 2.22; very low certainty evidence) and was downgraded due to imprecision and risk of bias.

Other outcomes

This study did not report adverse events relevant to this review, need for acute dialysis, neurological complications or death.

Urtoxazumab versus placebo

Lopez 2010 compared two doses of urtoxazumab (3.0 mg/kg and 1.0 mg/kg) to placebo and randomised 107 participants. See Summary of findings 4 and Summary of findings 5

Incidence of HUS

It is uncertain whether either 3.0 mg/kg urtoxazumab (Analysis 4.1.1 (71 participants): RR 0.34, 95% CI 0.01 to 8.14; very low

certainty evidence) or 1.0 mg/kg urtoxazumab (Analysis 4.1.2 (74 participants): RR 0.95, 95% CI 0.79 to 1.13; very low certainty evidence) reduced the incidence of HUS compared to placebo. The GRADE ratings were downgraded due to imprecision and risk of bias.

Adverse events

Treatment-emergent adverse events (non-serious)

Low certainty evidence showed there may be little or no difference in the number of treatment-emergent adverse events with either 3.0 mg/kg urtoxazumab (Analysis 4.1.1 (71 participants): RR 1.00, 95% CI 0.84 to 1.18) or 1.0 mg/kg urtoxazumab (Analysis 4.1.2 (74 participants): RR 0.95, 95% CI 0.79 to 1.13) compared to placebo. The GRADE ratings for these outcomes were downgraded based on risk of bias and imprecision.

Serious adverse events

Lopez 2010 reported a total of 25 serious adverse events in 18 patients: 10 in the placebo group, and 9 and 6 serious adverse events in the 1.0 mg/kg and 3.0 mg/kg urtoxazumab groups, respectively. It is unclear how many patients experienced these adverse events in each group, and how many patients experienced more than one event. It should be noted that only four serious adverse events were considered by Lopez 2010 to be remotely related to urtoxazumab. The serious adverse events included hypokalaemia, intussusception, and HUS in the 1.0 mg/kg urtoxazumab group and HUS in the placebo group.

Neurological complications

It is uncertain whether either 3.0 mg/kg urtoxazumab (Analysis 4.3.1 (71 participants): RR 2.06, 95% CI 0.20 to 21.68; very low certainty evidence) or 1.0 mg/kg urtoxazumab (Analysis 4.3.2 (74 participants): RR 2.84, 95% CI 0.31 to 26.08; very low certainty evidence) increased the risk of neurological complications compared to placebo. The GRADE ratings for these outcomes were downgraded due to imprecision and risk of bias.

Death (any cause)

It is uncertain whether either 3.0 mg/kg urtoxazumab (Analysis 4.4.1 (71 participants): RR 0.34, 95% CI 0.01 to 8.14; very low certainty evidence) or 1.0 mg/kg urtoxazumab (Analysis 4.4.2 (74 participants): RR 0.95, 95% CI 0.06 to 14.59; very low certainty evidence) compared to placebo. The GRADE ratings for these outcomes were downgraded for risk of bias and imprecision.

Other outcomes

Need for acute dialysis was not reported.

DISCUSSION

Summary of main results

This systematic review assessed interventions for secondary prevention of HUS in patients with gastrointestinal infection due to STEC. We identified four studies that examined four different interventions including antibiotics, anti-Shiga toxin antibodies containing bovine colostrum, a Shiga toxin binding agent (Synsorb Pk) and monoclonal antibodies (urtoxazumab) against Shiga toxin-2. The included studies reported a range of outcomes. However, the sample sizes were small, and given the small number of identified studies, no firm conclusions can be drawn at this time about the efficacy of these intervention for secondary prevention of HUS in patients with STEC. We identified a number of ongoing studies that could add significant contributions to the field in the future.

Overall completeness and applicability of evidence

The included studies that addressed four different interventions all had small sample sizes. The number of events was low for most of the outcomes in the included studies, resulting in wide 95% CIs around the summary estimates. The study with the largest sample size (Rowe 1995) included 364 children and tested Synsorb Pk versus placebo for the prevention of HUS in children with verotoxin-producing *E. coli* (VTEC) gastroenteritis. This study was available only in abstract form and the study has not as yet been published in a peer review journal. We tried to reach out to authors to obtain more details about the study however could not establish contact. Nonetheless, the number of events was low for incidence of HUS, hence no firm conclusions can be drawn from the summary estimate.

The second largest study (Lopez 2010) addressed pharmacokinetics of urtoxazumab in adults and its efficacy and safety in prevention of HUS and adverse events in 109 children. The number of events was low for incidence of HUS in the intervention and control group, and the 95% CI around the estimate was wide. The study reported treatment-emergent (non-serious) adverse event and serious adverse events. More than 85% of the patient population experienced at least one adverse event and these adverse events were present all three study groups, including the placebo group. There was no significant difference in prevalence of non-serious adverse events between any of the study group and the placebo. The most commonly reported adverse events were related to blood and lymphatic system disorders followed by infections and infestations and gastrointestinal disorders. Authors further described that among all the adverse events reported in the study about four patients had an adverse event thought to be related to the study medication. Three of these were mild in intensity and one was moderate. The mild adverse events included fever, headache and erythema, while the moderate event was vomiting.

This study also reported 25 serious events with almost equal proportion in all the study groups including the control group (Lopez 2010). A serious adverse event is defined by FDA 2016 as an adverse event in human drug trials that lead to any untoward medical occurrence that at any dose can results in death, is life threatening, and require hospitalisations, results in persistent or significant disability, may have caused congenital anomaly and require intervention to prevent permanent impairment and damage. Lopez 2010 describe that in their study 4/25 serious adverse events were considered to be remotely related to the study drug. This included hypokalaemia, intussusception and HUS (two cases, one in urtoxazumab 1 mg/kg/dose group and one case in the placebo group). There were two deaths reported, one in the urtoxazumab 1 mg/kg/dose group and other in the placebo group. Overall, the study did not show any convincing evidence of increased incidence of treatment-related (non-serious) adverse events or serious adverse events in the study groups versus the control groups.

Quality of the evidence

There were varying degrees of risk of bias among the four included studies. In Huppertz 1999 the risk of selection and detection bias was unclear, but the risk of performance, attrition, and reporting bias was low. The GRADE quality of evidence from this study was considered very low due to risk of bias and small sample size. In Lopez 2010 the risk of selection, performance, and detection bias was unclear, but the risk of attrition and reporting bias was low. The GRADE quality of evidence from this study was rated very low for incidence of HUS and low for adverse events. In Proulx 1992, the risk of performance and detection bias was high, the risk of selection bias was unclear, while the risk of attrition and reporting bias was low. The GRADE quality of evidence was rated very low. Finally, in Rowe 1995, the risk of performance bias was high, the risk of selection and detection bias was unclear, and the risk of attrition and reporting bias was low. The GRADE quality of evidence was rated as very low due to concern for risk of bias and low imprecision in the summary estimate due to low number of events in the intervention and control groups.

Potential biases in the review process

We followed the standard guidelines of Cochrane Kidney and Transplant Register. We performed a comprehensive search of multiple databases. Data were extracted by two authors using standard Cochrane standard data extraction forms. The risk of bias assessment was conducted in accordance with the Cochrane Handbook. We intentionally did not conduct a meta-analysis to quantitatively synthesize effect size given that the small number of included studies that examined different types of interventions for different patient populations and different outcome measures. We did, however, identify several ongoing studies examining secondary prevention of HUS on primary outcomes of interest; future updates to this review may permit meta-analyses given the accrual of additional evidence from these ongoing studies.

Agreements and disagreements with other studies or reviews

These results from this review differ from those of past Cochrane Reviews. In Michael 2009, methods for treating HUS and TTP were evaluated by conducting a meta-analysis of RCTs assessing the efficacy of different forms of treatment. The authors concluded that plasma exchange with fresh frozen plasma was the most effective treatment of these conditions. In this review, we attempted to evaluate methods for secondary prevention of HUS in paediatric patients who had contracted a diarrhoeal illness due to STEC. Thomas 2013 conducted a non-Cochrane systematic review looking at the prevention of diarrhoea-associated HUS and included animal and human studies. The authors of this review found three RCTs in humans, two that are reported in our review as well (Proulx 1992; Rowe 1995); and, one additional study that focused on the primary prevention of STEC infection rather than secondary prevention of HUS after the STEC infection. We report the similar findings from the two included studies that were included in both the reviews (Proulx 1992; Rowe 1995) and included two additional studies (Huppertz 1999; Lopez 2010).

AUTHORS' CONCLUSIONS

Implications for practice

We identified four different studies that assessed various interventions including the use of antibiotics, anti-Shiga toxin antibodies containing bovine colostrum, and Shiga toxin binding agent (Synsorb Pk) and monoclonal antibodies (Urtoxazumab) in the secondary prevention of HUS in children with *E. coli* O157:H7 enteritis. However, no conclusions regarding the implications for practice can be drawn at this time due to the small number of RCTs in this field of research and the relatively small sample sizes of those few existing trials.

Implications for research

The available studies target four different interventions that could be used to prevent secondary occurrence of HUS in patients with STEC. One small study tested a well-known antibiotic (trimethoprim-sulfamethoxazole) and showed no significant effect for secondary prevention of HUS in patients with STEC (Proulx 1992). Data from observational studies showed that use of antibiotics in patients with STEC might increase the risk of HUS hence the recommendation against their use by CDC and American gastroenterology association (CDC 2018a; Riddle 2016; Wong 2000; Wong 2012). Any future efforts to test antibiotics for prevention of HUS should start with determination of their safety profile in animal studies before a human trial is conducted on this intervention for prevention of HUS in patients with STEC.

Bovine colostrum containing anti-Shiga toxin antibodies is a potential therapy as colostrum has been used for protection against enteric pathogens (Brunser 1992; Mieten 1979). Use of bovine colostrum could be a cost-effective method however the included study had a small sample size with no HUS events, so it is hard to make any suggestion to test this intervention in the future studies.

Use of oral Shiga toxin binding agent like Synsorb Pk seems to be an attractive intervention that needs further investigations. Unfortunately, the included study (Rowe 1995) was only available as an abstract so details of the intervention were not readily available. Synsorb Pk had been tested in a RCT for the treatment of HUS (Trachtman 2003) and even though it did not show an effect for treatment of HUS, it was found to be safe for use. An interesting observation from the available data from Rowe 1995 was that effect of Synsorb Pk seems to be prominent if the intervention was given within four days of diagnosis. This observation, along with lack of effect of Synsorb Pk for treatment of HUS, indicate that there is a short window period in the early days of the disease where oral Synsorb Pk could be helpful to prevent subsequent development of HUS. The early use is attached to early diagnosis of STEC-associated diarrhoea. The early diagnosis can be potentially achieved with newly available, culture-independent, rapid diagnostic techniques (Buchan 2013), hence an opportunity to start the intervention early. We suggest that this medication should be further tested in the future studies and these studies should focus on early recruitment of the participants to assess the preventive effect of Synsorb Pk for HUS in patients with STEC.

Intravenous use of monoclonal antibodies against Shiga toxin is a potential strategy to mitigate the effect of Shiga toxin to induce the development of HUS. Even though the only included study on this intervention (Lopez 2010) did not show an effect

of urtoxazumab (a monoclonal antibody against Shiga toxin) on incidence of HUS however this study was not meant to define the efficacy of the intervention for secondary prevention of HUS but the pharmacokinetics and safety profile. Even though the number of adverse events, both serious and non-serious adverse events, were common in study participants, they were proportionally distributed in the two study groups and control group and there was no statistically significant difference between the study groups and the placebo. There was no increased risk of serious or non-serious adverse events in the intervention groups compared to the control group. We therefore think this intervention should be tested in further larger RCTs to assess its efficacy for secondary prevention of HUS in patients with STEC. This effort might require a multicentre potentially multi-country study as the incidence of STEC-associated HUS has been decreasing with better primary preventive strategies around safe food practices and early discovery and control of STEC-related outbreaks (CDC 2018b).

We also found two other studies that assessed efficacy of monoclonal antibodies against Shiga toxin for prevention of HUS in patients with STEC (NCT04132375; SHIGATEC 2011). We hope to include the results of these studies in a future update.

We aimed to assess the volume expansion with aggressive hydration at the time of STEC infection for secondary prevention of HUS in these patients. We did not find any published clinical study that addressed this intervention. The data from observational studies and meta-analysis of observational studies suggest that this intervention could be a potential intervention for secondary prevention of HUS in patients with STEC infection (Ardissino 2016; Grisaru 2017). This intervention should therefore be tested further in the future studies. We found an ongoing study (NCT03275792) addressing the secondary prevention of HUS in STEC infected patients and we hope to include the results of this study in the next update of this review.

ACKNOWLEDGEMENTS

We wish to thank the referees for their comments and feedback during the preparation of this protocol and the review.

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Huppertz 1999

Study characteristics

Methods	• Study design: parallel RCT • Study duration: July 1993 to June 1996

Huppertz 1999 *(Continued)*

	• Study follow-up period: 21 days
Participants	• Country: Germany • Setting: urban inpatient children's hospitals in the Wurzburg region • Inclusion criteria: children with diarrhoea whose stool cultures yielded *E. coli* containing *eae* which encodes intimin, in addition to *stx1*, *stx2*, or both or the EHEC-hemolysin gene • Number: treatment group (13); control group (14) • Mean age, range: treatment group (2 years, 5 months to 18 years); control group (1 years, 3 months to 12 years) • Sex (M/F): treatment group (5/8); control group (8/6) • Exclusion criteria: stool cultures positive for enteropathogenic *E. coli* adherence factor; unknown time of onset of diarrhoea; history of bovine milk intolerance; treatment of diarrhoea with drugs; breast-feeding; vomiting causing interference with drug administration
Interventions	Treatment group • Bovine colostrum preparation: 7 g prepared following the guidelines for the preparation of infant's milk and contained 80% protein with > 65% immunoglobulin, mainly IgG (Lactobin, Biotest Pharma, Dreieich, Germany), milk obtained from 100 carefully supervised cows not immunized against *E. coli* strains and containing high titres of antibodies against Stx1, Stx2, and EHEC-hemolysin) given before meals 3 times/day for 14 days Control group • Placebo: an innocuous preparation devoid of antibodies but similar in chemical composition and identical in appearance with bovine colostrum
Outcomes	• Occurrence of HUS: patients assessed every other day during admission by inpatient hospital staff, once weekly thereafter, and on day 15 and day 21 by parents for symptoms and laboratory evidence of HUS
Notes	• Funding source: not reported • Study also included two patients with HUS at the time of recruitment. These patients were not considered for outcome assessment in this review

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "Patients meeting the entry criteria and still in the hospital at the time of the bacteriologic diagnosis were randomly allocated to receive either bovine colostrum or placebo administered double-blind.." Comment: The authors do not provide any further details on how the randomisation was done
Allocation concealment (selection bias)	Unclear risk	Comment: Authors do not provide any details of concealment of allocation
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "an innocuous preparation devoid of antibodies but similar in chemical composition and identical in appearance with bovine colostrum, served as placebo..." Comment: Most likely done
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Comment: Even though the authors claimed that it was a double-blind study, no details were provided for blinding of the assessors

Huppertz 1999 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	Low risk	Two patients were lost to follow-up in the intervention group and one in the control group
Selective reporting (reporting bias)	Low risk	Comment: Authors seems to report all the relevant outcomes
Other bias	Low risk	Study also included two patients with HUS at the time of recruitment. These patients were not considered for outcome assessment in this review

Lopez 2010

Study characteristics	
Methods	• Study design: parallel RCT • Study duration: not reported • Study follow-up period: 4 months
Participants	• Country: Argentina • Setting: not reported • Inclusion criteria: children age 1 to 15 years with bloody diarrhoea for 72 hours at the time of study drug administration; and were positive for STEC infection, as determined by assessing a stool sample by the use of diagnostic test kits and/or a PCR assay • Number: treatment group 1 (38); treatment group 2 (35); control group (36) • Mean age ± SD (years): treatment group 1 (2.9 ± 2.4); treatment group 2 (2.8 ± 2.4); control group (2.7 ± 2.5) • Sex (M/F): treatment group 1 (10/9); treatment group 2 (17/18); control group (5/4) • Exclusion criteria: 2 or more symptoms indicative of the early stages of HUS (haemolytic anaemia, red blood cell fragmentation, decreased platelet count, advanced haematuria, or highly elevated SCr; were anuric or oliguric; evidence of clinically significant gastrointestinal disease; history of anaemia, kidney disease, thrombocytopenia, inflammatory bowel disease, or immunodeficiency; severely dehydrated; medical history of exposure to murine or human MAbs; administered antibiotics or antimotility or laxative drugs
Interventions	Treatment group 1 • Urtoxazumab infusion: 1.0 mg/kg (1:2 ratio) in a total volume of 10 mL/kg for those with body weights of 10 kg or up to a maximum infusion volume of 100 mL for those with body weights of 10 kg or more given over 1 hour Treatment group 2 • Urtoxazumab infusion: 3.0 mg/kg (1:2 ratio) in a total volume of 10 mL/kg for those with body weights of 10 kg or up to a maximum infusion volume of 100 mL for those with body weights of 10 kg or more given over 1 hour Control group • Placebo: Infusion: in a total volume of 10 mL/kg for those with body weights of 10 kg or up to a maximum infusion volume of 100 mL for those with body weights of 10 kg or more given over 1 hour
Outcomes	• Occurrence of HUS: measured at follow-up on the first day (up to 8 hours following the infusion); on days 2, 4, 6, 8, 22, and 57; and at month 4

Lopez 2010 *(Continued)*

Notes	• Funding source: the generation of urtoxazumab from the parental murine monoclonal antibody and its characterization were financially supported by the Program of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research of Japan

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "STEC-infected pediatric patients were sequentially enrolled in double-blind randomised fashion into one of two cohorts of 12 patients each." Comment: The authors do not provide any further details on how the randomisation was done
Allocation concealment (selection bias)	Unclear risk	Comment: Authors do not provide any details of concealment of allocation
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Quote: "The patients in the first cohort were stratified to receive an i.v. infusion of either placebo or 1.0 mg/kg urtoxazumab (1:2 ratio) in a total volume of 10 ml/kg." Comment: Unclear whether placebo appeared different from study drug preparation
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Comment: Even though the authors claimed that it was a double-blind study, no details were provided for blinding of the assessors
Incomplete outcome data (attrition bias) All outcomes	Low risk	Comment: The authors report outcomes for all patients enrolled at the start of the study
Selective reporting (reporting bias)	Low risk	Comment: Authors seems to report all the relevant outcomes
Other bias	Low risk	No other risk of bias was noted

Proulx 1992

Study characteristics

Methods	• Study design: parallel RCT • Study duration: 1 June 1989 to 1 June 1990 • Study follow-up period: 1 month
Participants	• Country: Canada • Setting: inpatients • Relevant health status: children admitted to St. Justine Pediatric Hospital for diarrhoeal illness or seen in emergency department for diarrhoeal illness with positive stool screen for *E. coli* O157:H7 • Number: treatment group (22); control group (25) • Mean age ± SD (years): treatment group (4.91 ± 3.88); control group (5.73 ± 4.63) • Sex (M/F): not reported • Exclusion criteria: children who already had HUS at start of trial; could not be enrolled within 24 hours of obtaining culture results; history of allergy to sulfonamide antibiotics

Proulx 1992 *(Continued)*

Interventions	Treatment group • • Trimethoprim-sulfamethoxazole (oral): 4/20 mg/kg twice/day for 5 days Control group • No treatment
Outcomes	• Occurrence of HUS: HUS was defined as the presence of anaemia with a haemoglobin value at less than the 3rd percentile for age, the presence of thrombocytopenia (platelet count < 100 x 10^9/L), the presence of schistocytes on blood smear, and AKI (with a creatinine value greater than the 90th percentile for age), measured at Initial point of specimen testing positive of O157:H7 to 1 month following end treatment by inpatient hospital staff during hospitalisation and parents after discharge
Notes	• Funding source: not reported

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Patients were randomly selected, according to a computer-generated list of random numbers, to receive either a standard dose of TMP-SMX twice daily for 5 days or to receive no treatment."
Allocation concealment (selection bias)	Unclear risk	Comment: Authors do not provide any details of allocation concealment
Blinding of participants and personnel (performance bias) All outcomes	High risk	Quote: "No placebo was available, neither patients nor treating physicians were unaware of the treatment."
Blinding of outcome assessment (detection bias) All outcomes	High risk	Quote: "No placebo was available, neither patients nor treating physicians were unaware of the treatment."
Incomplete outcome data (attrition bias) All outcomes	Low risk	Quote: "No patients were lost to follow up."
Selective reporting (reporting bias)	Low risk	Comment: Authors seem to report all relevant outcomes
Other bias	Low risk	No other risk of bias was noted

Rowe 1995

Study characteristics

Methods	• Study design: parallel RCT • Study duration: not reported • Study follow-up period: 7 days
Participants	• Country: Canada • Setting: inpatient

Rowe 1995 *(Continued)*

	• Inclusion criteria: children with diarrhoeal illness and documented STEC O157 (or other STEC serogroup) infection, close contact with an individual with HUS or STEC infection, or symptoms consistent with STEC infection (abdominal cramping, bloody diarrhoea, rectal prolapse) • Number: treatment group (174); control group (179) • Mean age ± SD (years): not reported • Sex (M/F): not reported • Exclusion criteria: lab evidence of mild HUS or haemolytic anaemia at start of trial
Interventions	Treatment group • Synsorb-Pk: 500 mg/kg mixed in baby food given orally divided into 2 doses/day for 7 days Control group • Placebo: equal volume of corn meal given in same dose as treatment group
Outcomes	• Occurrence of HUS: lab evidence of HUS assessed at day 7 of treatment
Notes	• Funding source: Synsorb Biotech Inc. Calgary

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "Eligible subjects received 500 mg/kg of Synsorb-Pk mixed in baby food divided BID for 7 days or an equal volume of corn meal." Comment: Authors do not provide any further details on how the randomisation was done. Also, full text was not available for further evaluation
Allocation concealment (selection bias)	Unclear risk	Comment: Authors do not provide any details of allocation concealment
Blinding of participants and personnel (performance bias) All outcomes	High risk	Quote: "Eligible subjects received 500 mg/kg of Synsorb-Pk mixed in baby food divided BID for 7 days or an equal volume of corn meal." Comment: The control seems to be different from the intervention group in appearance and taste
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Comment: No details were provided on blinding of assessors
Incomplete outcome data (attrition bias) All outcomes	Low risk	11 subjects were excluded after randomisation, 7 from the treatment group and 4 from the control group. Overall loss to follow up was 3.1%
Selective reporting (reporting bias)	Low risk	Comment: Authors seem to report all relevant outcomes
Other bias	Low risk	No other risk of bias was noted

AKI - acute kidney injury; HUS - haemolytic uraemic syndrome; M/F - male/female; RCT - randomised controlled trial; SCr - serum creatinine

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Caletti 2011	The intervention was given after the HUS was developed and not as a secondary prevention
HUS-SYNSORB Pk 1998	The intervention was given during an episode of HUS and not as a secondary prevention
NCT02205541	The study aims to give the intervention during an episode of HUS and not as secondary prevention
Pape 2009	The intervention was given as a treatment for HUS and not as a secondary prevention

HUS - haemolytic uraemic syndrome

Characteristics of studies awaiting classification *[ordered by study ID]*

McLaine 1995

Methods	• Study design: parallel RCT • Study duration: 1 June 1994 to 1 June 1996 • Study follow-up period: not reported
Participants	• Country: Canada • Setting: not reported • Inclusion criteria: children age 6 months to 6 years with diarrhoea defined as 2 or more loose stools in the 24-hour period prior to enrolment and one of the following: bloody diarrhoea, non-bloody, diarrhoea with severe abdominal cramps, rectal prolapse, evidence of *E. coli* 0157 or other STEC serotypes from stool culture, or diarrhoea in someone who was in close contact with an individual with HUS or proven STEC infection • Number: 135 total enrolled • Mean age ± SD (years): not reported • Sex (M/F): not reported • Exclusion criteria: not reported
Interventions	Treatment group • Synsorb Pk: details not reported Control group • Placebo: details not reported
Outcomes	• Occurrence of HUS: methods for measurement of outcome not reported
Notes	• Funding source: not reported

HUS - haemolytic uraemic syndrome; RCT - randomised controlled trial

Characteristics of ongoing studies *[ordered by study ID]*

NCT03275792

Study name	Shiga toxin producing Escherichia coli (STEC) volume expansion
Methods	• Study design: parallel RCT • Study duration: May 2019 to April 2020 • Study follow-up period: 24 months

NCT03275792 *(Continued)*

Participants	• Country: Canada • Setting: urban inpatient children's hospital • Inclusion criteria: children age 6 months to 18 years with STEC infection determined by positive culture, antigen, or PCR for Stx/gene, and are in day 1-10 of illness 1-10 on presentation • Number: 30 participants (estimated enrolment) • Mean age ± SD (years): not reported • Sex (M/F): not reported • Exclusion criteria: evidence of evolving HUS determined by HCT < 30% or platelet count < 150 x 10^9/L; physician desires patient admission (therefore unable to randomise); unable to contact family within 48 hours of positive stool test; history of atypical HUS; chronic disease limiting fluid volumes administered
Interventions	Treatment group • Infusion of 40 mL/kg of 0.9% normal saline IV over 60 minutes, 0.9% normal saline with 5% dextrose at 150% of standard maintenance volume. If urine output is < 0.5 mL/kg/hr over a 12-hour period (AKI stage 2), repeat 20 mL/kg bolus or boluses of 0.9% normal saline will be infused as long as there are no signs of central volume overload. Oral fluids ad lib along with strict input/output documentation. • Intervention length 2 to 4 days Control group • Following standard ED care (volume status assessed; dehydration corrected employing oral rehydration in children with mild to moderate dehydration (most common); IV if severe (rarely)), children are discharged with saline lock IV (routine procedure across Canadian pediatric EDs) • Oral fluids (preferably electrolyte maintenance solutions) ad lib following ED discharge
Outcomes	• Occurrence of HUS: urine biomarkers to predict progression to AKI and HUS at the end of 24 months
Starting date	May 2019
Contact information	Stephen Freedman, MDCM, MSc, 403-955-7740, stephen.freedman@ahs.ca Karen Lowerison, 403-955-3186, karen.lowerison@ahs.ca
Notes	• Funding source: not reported

NCT04132375

Study name	Phase 2/3 study to evaluate PK, safety & efficacy of INM004 in STEC positive pediatric patients for prevention of HUS
Methods	• Study design: parallel RCT • Study duration: 17 July 2019 to 1 September 2022 • Study follow-up period: 4 weeks
Participants	• Country: Argentina • Setting: urban inpatient children's hospital • Inclusion criteria: children aged 1 to 10 years with bloody diarrhoea based upon history or presentation, with detection of Stx2 in stool based on enzyme immunoassay and/or stx2 based on PCR before randomisation • Number: 396 participants estimated • Mean age ± SD (years): not reported

NCT04132375 *(Continued)*

	• Sex (M/F): not reported • Exclusion criteria: Any laboratory findings compatible with the development of HUS: microangiopathic haemolytic anaemia defined as LDH above the upper limit of normal for age with the finding of schistocytes on peripheral smear and a negative Coombs' test, and/or thrombocytopenia: platelet count < 150 × 10^3/μL, and/or kidney failure: SCr > upper limit of normal adjusted for age and gender criteria despite correction of hypovolaemia, and/or haematuria, and/or proteinuria, history of chronic/recurrent haemolytic anaemia, thrombocytopenia, or CKD, family history of HUS, evidence of clinically significant chronic active disease not medically controlled, history of anaphylaxis, prior administration of equine serum (e.g. antitetanus serum or anti-ophidic serum, or anti-arachnid toxin serum), or allergic reaction to contact with, or exposure to, horses, family relation or work relation with a member of the personnel of the research group
Interventions	Treatment group • Subjects will receive a 1st IV dose of 4 mg/kg INM004 (anti-Stx hyperimmune equine immunoglobulin F[ab']2 fragments) and a 2nd IV dose of 4 mg/kg of INM004. Each dose will be separated by 24 h (± 2 h) Control group • Subjects will receive a 1st IV dose of placebo and a 2nd IV dose of placebo. Each dose will be separated by 24 h (± 2 h)
Outcomes	• Occurrence of HUS: methods for evaluation not specified
Starting date	July 17, 2019
Contact information	Mariana Colanna, Bioch, + 54 9 1161718697, mcolonna@inmunova.com Santiago Sanguineti, PhD, +54 9 11 4564-3625, ssanguineti@inmunova.com
Notes	Funding source: not reported

SHIGATEC 2011

Study name	Shigatec: a phase II study assessing monoclonal antibodies against Shiga toxin 1 and 2 in Shiga toxin-producing *E. coli*-infected children
Methods	• Study design: parallel, double blind, placebo-controlled RCT • Study duration: November 2010 to February 2011 • Study follow-up period: 12 months
Participants	• Country: Argentina • Setting: urban inpatient children's hospital • Inclusion criteria: children aged 6 months to 18 years with bloody diarrhoea (by visual inspection) for no more than 36 hours prior to screening and detection of Shiga toxin (Stx1 and/or Stx2) in stool • Number: 22 patients enrolled in low dose cohort, no data available for control group • Mean age (years): treatment group (3 years and 4 months) • Sex (M/F): treatment group (6/5) • Exclusion criteria: laboratory findings compatible with development of at least 2 out of 3 following criteria that define HUS: haemolytic anaemia: haematocrit < 30% with evidence of haemolysis (as indicated by LDH > upper limit of normal for age or the finding of schistocytes on peripheral smear); thrombocytopenia: platelet count < 150 x 10^3/μL; nephropathy: SCr > upper limit normal adjusted for age and gender; bloody-diarrhoea suspected not to be caused by Shiga toxin-producing bacteria but by other organisms or pre-existing diseases; family history of proven or suspected hereditary HUS or thrombotic thrombocytopenic purpura, history of chronic/recurrent haemolytic anaemia or thrombocytopenia

SHIGATEC 2011 *(Continued)*

Interventions	Treatment group • Shigamabs: 2 doses (1 and 3 mg/kg) are being tested in 2 sequential cohorts of 21 children. Shigamabs is infused over one hour as a single infusion Control group • Placebo infused in same manner as intervention
Outcomes	• Occurrence of HUS: lab evidence of HUS assessed throughout follow up period
Starting date	November 2010
Contact information	No contact information listed
Notes	The data were reported for 23 patients from low dose arm. No data were available from the control group. The study is ongoing and no final results are available

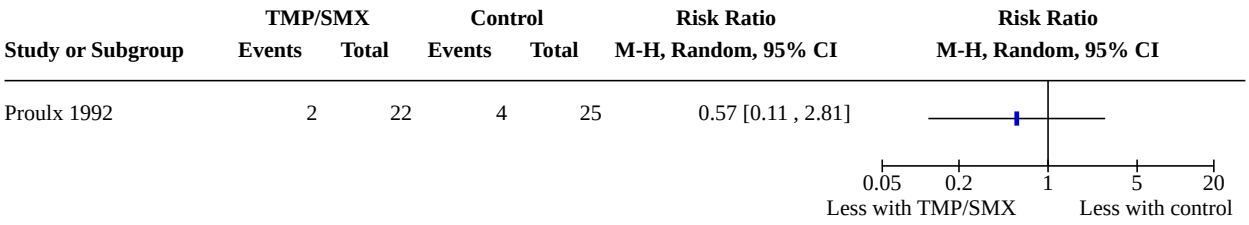
AKI - acute kidney injury; CKD - chronic kidney disease; ED - emergency department; HCT - haematocrit; HUS - haemolytic uraemic syndrome; IV - intravenous; LDL - lactate dehydrogenase; M/F - male/female; PCR - polymerase chain reaction; RCT - randomised controlled trial; SCr - serum creatinine; STEC - Shiga toxin producing Escherichia coli

DATA AND ANALYSES

Comparison 1. Trimethoprim/sulfamethoxazole versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Incidence of HUS	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected

Analysis 1.1. Comparison 1: Trimethoprim/sulfamethoxazole versus control, Outcome 1: Incidence of HUS

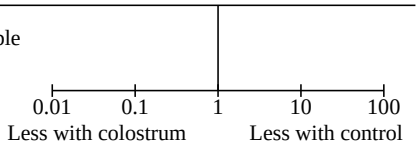


Comparison 2. Bovine colostrum versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 Incidence of HUS	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected
2.2 Adverse events	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected

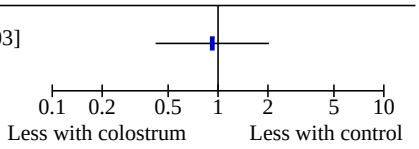
Analysis 2.1. Comparison 2: Bovine colostrum versus control, Outcome 1: Incidence of HUS

Study or Subgroup	Colostrum Events	Colostrum Total	Control Events	Control Total	Risk Ratio M-H, Random, 95% CI
Huppertz 1999	0	13	0	14	Not estimable



Analysis 2.2. Comparison 2: Bovine colostrum versus control, Outcome 2: Adverse events

Study or Subgroup	Colostrum Events	Colostrum Total	Control Events	Control Total	Risk Ratio M-H, Random, 95% CI
Huppertz 1999	6	13	7	14	0.92 [0.42 , 2.03]

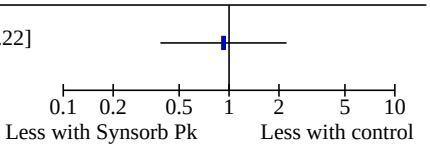


Comparison 3. Synsorb Pk versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.1 Incidence of HUS	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected

Analysis 3.1. Comparison 3: Synsorb Pk versus control, Outcome 1: Incidence of HUS

Study or Subgroup	Synsorb Pk Events	Synsorb Pk Total	Control Events	Control Total	Risk Ratio M-H, Random, 95% CI
Rowe 1995	9	174	10	179	0.93 [0.39 , 2.22]

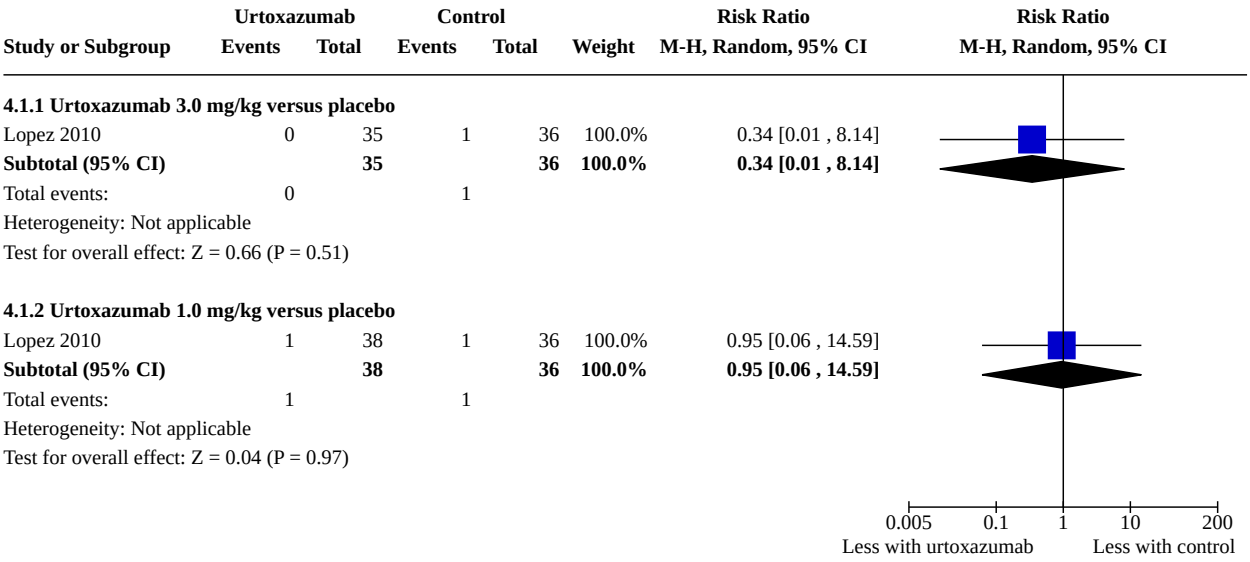


Comparison 4. Urtoxazumab versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.1 Incidence of HUS	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.1.1 Urtoxazumab 3.0 mg/kg versus placebo	1	71	Risk Ratio (M-H, Random, 95% CI)	0.34 [0.01, 8.14]

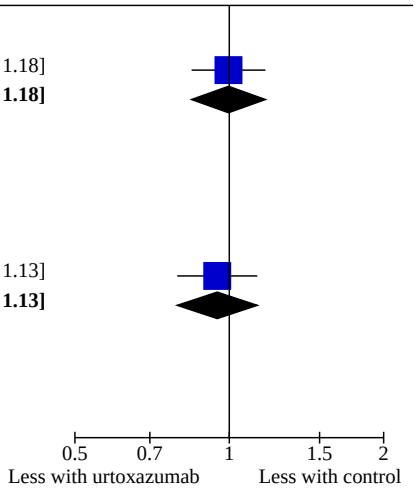
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.1.2 Urtoxazumab 1.0 mg/kg versus placebo	1	74	Risk Ratio (M-H, Random, 95% CI)	0.95 [0.06, 14.59]
4.2 Adverse events: treatment emergent	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.2.1 Urtoxazumab 3.0 mg/kg versus placebo	1	71	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.84, 1.18]
4.2.2 Urtoxazumab 1.0 mg/kg versus placebo	1	74	Risk Ratio (M-H, Random, 95% CI)	0.95 [0.79, 1.13]
4.3 Neurological complications	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.3.1 Urtoxazumab 3.0 mg/kg versus placebo	1	71	Risk Ratio (M-H, Random, 95% CI)	2.06 [0.20, 21.68]
4.3.2 Urtoxazumab 1.0 mg/kg versus placebo	1	74	Risk Ratio (M-H, Random, 95% CI)	2.84 [0.31, 26.08]
4.4 Death (any cause)	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.4.1 Urtoxazumab 3.0 mg/kg versus placebo	1	71	Risk Ratio (M-H, Random, 95% CI)	0.34 [0.01, 8.14]
4.4.2 Urtoxazumab 1.0 mg/kg versus placebo	1	74	Risk Ratio (M-H, Random, 95% CI)	0.95 [0.06, 14.59]

Analysis 4.1. Comparison 4: Urtoxazumab versus control, Outcome 1: Incidence of HUS



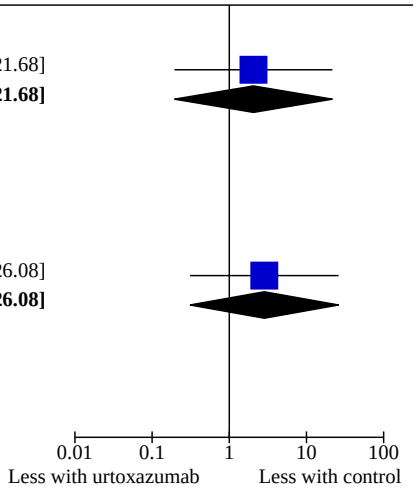
Analysis 4.2. Comparison 4: Urtoxazumab versus control, Outcome 2: Adverse events: treatment emergent

Study or Subgroup	Urtoxazumab Events	Urtoxazumab Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
4.2.1 Urtoxazumab 3.0 mg/kg versus placebo						
Lopez 2010	31	35	32	36	100.0%	1.00 [0.84 , 1.18]
Subtotal (95% CI)		**35**		**36**	**100.0%**	**1.00 [0.84 , 1.18]**
Total events:	31		32			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.04 (P = 0.97)						
4.2.2 Urtoxazumab 1.0 mg/kg versus placebo						
Lopez 2010	32	38	32	36	100.0%	0.95 [0.79 , 1.13]
Subtotal (95% CI)		**38**		**36**	**100.0%**	**0.95 [0.79 , 1.13]**
Total events:	32		32			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.59 (P = 0.56)						

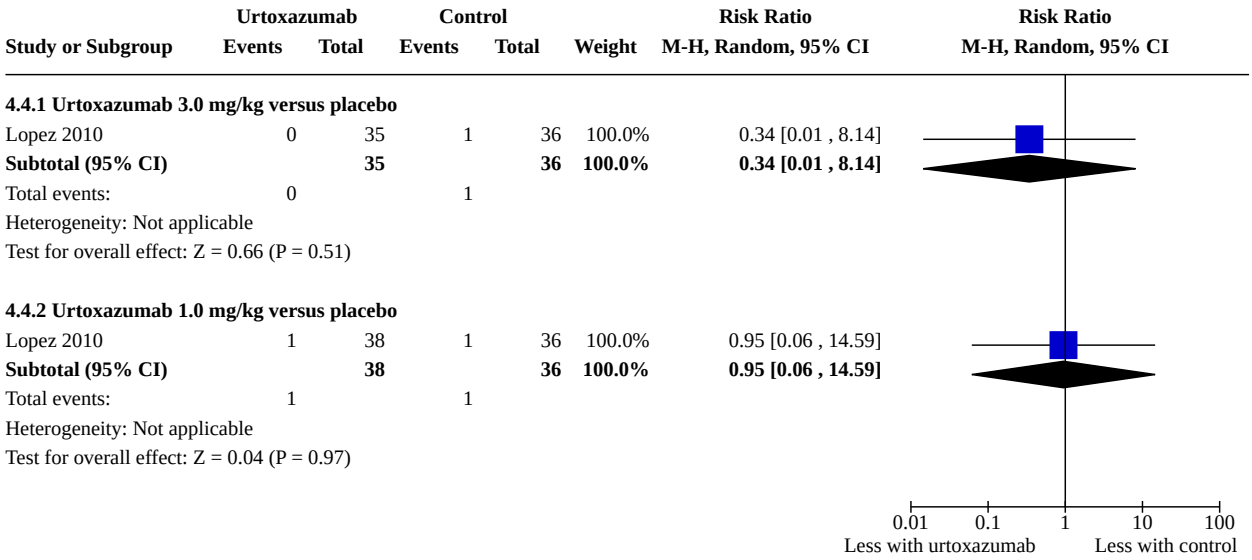


Analysis 4.3. Comparison 4: Urtoxazumab versus control, Outcome 3: Neurological complications

Study or Subgroup	Urtoxazumab Events	Urtoxazumab Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
4.3.1 Urtoxazumab 3.0 mg/kg versus placebo						
Lopez 2010	2	35	1	36	100.0%	2.06 [0.20 , 21.68]
Subtotal (95% CI)		**35**		**36**	**100.0%**	**2.06 [0.20 , 21.68]**
Total events:	2		1			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.60 (P = 0.55)						
4.3.2 Urtoxazumab 1.0 mg/kg versus placebo						
Lopez 2010	3	38	1	36	100.0%	2.84 [0.31 , 26.08]
Subtotal (95% CI)		**38**		**36**	**100.0%**	**2.84 [0.31 , 26.08]**
Total events:	3		1			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.92 (P = 0.36)						



Analysis 4.4. Comparison 4: Urtoxazumab versus control, Outcome 4: Death (any cause)



APPENDICES

Appendix 1. Electronic search strategies

Database	Search terms
CENTRAL	1. MeSH descriptor: [Hemolytic-Uremic Syndrome] this term only 2. MeSH descriptor: [Shiga-Toxigenic Escherichia coli] explode all trees 3. STEC-HUS:ti,ab,kw (Word variations have been searched) 4. "D+HUS":ti,ab,kw (Word variations have been searched) 5. "O157:H7":ti,ab,kw (Word variations have been searched) 6. diarrh*ea-associated h*emolytic ur*emic:ti,ab,kw (Word variations have been searched) 7. {or #1-#6}
MEDLINE	1. Hemolytic-Uremic Syndrome/ 2. STEC-HUS.tw. 3. exp Shiga-Toxigenic Escherichia coli/ 4. diarrh?ea-associated h?emolytic ur?emic.tw. 5. D+HUS.tw. 6. shiga-toxin$.tw. 7. "O157:H7".tw. 8. or/1-7
EMBASE	1. diarrh?ea-associated h?emolytic ur?emic.tw. 2. hemolytic uremic syndrome/ 3. STEC-HUS.tw. 4. shiga toxin producing escherichia coli/ 5. "O157:H7".tw. 6. D+HUS.tw. 7. or/1-6

Appendix 2. Risk of bias assessment tool

Potential source of bias	Assessment criteria
Random sequence generation Selection bias (biased allocation to interventions) due to inadequate generation of a randomised sequence	*Low risk of bias:* Random number table; computer random number generator; coin tossing; shuffling cards or envelopes; throwing dice; drawing of lots; minimisation (minimisation may be implemented without a random element, and this is considered to be equivalent to being random).
	High risk of bias: Sequence generated by odd or even date of birth; date (or day) of admission; sequence generated by hospital or clinic record number; allocation by judgement of the clinician; by preference of the participant; based on the results of a laboratory test or a series of tests; by availability of the intervention.
	Unclear: Insufficient information about the sequence generation process to permit judgement.
Allocation concealment Selection bias (biased allocation to interventions) due to inadequate concealment of allocations prior to assignment	*Low risk of bias:* Randomisation method described that would not allow investigator/participant to know or influence intervention group before eligible participant entered in the study (e.g. central allocation, including telephone, web-based, and pharmacy-controlled, randomisation; sequentially numbered drug containers of identical appearance; sequentially numbered, opaque, sealed envelopes).
	High risk of bias: Using an open random allocation schedule (e.g. a list of random numbers); assignment envelopes were used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or not sequentially numbered); alternation or rotation; date of birth; case record number; any other explicitly unconcealed procedure.
	Unclear: Randomisation stated but no information on method used is available.
Blinding of participants and personnel Performance bias due to knowledge of the allocated interventions by participants and personnel during the study	*Low risk of bias*: No blinding or incomplete blinding, but the review authors judge that the outcome is not likely to be influenced by lack of blinding; blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken.
	High risk of bias: No blinding or incomplete blinding, and the outcome is likely to be influenced by lack of blinding; blinding of key study participants and personnel attempted, but likely that the blinding could have been broken, and the outcome is likely to be influenced by lack of blinding.
	Unclear: Insufficient information to permit judgement
Blinding of outcome assessment Detection bias due to knowledge of the allocated interventions by outcome assessors.	*Low risk of bias:* No blinding of outcome assessment, but the review authors judge that the outcome measurement is not likely to be influenced by lack of blinding; blinding of outcome assessment ensured, and unlikely that the blinding could have been broken.
	High risk of bias: No blinding of outcome assessment, and the outcome measurement is likely to be influenced by lack of blinding; blinding of outcome assessment, but likely that the blinding could have been broken, and the outcome measurement is likely to be influenced by lack of blinding.
	Unclear: Insufficient information to permit judgement
Incomplete outcome data Attrition bias due to amount, nature or handling of incomplete outcome data.	*Low risk of bias:* No missing outcome data; reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardised difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size; missing data have been imputed using appropriate methods.

(Continued)

	High risk of bias: Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; 'as-treated' analysis done with substantial departure of the intervention received from that assigned at randomisation; potentially inappropriate application of simple imputation.
	Unclear: Insufficient information to permit judgement
Selective reporting Reporting bias due to selective outcome reporting	*Low risk of bias:* The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way; the study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon).
	High risk of bias: Not all of the study's pre-specified primary outcomes have been reported; one or more primary outcomes is reported using measurements, analysis methods or subsets of the data (e.g. sub-scales) that were not pre-specified; one or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); one or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis; the study report fails to include results for a key outcome that would be expected to have been reported for such a study.
	Unclear: Insufficient information to permit judgement
Other bias Bias due to problems not covered elsewhere in the table	*Low risk of bias:* The study appears to be free of other sources of bias.
	High risk of bias: Had a potential source of bias related to the specific study design used; stopped early due to some data-dependent process (including a formal-stopping rule); had extreme baseline imbalance; has been claimed to have been fraudulent; had some other problem.
	Unclear: Insufficient information to assess whether an important risk of bias exists; insufficient rationale or evidence that an identified problem will introduce bias.

WHAT'S NEW

Date	Event	Description
5 July 2021	Amended	Minor edits

HISTORY

Protocol first published: Issue 4, 2018
Review first published: Issue 7, 2021

CONTRIBUTIONS OF AUTHORS

1. Draft the protocol: AI, TS, ETS
2. Study selection: AI, SPM, DMU
3. Extract data from studies: AI, SPM, DMU
4. Enter data into RevMan: AI, SPM, DMU
5. Carry out the analysis: AI

6. Interpret the analysis: AI, ETS, OG, DH
7. Draft the final review: AI, SPM, DMU, ETS
8. Disagreement resolution: OG, ETS, DH
9. Update the review: AI

DECLARATIONS OF INTEREST

All authors declare they do not have any conflict of interest. None of the authors listed in this review have any present or past affiliations or other involvement in any organization or entity with an interest in the outcome of the review. There have been no reported relationships present during the past 36 months, including, but not restricted to, financial remuneration for lectures, consultancy, travel or authorship of a study that might be included in this review.

SOURCES OF SUPPORT

Internal sources

- No sources of support provided

External sources

- No sources of support provided

INDEX TERMS

Medical Subject Headings (MeSH)

Antibodies, Monoclonal, Humanized [adverse effects] [therapeutic use]; Bias; Cattle; Colostrum [immunology]; Diarrhea [*complications] [microbiology] [therapy]; Escherichia coli Infections [*therapy]; Hemolytic-Uremic Syndrome [epidemiology] [*prevention & control]; Incidence; Organosilicon Compounds [adverse effects] [therapeutic use]; Placebos [therapeutic use]; Randomized Controlled Trials as Topic; Secondary Prevention [*methods]; *Shiga-Toxigenic Escherichia coli; Trimethoprim, Sulfamethoxazole Drug Combination [therapeutic use]; Trisaccharides [adverse effects] [therapeutic use]

MeSH check words

Adult; Animals; Child; Humans