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Review

Shiga toxin-induced haemolytic uraemic syndrome and the role of antibiotics: a global overview

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SUMMARY

Objectives: The administration of antibiotics in infections caused by Shiga toxin producing *E. coli* (STEC) strains, such as O157:H7, was and remains controversial, as it has been associated with the development of haemolytic uraemic syndrome (HUS). We conducted a literature review to better examine this association.

Methods: We searched the PubMed and Google Scholar databases for relevant articles, using the key words: "haemolytic uraemic syndrome", "Shiga toxin", "*E. coli* O157:H7", "*E. coli* O104:H4", "STEC colitis", "STEC antibiotics", "STEC fosfomycin", "STEC trimethoprim sulfamethoxazole", "STEC fluoroquinolones", "STEC ciprofloxacin", "STEC rifaximin", "STEC gentamycin", "STEC colistin", "Shiga toxin binding agent", "Shiga toxin monoclonal antibody" and "STEC Japan epidemic".

Results: Numerous studies report that antibiotics increase the risk of HUS development, while others report that antibiotics do not have any effect or can even reduce the rate of HUS development in STEC infections. The infecting STEC strain, the type of antibiotic as well as the timing of its administration appear to significantly affect the development of HUS in a STEC infected patient.

Conclusions: It appears that, while some antibiotics such as b-lactams and TMP/SMX may be detrimental, others appear to be safe and can prevent the development of HUS. Of note, fosfomycin appears to be the antibiotic with the most positive results from clinical studies, and may be able to avert HUS development, especially if administered within the first two or three days from diarrhoea onset. Fluoroquinolones have also shown positive outcomes in clinical studies, despite demonstrating unfavourable results in in vitro studies. Other agents, such as colistin, gentamycin and rifamycins, have shown promising results in in vitro studies and require further evaluation.

Introduction

Antibiotic administration in Shiga toxin producing *E. coli* (STEC) infections is a topic of great controversy. As the role of shiga toxin (Stx) is central to the development of haemolytic uraemic syndrome (HUS), it is not surprising that an increase in its production or release would be expected to have catastrophic consequences. The two toxins produced by STEC, Stx1 and Stx2, appear to differ significantly in their potency to induce protein synthesis inhibition and cytotoxicity, with some subtypes of Stx2 being more potent than Stx1, while other subtypes have similar potency.[1] Unless specified, the term Stx will be used throughout the article for both Stx1 and Stx2.

The detrimental effects of antibiotic administration in STEC infections has been attributed at large to secondary effects that lead to an increase of Stx production or release, such as[2]:

- The creation of an advantageous environment for STEC in the colon, by eliminating competing bowel flora, especially in cases in which the STEC strain is not susceptible to the antibiotic administered.
- The antibiotic induced lysis of the bacterial cell wall results in release of preformed toxin, increasing the amount of toxin available for interaction with the bowel wall.
- The induction of phage production and expression of Stx genes.

The latter mechanism involves the bacterial SOS response, an inducible DNA repair mechanism. This process is governed by two proteins: a repressor, named LexA, which binds to the promoter

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region of the SOS response genes, preventing their transcription and an inducer, the RecA filament, which enables the cleavage of LexA upon recognition of DNA damage.[3] RecA filament expression also promotes cleavage of a phage gene repressor, activating the transcription of late phage genes, which include the genes for Stx. As Stx2 has been found to be produced only when the phage enters the lytic cycle, factors that trigger the SOS response such as antibiotics or exposure of STEC to neutrophils or hydrogen peroxide, increase the release of Stx2. On the other hand, regulation of Stx1 production appears to be affected by iron concentrations, as high iron concentrations decrease the activity of a promoter for the gene of Stx1.[4]

Nevertheless, regardless of the infecting STEC strain and the toxin produced by it, the administration of antibiotics in STEC infections remains controversial, as some studies show it can be of benefit, whereas others demonstrate the detrimental effects that such treatment has on the patient's clinical course. This issue has been addressed by two meta-analyses.[5,6] The first, conducted in 2002 by Safdar et al.,[5] included 9 studies and did not find an association between antibiotic administration and an increased risk of HUS. The more recent one, published in 2016, concluded that when all 17 studies referenced were included in the meta-analysis, no definite association between antibiotic administration and the development of HUS was identified. However, if the analysis was restricted to the 5 studies which the authors considered had a low risk of bias, an association was found with an odds ratio of 2.24 (95% CI, 1.45–3.46; $I2 = 0\%$), leading the authors to recommend against the use of antibiotics in STEC infections.[6]

The use of antibiotics in STEC infections has also been addressed in the Infectious Disease Society of America (IDSA) guidelines for the management of infectious diarrhoea. Their previous edition, published in 2001, stated that antibiotic administration should be avoided in suspected STEC infections, as their role remained unclear.[7] However, the recommendation does not appear to be widely implemented, as the rate of antibiotic administration in this setting remains high.[8] The latest edition of the IDSA guidelines, published in October of 2017, strongly recommends against the use of antibiotics in infections caused by Stx2 producing STEC and considers the evidence insufficient for an analogous recommendation to be made for cases caused by non-Stx2 producing STEC strains.[9]

The aim of this review was to provide an up to date presentation of data regarding the effects of antibiotics in Stx production and HUS development in STEC infected patients. The results of in vitro, in vivo and clinical studies, regarding antibiotic administration in STEC infections are presented and discussed.

Materials and methods

We searched the PubMed and Google Scholar databases for relevant articles, using the key words: "haemolytic uraemic syndrome", "Shiga toxin", "*E. coli* O157:H7", "*E. coli* O104:H4", "STEC colitis", "STEC antibiotics", "STEC fosfomycin", "STEC trimethoprim sulfamethoxazole", "STEC fluoroquinolones", "STEC ciprofloxacin", "STEC rifaximin", "STEC gentamycin", "STEC colistin", "Shiga toxin binding agent", "Shiga toxin monoclonal antibody" and "STEC Japan epidemic". Additional articles relevant to our theme were extrapolated from the references of the articles found during this search.

Results

In vitro studies

The results of in vitro studies examining the effects of antibiotic administration on toxin production are summarised in Table 1. Nassar et al.[10] have examined the effects of subinhibitory concentrations of norfloxacin, azithromycin, rifampicin, imipenem and gentamycin on two STEC strains, one producing only Stx2 and one producing both Stx1 and Stx2. While all antibiotics tested increased the release of Stx2 in both strains, only norfloxacin and azithromycin did so in an SOS dependent manner. In the case of gentamycin, it has been postulated that the increase in toxin release might arise from the destabilisation of lipid membranes caused by the antibiotic, resulting in the release of preformed Stx2 from membrane vesicles. None of the antibiotics tested increased the release of Stx1 in culture supernatants. This discrepancy has been noted also by McGannon et al.,[11] as one of the strains examined by their group preferentially expressed Stx2 when exposed to ciprofloxacin or trimethoprim/sulfamethoxazole (TMP/SMX), and by Zhang et al.,[12] who found that ciprofloxacin induced Stx2 production in an Stx2 producing strain and in an Stx1 & Stx2 producing strain, while it inhibited Stx1 production in an Stx1 producing strain and in the aforementioned Stx1 & Stx2 producing strain.

Nassar et al. postulated that a difference in localisation of the two toxins might be responsible for the difference observed in toxin release,[10] as the toxins are located in different regions of the bacterial cell. Stx1 is located in the periplasmic space, while Stx2 is located in the extracellular fraction.[13] In addition to this, the active site of Stx2 appears to be more accessible than the active site of Stx1, which may contribute to its increased virulence.[14]

The effects of subinhibitory concentrations of antibiotics on toxin release have been studied by various groups.[10,11,15–21] Walterspiel et al.[15] studied the effects of administration of high and low subinhibitory concentrations of ciprofloxacin, TMP/SMX, cefixime and tetracycline on Stx1 production in 5 strains of STEC. The results differed significantly among the strains tested and the concentrations of antibiotics. Only ciprofloxacin demonstrated an increase in Stx1 production in all strains at both concentrations. At high subinhibitory concentrations, TMP/SMX, cefixime and tetracycline increased Stx1 production in most strains, while they had no effect on toxin production at low subinhibitory concentrations in most, but not all, strains. In one strain, high subinhibitory concentration of tetracycline resulted in a decrease in toxin production. In conclusion, with the exception of ciprofloxacin, the effects of antibiotics on Stx1 production varied among strains and depended on the type and the concentration of antibiotic used. Similar findings were reported by other groups,[11,16,18,20,22] which all demonstrated that the effect on toxin release depends on the antibiotic and the concentration at which it is used and differs among STEC strains.

However, within each study, some antibiotics elicit homogenous responses by all strains. Mohsin et al.[18] found that gentamycin had no effect on toxin production at subinhibitory concentrations and decreased toxin production at Minimum Inhibitory Concentration (MIC), in all strains tested. In addition, gentamycin decreased the cytotoxicity of all tested STEC strains to Vero cells. The same was true for cefotaxime, with the exception of one strain, on which cefotaxime at MIC had no effect on toxin production. Yoshimura et al.[17] found that toxin production remained unchanged when the strains examined were exposed to fosfomycin at a concentration equal to 8 times the MIC and decreased when exposed to kanamycin at MIC, minocycline at subinhibitory concentration and norfloxacin at a concentration equal to 8 times the MIC. Zhang et al.[12] found that cephalexin, fosfomycin, azithromycin and gentamicin reduced the production of Stx1 and Stx2, while ciprofloxacin induced the production of Stx2 but inhibited the production of Stx1. Amran et al.[23] showed that Stx2 production was increased by norfloxacin, ofloxacin, ciprofloxacin, kanamycin, decreased by azithromycin and was not affected by fosfomycin. McGannon et al.[11] demonstrated that after exposure to subinhibitory concentrations of antibiotics, toxin production remained unchanged by ceftriaxone and rifampicin and was decreased by doxycycline, fosfomycin and gentamycin in all strains examined.

Table 1
In vitro studies (sub denotes subinhibitory concentrations as used) (x4MIC denotes levels 4 times above the MIC).

Study (first author, year of publication)	Effect studied	Number of serotypes used[a]	Antibiotic increased Stx production (number of isolates)	Antibiotic decreased Stx production (number of isolates)	Antibiotic had no effect on Stx production (number of isolates)
Walterspiel, 1992[15]	Stx 1 production	5 EHEC stx1+	ciprofloxacin sub high (5), ciprofloxacin sub low (5), cotrimoxazole sub high (5), cotrimoxazole sub low (1), tetracycline sub high (4), tetracycline sub low (1), cefixime sub high (4), cefixime sub low (1)	tetracycline sub high (1)	cefixime sub high (1), cotrimoxazole sub low (4), tetracycline sub low (4), cefixime sub low (4)
Grif, 1998[16]	Stx production	3 *E. coli* O157 (1 stx1+ & stx2+, 1 stx1 +, 1 stx2+)	azithromycin sub (3) cotrimoxazole sub (3) trimethoprim sub (3) gentamicin sub (3) cefixime sub (1 stx2), ceftriaxone sub (1 stx2), erythromycin sub (1 stx2), ampicillin sub (1 stx2) penicillin G sub (2), streptomycin sub (2), ciprofloxacin sub (2), fosfomycin sub (2), sulfamethoxazole sub (2)	cefixime sub (2), ceftriaxone sub (2), erythromycin sub (2), penicillin G sub (1), streptomycin sub (1), ciprofloxacin sub (1), fosfomycin sub (1), sulfamethoxazole sub (1)	ampicillin sub (2)
Yoshimura, 1999[17]	Stx production	*E. coli* O157:H strain E32511/HSC and streptomycin and mitomycin C resistant strain of E32511/HSC		minocycline sub kanamycin norfloxacin x8MIC	fosfomycin x8MIC
Ochoa, 2007[24]	Phage induction, drug-induced bacteriolysis, and toxin release	57 STEC strains (26 O157 and 31 non-O157)	ciprofloxacin (caused bacteriolysis and release of toxin in 25/26 O157 strains and 15/31 non-O157 strains)		rifamixin
Pedersen, 2008[22]		29 STEC (4 stx1+, 10 stx2+, 4 stx2b+, 6 stx2c+, 5 stxd+)	ciprofloxacin (1 stx1, 4 stx2), gentamycin (1 stx2), azithromycin (1 stx1, 1 stx2),	ciprofloxacin (7 stx2), gentamycin (22 stx2), azithromycin (22 stx2), telithromycin (1 stx1, 24 stx2)	ciprofloxacin (3 stx1, 14 stx2), gentamycin (4 stx1, 2 stx2), azithromycin (3 stx1, 2 stx2), telithromycin (3 stx1, 1 stx2)
Zhang, 2009[12]	Phage induction and Stx1 and Stx2 production	3 *E. coli* O157:H7 strains: 933 (Stx1 and Stx2 producer), 933-1C (Stx1 producer) and 933-2 (Stx2 producer)	ciprofloxacin (Stx 2 production both in the stx2 producing strain and in the stx1 and stx2 producing strain)		cephalexin (3) fosfomycin (3) azithromycin (3) gentamicin (3) ciprofloxacin (Stx 1 production both in the stx1 producing strain and in the stx1 and stx2 producing strain)
Ichinohe, 2009[26]	Copy numbers and mRNA expression levels of the stx genes	1 *E. coli* O157:H7 stx1+ & stx2+	norfloxacin increased stx2 DNA by 10^3	fosfomycin and panipenem decreased stx2 DNA	ceftazidime, aztreonam
McGannon, 2010[11]	Stx production	4 (1 stx1+ & stx2+, 1 stx1 +, 2 stx2+)	ciprofloxacin sub (1), cotrimoxazole sub (1) both on the stx1+ & stx2+ strain, azithromycin (1) (stx2+ strain)	azithromycin sub (3), doxycycline sub (4), fosfomycin sub (4), gentamycin sub (4)	ceftriaxone sub (4), rifampin sub (4)
Mohsin, 2010[18]	Stx 1 & Stx2 production and verotoxicity	4 STEC (1 stx1+, 2 stx2+, 1 stx1+ & stx2+)	ampicilin sub (3)	ampicilin (3), ampicilin sub (1), gentamycin (4), cefotaxime (3),	gentamycin sub (4), cefotaxime sub (4), cefotaxime (1), ampicilin (1)
Rahal, 2011[27]	Stx1 & Stx2 transcription and release	5 *E. coli* O157		rifampicin (5)	
Rahal, 2011[28]	Stx1 & Stx2 transcription and release	1 *E. coli* O157:H7 stx1+ & stx2+		gentamycin at MBC caused 50-75% decrease in Shiga toxin transcription and release, rifampicin at MIC followed by gentamycin at MBC caused 99% decrease in Shiga toxin transcription and release	

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Table 1 (*continued*)

Study (first author, year of publication)	Effect studied	Number of serotypes used[a]	Antibiotic increased Stx production (number of isolates)	Antibiotic decreased Stx production (number of isolates)	Antibiotic had no effect on Stx production (number of isolates)
Bielaszewska, 2012[19]	Phage induction and Stx2 production	2 *E. coli* O104:H4, 1 *E. coli* 157:H7 for comparison	ciprofloxacin sub (3)	chloramphenicol sub (2 O104:H4), meropenem sub (2 O104:H4), azithromycin sub (2 O104:H4), rifaximin sub (2 O104:H4), tigecycline sub (2 O104:H4)	fosfomycin sub (3), gentamicin sub (3), kanamycin sub (3)
Corogeanu, 2012[20]	Comparisson of antibiotic induced stx release in 157:H7 vs O104:H4. Stx2 RNA transcripts were quantified.	3 STEC: 1 *E. coli* 157:H7, 2 *E. coli* O104:H4	ciprofloxacin (1 O157), ciprofloxacin sub (1 O157), fosfomycin (1 O157), fosfomycin sub (1 O157), meropenem (1 O157), meropenem x4MIC (1 O157), rifampicin sub (3), rifampicin x4MIC (3), chloramphenicol (1 O157)	ciprofloxacin x4MIC (2 O104), fosfomycin x4MIC (2 104), meropenem x4MIC (2 104), chloramphenicol x4MIC (3), chloramphenicol (2)	fosfomycin (2 104), meropenem (2 104), gentamycin (3)
Nassar 2013[10]	Evaluation of SOS responce induction by measuring expression of recA (SOS-inducer), Q (late gene of Stx-phage), stx1, and stx2 genes	2 *E. coli* O157:H7	azithromycin sub (2), norfloxacin sub (2), imipenem sub (2), rifampicin sub (1) gentamycin low sub (2), gentamycin sub (1)	rifampicin sub (1)	gentamycin sub (1)
Amran, 2013[23]	Stx2 production	*E. coli* O157: H- strain E32511	norfloxacin, ofloxacin, ciprofloxacin, kanamycin	azythromycin	fosfomycin
Percivalle, 2016[21]	Verotoxicity	2 (1 *E. coli* O157:H7 stx1+ & stx2+, 1 stx-)		colistin, colistin sub	

[a] Inconsistencies regarding metadata between identical serotypes can be attributed to the differences between strains of the same serotype.

In addition to antibiotics, McGannon et al.[11] also examined the role of the intestinal flora on toxin production. They found that, when *E. coli* O157:H7 STEC was coincubated with phage-sensitive, Stx-negative, antibiotic resistant strain of *E. coli*, Stx production increased, compared to that produced by *E. coli* O157:H7 STEC alone after incubation with antibiotics. The authors postulated that as the induction of SOS response by antibiotics can cause the switch from the lysogenic stage to the lytic cycle, antibiotic administration can precipitate the release of Stx-encoding phages and, consequently, the infection of the non-pathogenic *E. coli* of the intestinal flora. These secondary infected strains can then enter the lytic cycle and produce very high quantities of Stx. In the event that the intestinal flora of the host happens to be resistant to the administered antibiotic, to which the STEC strain is susceptible, the results can be catastrophic.

Bielaszewka et al.[19] examined the effects of subinhibitory concentrations of antibiotics on phage induction and Stx2 production. Both parameters were significantly increased by ciprofloxacin, but were not affected significantly by fosfomycin, gentamicin and kanamycin and were significantly decreased by chloramphenicol, meropenem, azithromycin, rifaximin and tigecycline.

Ochoa et al.[24] examined the effects of ciprofloxacin and rifaximin on phage induction, drug-induced bacteriolysis and toxin release in 57 STEC strains. Ciprofloxacin caused bacteriolysis and release of toxin in 39 strains. In contrast, rifaximin did not induce phage replication or lysis in any strain and did not increase toxin release, when compared to the absence of antibiotic. The researchers examined rifaximin as a potential therapeutic agent, as its mechanism of action suggested that it would not lead to phage induction. Rifaximin binds to the beta subunit of prokaryotic RNA polymerase and inhibits transcription. For transcription to begin, sigma (σ) factors of bacterial RNA polymerase must first recognise their promoters. Two important σ factors of *E. coli* are σ70 and σ32. Stx gene transcription is mediated by σ70-dependent promoters, which utilise the main σ factor of *E. coli*, σ70. Genes of heat shock proteins,[24] which protect the bacterium from cell wall lysis,[25] are transcribed by σ32-dependent promoters.[24] Rifampin, another rifamycin, preferentially inhibits σ70-dependent promoters more than σ32-dependent promoters,[24] therefore permitting the transcription of heat-shock genes.[10]

Other researchers have also attributed their results to the mechanism of action of the antibiotics tested. Ichinohe et al.[26] found that the copy numbers of Stx2 genes were increased by norfloxacin, decreased by fosfomycin and panipenem and remained unaffected by ceftazidime and aztreonam. They ascribed these results to the rapid bacterial cell wall lysis caused by fosfomycin and panipenem.

Two studies conducted by Rahal et al.,[27,28] measured in vitro the transcription and release of Stx1 and Stx2 after the administration of antibiotics, as well as the effect of antibiotic administration on the survival of mice infected with STEC strains, which will be discussed in the following section. The first in vitro experiment[27] showed that the administration of rifampicin resulted in a decrease of toxin transcription and release, in all 5 strains examined. In the second experiment,[28] the group examined the effect of the combination of rifampicin at MIC followed by gentamycin at Minimum Bactericidal Concentration (MBC) on toxin production, in a single strain of *E. coli* O157:H7. The combination resulted in a 99% decrease in toxin transcription and release, while gentamycin alone at MBC caused a 50–75% decrease in the same parameters.

Finally, Percivalle et al.,[21] examined the effects of colistin on toxin production and survival of Vero cells co-incubated with *E. coli* O157:H7. Colistin reduced the release of Stx in a concentration-dependent manner, even at subinhibitory concentrations. At concentrations above the MIC, colistin also enhanced the survival of Vero cells.

In vivo studies

Parameters examined by in vivo studies regarding STEC infections are summarised in Table 2. They include the effects of antibiotic administration on survival and on fecal pathogen excretion

Table 2
In vivo studies (sub denotes subinhibitory concentrations were used) (x4MIC denotes at levels 4 times above the MIC).

Study (first author, year)	Serotypes used[a]	Animals Used	Effect studied	Route of infection	Antibiotics studied	Timing of antibiotic administration (hours after infection)	Duration of antibiotic administration	Mortality rate in antibiotic group	Mortality rate in untreated group
Yoshimura,1999[17]	*E. coli* O157:H strain E32511/HSC and streptomycin and mitomycin C resistant strain of E32511/HSC	Six-week-old male ICR mice	Mortality rate at 2 weeks	Simultaneous per os (p.o.) and intraperitoneal (i.p.). Antibiotic were administered either p.o. or i.p.	fosfomycin, minocycline, kanamycin, norfloxacin	2 h after p.o. Infection	3 days (day 0 through day 2)	fosfomycin p.o./i.p., 58%/80%, minocycline p.o./i.p. 15%/25%, kanamycin p.o., 25%, i.p. 70% norfloxacin p.o./i.p., 0%/10%	70%
Sawamura, 1999[29]	*E. coli* O157 TI001	BALB/c mice	Survival at 15d	Per os	fosfomycin 500 mg/kg/d b.i.d.	fosfomycin at 3 h postinfection vs 6 h postinfection vs 12 h postinfection vs 24 h postinfection	5 days	fosfomycin at 3h: 0% fosfomycin at 6h: 0% fosfomycin at 12h: 100% fosfomycin at 24h: 100%	100,00%
	E. coli O157 TI001	BALB/c mice	Survival at 15d	Per os	fosfomycin 500 mg/kg/d b.i.d. norfloxacin 50 mg/kg/d b.i.d.	fosfomycin at 3 h postinfection, norfloxacin at 3 h postinfection	5 days	fosfomycin at 3h: 17.7% norfloxacin at 3h: 0%	100,00%
Kurioka, 1999[30]	*E. coli* O157:H7, strain N-9, producing Stx 1 and Stx 2	3-week-old female C57BL/6 NCrj mice, fed a low-protein calorie diet	Survival at 3 weeks, duration of fecal pathogen excretion, toxin level in stool and blood	Intragastrically	norfloxacin, fosfomycin, kanamycin, ampicillin, clarithromycin, TMP/SMX	Protocol A: days 1–3 b.i.d. Protocol B: days 3-5 b.i.d.	3 days	Protocol A: 0% for all antibiotics Protocol B: 0% for all antibiotics except TMP/SMX treated mice who had a 95% mortality rate by day 6 postinfection.	85% mortality between days 10 and 12
Hiramatsu, 2003[32]	*E. coli* SS812 serotype O157:H7	C3H/HeN mice	Survival at day 7 postinfection, bacterial counts in stool, toxin level in stool	Intragastrically	rokitamycin 20 mg/kg b.i.d. levofloxacin 1.2 mg/kg b.i.d.	1 day postinfection	7 days	rokitamycin: 19% levofloxacin: 80%	93,00%
Zhang, 2009[12]	3 *E. coli* O157:H7 strains: 933 (Stx1 and Stx2 producer), 933-1C (Stx1 producer) and 933-2 (Stx2 producer)	Gnotobiotic piglets	Survival at 2 weeks, duration of fecal pathogen excretion, bacterial counts in cecum, toxin level in stool, presence of hemorrhagic cerebellar lesions	Per os	ciprofloxacin, azithromycin	1 day postinfection	Single dose	azithromycin 25% ciprofloxacin 100%	100%
Rahal, 2011[27]	*E. coli* O157 cultures, live mice	BALB/c mice	Survival and weight variations for 6 days	Intraperitoneal injection	rifampicin by intraperitoneal injection	1h	Single dose	rifampicin 75%	100%

(*continued on next page*)

Table 2 (*continued*)

Study (first author, year)	Serotypes used[a]	Animals Used	Effect studied	Route of infection	Antibiotics studied	Timing of antibiotic administration (hours after infection)	Duration of antibiotic administration	Mortality rate in antibiotic group	Mortality rate in untreated group
Rahal, 2011[28]	*E. coli* O157	BALB/c mice	Mortality rate at 2 weeks	Intraperitoneal injection	Intraperitoneal injections of either gentamycin at MBC, rifampicin at MIC, rifampicin at MIC followed by rifampicin ant MBC or rifampicin at MIC followed by gentamycin at MBC	1 h after i.p. Infection. In 2 of the 4 intervention groups who received rifampicin at MIC 1 h post-infection, a second injection of either gentamycin at MBC or rifampicin at MBC was administered at 17 h post-infection.	1 day	gentamycin only 100%, rifampicin at MIC followed by gentamycin at MBC 50%, rifampicin at MIC 75%, rifampicin at MIC followed by rifampicin at MBC 87.5%	100% by day 4
Amran, 2013[23]	*E. coli* E32511(Stx2c)	4-week-old female ICR outbred mice	Survival at 2 weeks	Per os	azithromycin, norfloxacin, ofloxacin, ciprofloxacin fosfomycin, kanamycin	2 h postinfection	Single dose	azithromycin: 0% norfloxacin: 20% ofloxacin: 60% ciprofloxacin: 80% ofloxacin x75mic: 70% ciprofloxacin x333mic: 60% fosfomycin: 100% kanamycin: 20%	100,00%
	E. coli E32511(Stx2c)	4-week-old female ICR outbred mice	Survival and weight gain at 2 weeks	Per os	azithromycin 200 µg/g	2 h postinfection vs 6 h postinfection vs 24 h postinfection	Single dose	azithromycin at 2h: 20% azithromycin at 6h: 60% azithromycin at 24h: 100%	100%
	E. coli E32511(Stx2c)	4-week-old female ICR outbred mice	Survival and weight gain at 2 weeks	Per os	azithromycin at several doses, with a two- fold serial dilution from 200 µg/g to 1,6 µg/g	2 h postinfection	Single dose	All azithromycin doses from 6,25 µg/g or higher: 0% azithromycin at 3,1 or at 1,6 µg/g: 20%	100%
Amran, 2013[31]	*E. coli* O91:H21 strain B2F1 (Stx2d)	4-week-old female ICR outbred mice	Survival at 2 weeks, fecal pathogen excretion	Per os	azithromycin, daio (traditional chinese medicine, containing anthranoids and anthraquinone glycosides, which have laxative and purgative effects)	azithromycin 24 h postinfection as a single dose or once a day for 3 days daio 24 h postinfection once a day for 3 or 5 days	azithromycin: 1 or 3 days daio: 3 or 5 days	azithromycin single dose 35%, azithromycin for 3 days 47%, daio for 5 days 0%, daio for 3 days 50%	100% by day 8 in the group serving as the controls of azithromycin, 67% by day 13 in the group serving as the controls of daio.
	E. coli E32511(Stx2c)	4-week-old female ICR outbred mice	Survival at 2 weeks, fecal pathogen excretion	Per os	azithromycin, daio (traditional Chinese medicine, containing anthranoids and anthraquinone glycosides, which have laxative and purgative effects)	Combination of azithromycin 6 h postinfection and daio at 2 h and at 24 h postinfection	1 day	20%	100%

[a] Inconsistencies regarding metadata between identical serotypes can be attributed to the differences between strains of the same serotype.

as well as the importance of the timing of antibiotic administration. The latter is evident in a study by Sawamaura et al.,[29] in which mice infected with STEC were administered fosfomycin 500 mg/kg/day b.i.d. for 5 days beginning at either 3 h, 6 h, 12 h or 24 h postinfection. All the mice in the 3-h group and in the 6-h group survived, while all of the mice of both other groups died, as did the mice in the control group. The same group compared the effects of fosfomycin 500 mg/kg/day b.i.d. for 5 days, beginning at either 3 h postinfection, with norfloxacin 500 mg/kg/day b.i.d. for 5 days, also beginning at 3 h postinfection. The mortality rate at 2 weeks was 17,7% for fosfomycin, 0% for norfloxacin and 100% for the control group. Of note is that Stx could not be detected in the feces of the mice in either group which, according to the authors, suggested that neither of these antibiotics induced the release of verotoxin.

In a study by Kurioka et al.,[30] mice infected with STEC received a 2-day course of either norfloxacin, fosfomycin, kanamycin, ampicillin, clarithromycin or TMP/SMX, starting at either day 1 postinfection or at day 3 postinfection. At 3 weeks, all animals in both treatment groups had survived, with the exception of TMP/SMX treated mice in the late treatment group, which had a 95% mortality rate by day 6 postinfection. Untreated mice had an 85% mortality rate between days 10 and 12. Mice treated with TMP/SMX in the late treatment group developed neurologic symptoms and had a positive Stx immunoreaction in their brains, as did untreated mice and had significantly higher leukocyte counts and Blood Urea Nitrogen (BUN) than untreated mice. Mice treated with TMP/SMX at day 1 and mice treated with norfloxacin or fosfomycin in both treatment groups, did not develop neurologic symptoms and had significantly lower leukocyte counts and BUN than untreated mice.

A study by Amran et al.,[23] examining the effects of antibiotic administration timing, compared the mortality rates of STEC infected mice after the administration of a single dose of azithromycin 200 μg/g at 2 h postinfection, at 6 h postinfection or at 24 h postinfection. Mortality rates at 2 weeks were 20%, 60% and 100%, respectively. Compared to mice treated at 6 h, mice treated at 2 h had a significantly higher weight at 2 weeks. The study also examined the effects of different doses of azithromycin on survival, with a twofold serial dilution from 200 μg/g to 1.6 μg/g. A single dose of the drug was administered. All the mice that received 6.25 μg/g or higher survived, while 20% of mice that received 3.1 μg/g or at 1.6 μg/g died, as did all of the mice in the control group. There was a statistically significant correlation between the dose of azithromycin and the increase in the body weight. Finally, the study compared survival of mice at 2 weeks following a single dose of either azithromycin, norfloxacin, fosfomycin, kanamycin, ofloxacin or ciprofloxacin at 2 h postinfection, the results of which are summarised on Table 2.

The same group examined the use of Daio, a Chinese medicine containing anthranoids and anthraquinone glycosides, alone or in combination with azithromycin, in mice infected with STEC.[31] Survival of mice at 2 weeks was superior in the group that received Daio than both control and the group that received azithromycin. The combination of azithromycin and Daio was superior to azithromycin alone in terms of survival and fecal excretion of STEC. However, the survival of the group that received Daio alone was superior than the group that received the combination.

Hiramatsu et al.[32] compared the administration of levofloxacin vs rokitamycin 1-day postinfection for 7 days, in mice infected intragastrically with STEC. Mortality rates at 1 week were 93% for levofloxacin, 20% for rokitamycin and 80% for control. On autopsy, mice treated with levofloxacin showed renal involvement with necrotic tubular epithelial cells, while their colons were infiltrated by inflammatory cells. No such lesions were evident in the rokitamycin group.

Zhang et al.[12] compared the survival at 2 weeks of STEC infected gnotobiotic piglets after a single dose of either azithromycin or ciprofloxacin, administered at day 1 postinfection. At 1 week, the mortality rates were 25% for azithromycin and 100% for both ciprofloxacin and control. CNS lesions were evident in 87% of untreated and in 88% of ciprofloxacin treated piglets, whereas only 36% of azithromycin treated piglets had CNS lesions. The level of toxin and the amount of measurable organisms in gut contents were decreased in the azithromycin group compared to the untreated group. In the ciprofloxacin group, most animals had no measurable organisms and reduced toxin levels in their gut contents. However, in the few animals that did have measurable organisms, toxin levels were much higher than in the control group.

Yoshimura et al.[17] studied the survival of mice infected simultaneously intraperitoneally and per os with STEC, after which they were administered a 3-day course either fosfomycin, minocycline, kanamycin or norfloxacin, starting at 2 h postinfection. Each antibiotic was administered either intraperitoneally or per os. Mice treated with minocycline and norfloxacin administered from either route as well as per os administered kanamycin had significantly lower mortality rates than controls.

Finally, the two studies conducted by Rahal et al.[27,28] included in vivo experiments. In the first experiment,[27] the administration of a single dose of rifampicin intraperitoneally 1-h postinfection, resulted in a decrease of mortality at 6 days, from 100% in the control group to 75% to the rifampicin group. In the second experiment,[28] mice that received the combination of rifampicin at MIC, at 1-h postinfection, followed by gentamycin at MBC, at 17 h postinfection, had a greater survival rate at 2 weeks compared to mice that received either drug alone.

Clinical studies

The impact of antibiotic administration in patients with STEC infections has been studied in numerous clinical studies. Table 3 summarises the results of these studies, while Table 4 presents the conclusions drawn by the authors of each study.

Only one randomised control trial has been conducted on the matter,[33] which concluded that antibiotic administration, namely TMP/SMX for 5 days, had no effect in the occurrence of HUS, the duration of symptoms or the fecal excretion of the pathogen. Antibiotics were administered rather late in the course of illness, a mean of 7.4 days after the onset of diarrhoea, whereas the mean onset of HUS is 6 days after the onset of diarrhoea.[6,34] Therefore, it is possible that the antibiotics were administered too late to have any effect on the clinical course of the patients.

Studies that do not show an association between antibiotics and the development of HUS in STEC infections

The conclusion that there is no association between antibiotics and the development of HUS is not uncommon, as 19 studies, of the 36 included in this review, concluded that antibiotics in general had no effect on the development of HUS.[33,35–52] However, in 7 of these studies,[38–41,49,51,52] specific exceptions were noted.

Ohnishi and Nakamura-Uchiyama[41] studied 15 patients with STEC colitis admitted at their hospital between 1997 and 2010. Six patients had received levofloxacin for the treatment of STEC colitis, while the remaining 9 patients did not receive any antibiotics. Only 1 case of HUS occurred and was among the patients that did not receive treatment. All patients were above 16 years of age. While no significant difference was observed between the two groups, the authors suggested that fluoroquinolone therapy might be effective in preventing the development of HUS in cases where it is administered early.

While Ostroff et al.[49] did not find that antibiotics in general increase the risk of developing HUS/TTP, they noted that patients

Table 3
Clinical studies regarding the incidence of HUS or TTP following antibiotic administration in STEC infected patients.

Study (first author, year)	n1: pts on antibiotic	n2: pts not on antibiotic	Age (years)	Administered antibiotics (number of pts who received them)	Onset (and duration) of antibiotic administration	Duration of illness/ diarrhoea/bloody diarrhoea	Total HUS/TTP, and HUS after antibiotic administration (antibiotic and number of pts)	Number of deaths
Riley, 1983[44]	11	32	4 – 76	After onset of illness: tetracycline (8) erythromycin (3) Before onset of illness: penicillin (1)	Before (penicillin) or after (tetracycline, erythromycin) onset of illness	Similar mean duration of illness between n1 and n2		0
Remis, 1984[45]	9	15	1 – 80	NS		Similar duration of illness between n1 and n2: 7.5 (n1) vs. 8.5 days (n2)	1 HUS	
Ryan, 1986[46]	25	9	86 – 87 (mean)		For 5 days	Similar duration of diarrhoea between n1 and n2	1 HUS	4 (1: sepsis, 1: congestive heart failure, 2: fever 39 & unidentified infection source)
Carter, 1987[54]	20	53	16 – 101	Before onset of symptoms of secondary infection: tetracycline (1) amoxicillin (4)	Within 2 days before serving the suspect meal or during the outbreak (after onset of 54 primary infections, before onset of 19 secondary infections)	n of pts with bloody diarrhoea: n1 > n2 (P 1/4 0.02)	12 HUS	19 (11 with HUS, deaths: n1 > n2)
MacDonald, 1988[47]	4	21	1 – 70	TMP/SMX+ erythromycin (2) ampi-cillin + gentamicin + metronidazole (1) cefoxitin (1) sulphasalazine (2)		Similar duration of illness between n1 and n2 [n1: 10.5 days (mean)]	0	
Griffin, 1988[48]	9	18	11 months – 78 years	NS, sulphasalazine (1)	first 3 days of illness	Similar duration of diarrhoea between n1 and n2: mean +/- SD: 7.8 +/- 1.6 (n1) vs.9.7 +/- 8.4 (n2)	1 HUS (age: 4) 3 TTP (age ≥ 70)	2 (with TTP)
Ostroff, 1989[49]	37	43	11 months – 78 years (mean: 22.3 years)	erythromycin (13) ampicillin (12) TMP/SMX (9) metronidazole (3) gentamicin (2) tetracycline (1) neomycin (1) cefotaxime (1)	4.3 days (mean) after disease onset	Illness (mean +/- SD): 7 +/- 3.1 (n1) vs.7 +/- 2.8 (n2) days, $P > 0.05$ Diarrhoea (mean): 5.8 (n1) vs. 6.2 (n2) days, $P > 0.05$ Bloody diarrhoea (mean): 3.6 (n1) vs. 2.7 (n2) days, $P < 0.05$	9 HUS, 2 TTP [5 of n1: HUS/TTP vs. 32 of n1: no HUS/TTP (RR 1/4 1, CI 1/4 0.3–4.1), TMP/SMX: 3: HUS/TTP vs. 6: no HUS/TTP, (RR 1/4 3.1, CI 1/4 0.6–9.8) gentamicin: 2: TTP vs. 0: no TTP, (RR 1/4 9.1, low CI 1/4 1.8)]	1 (with TTP)
Pavia, 1990[55]	7	7	6 – 39	Initial phase of outbreak: TMP/SMX (5) Later phase: ampicillin (2) [vs. placebo (2)]	Within 72 h after onset of diarrhoea	TMP/SMX: longer duration of diarrhoea & bloody diarrhoea than n2; ampicillin: duration similar with n2	8 HUS (TMP/SMX: 5/8 with HUS vs. 0/7 without HUS, P 1/4 0.026). 1 HUS after sulfasalazine administration.	4 (3 of 5 on TMP/SMX vs.1 of n2, $P = 0.22$)
Martin, 1990[58]	20	97	<18	NS	During prodromal illness		117 HUS	4 (0 of n1)
Cimolai, 1990[59]	51	50	8 months – 17 years	"Appropriate" (36): ampicillin TMP/SMX etc. "inappropriate" (15): not specified	For ≥24 h or <24 h	Similar duration of diarrhoea/bloody diarrhoea between n1 and n2	28 HUS ("appropriate" antibiotics for ≥24 h: 2 HUS vs 29 no HUS, $P = 0.001$)	

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Table 3 (*continued*)

Study (first author, year)	n1: pts on antibiotic	n2: pts not on antibiotic	Age (years)	Administered antibiotics (number of pts who received them)	Onset (and duration) of antibiotic administration	Duration of illness/ diarrhoea/bloody diarrhoea	Total HUS/TTP, and HUS after antibiotic administration (antibiotic and number of pts)	Number of deaths
Proulx, 1992[33]	22	25	64 +/- 52 (mean +/- SD)	TMP/SMX (22)	Onset 7.4 days (mean) after onset of diarrhoea (for 5 days)	Mean duration of diarrhoea (>5/day):4.95 (n1) vs. 4.1 days (n2), $P > 0.05$	6 HUS [2 of n1 (9%) vs. 4 of n2 (16%), $P = 0.67$]	
Bell, 1997[50]	50	218	< 16	TMP/SMX (31) ampicillin or amoxicillin (13) cephalosporin (6) metronidazole (4) tetracycline (1) gentamicin (1) erythromycin (1) ciprofloxacin (1)	Within 3 days from onset of illness	Diarrhoea (mean): 6.9 (n1) vs. 7.7 days (n2), $P = 0.2$ Bloody diarrhoea (mean): 4.5 (n1) vs. 4.6 (n2) days, $P = 0.6$	37 HUS [8 of n1 (16%) vs. 28 of n2 (13%), $P = 1.56$]	
Slutsker, 1998[51]	39	54	4 months – 87 years	SMX (5 younger than 13, ≤3 days after diarrhoea onset) Other NS (34) (NS whether controls had taken antibiotics)			**7 HUS (4/7 1/45 7% took antibiotics vs. 40% of 86 pts without HUS who took antibiotics, HUS among pts aged <13: 3/6 who took antibiotics ≤3 days after diarrhoea onset vs. 1/23 who took antibiotics >3 days after diarrhoea onset or did not take antibiotics, $P = 0.02$)**	1 (with HUS)
Takeda, 1998[62]	310	19	7.7 +/- 2.4	fosfomycin in 92% of cases	221 within 3 days of onset; 89 on or after 4 days of onset		68 HUS. 57 (18.4%) from n1 and 11 (57.9%) from n2. Of the 221 patients who had taken an antibiotic within 3 days of onset HUS developed in 34 (15.4%), while from the 89 that received an antibiotic on or after 4 days of onset, 23 (25.8%) developed HUS.	3 (with HUS, n1)
Ikeda, 1999[64]	290	2	6 – 11	fosfomycin (257) cephalosporins (101) quinolones (42) penicillins (32) tetracyclines (17) aminoglycosides (16) Grouping for analysis: fosfomycin alone (128) vs. other antibiotics (162)	Within the first 5 days of illness		36 HUS (14 HUS among 128 pts taking only fosfomycin within the first 5 days, 22 HUS among 162 pts taking other antibiotics within the first 5 days of illness)	

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Table 3 (*continued*)

Study (first author, year)	n1: pts on antibiotic	n2: pts not on antibiotic	Age (years)	Administered antibiotics (number of pts who received them)	Onset (and duration) of antibiotic administration	Duration of illness/ diarrhoea/bloody diarrhoea	Total HUS/TTP, and HUS after antibiotic administration (antibiotic and number of pts)	Number of deaths
Hashimoto, 1999[53]	56	70		fosfomycin (27), norfloxacin (17), enoxacin (5), lomefloxacin (4), levofloxacin (4), tosufloxacin (2), ciprofloxacin (1), clarithromycin (2), josamycin (1), cephalosporins (3), minocycline (1)			0	
Fukushima, 1999[63]	425	0		fosfomycin and bactericidal antibiotics in all			12 HUS. All after antibiotic administration	0
Shiomi, 1999[61]	42	0	6 – 12	Oral norfloxacin or sparfloxacin (15) fosfomycin per os + cefotaxime iv (12) fosfomycin iv (15)	Onset within 3 days from onset of disease (for 5 days)	Duration of frequent diarrhoea (>10/day): pts on fluoroquinolones > pts on fosfomycin per os + cefotaxime iv or fosfomycin iv	5 HUS (3: iv fosfomycin, 2: iv cefotaxime + fosfomycin per os)	
Wong, 2000[57]	9	62	< 10	TMP/SMX (3) cephalosporins (4) amoxicillin (2)			10 HUS (5 of n1 vs. 5 of n2, $P < 0.001$). 2 HUS TMP/SMX. 3 HUS cephalosporins.	
Dundas, 2001[52]	14 (before symptom onset) 15 (after symptom onset)	72 (before symptom onset) 104 (after symptom onset)	1.5 – 94	Various NS antibiotics (14) ciprofloxacin (15)	≤ 4 weeks before (various antibiotics) or ≤ 4 days after (ciprofloxacin) symptoms onset		34 HUS (HUS in pts on antibiotics vs. pts not on antibiotics before symptom onset: 8/14 vs. 15/72, HUS in pts on ciprofloxacin vs. not on ciprofloxacin after symptom onset: 7/15 vs. 26/104)	16 (aged>65, deaths of pts on antibiotics vs. pts not on antibiotics before symptom onset: 4/10 vs. 6/72, $P > 0.05$)
Cadwgan, 2002[43]	10	22	Median age 58 years, range 16 – 93 years	ciprofloxacin (6), cefotaxime plus metronidazole (4)			6 HUS. 2 after antibiotic administration (unspecified)	
Tserenpuntsag, 2005[42]	89	142					36 HUS (11 after antibiotic administration) $P = 0,38$	
Lynn, 2005[36]	63	332	< 16	ciprofloxacin (8) penicillin, metronidazole, cephalosporins (2nd or 3rd generation)	("before admission")		395 HUS, of which 63 received antibiotics.	7 (3 of n1 vs. 4 of n2, $P > 0.05$)
Matsel, 2009[68]	3	19	4.8 +/- 3.3	Unknown	Before development of HUS		22 HUS. 3 after antibiotic administration	1 (HUS)
Rivero, 2010[56]	14	30	Median age 21,5 months, range 6-72 months	Unknown			16 HUS. 7 after antibiotic treatment	1 (HUS)

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Table 3 (*continued*)

Study (first author, year)	n1: pts on antibiotic	n2: pts not on antibiotic	Age (years)	Administered antibiotics (number of pts who received them)	Onset (and duration) of antibiotic administration	Duration of illness/ diarrhoea/bloody diarrhoea	Total HUS/TTP, and HUS after antibiotic administration (antibiotic and number of pts)	Number of deaths
Piercefield, 2010[37]	42	30			After the onset of symptoms but before the diagnosis of HUS		42 HUS. 15 after antibiotic administration.	1 (HUS)
Matano, 2011[67]	4	0		fosfomycin p.o. (4), ceftriaxone i.v.(1), cefmetazole i.v. (2), meropenem i.v. (1), kanamycin p.o. (1), sulbactam/cefoperazone i.v. (1)	fosfomycin on admission in all patients, continued until day 8 post-admission in the 2 surviving patients. IV antibiotics were administered 2-3 days after admission, with the exception of a single dose of cefmetazole administered on admission to the 1 surviving patient.		4 HUS. fosfomycin (4), cefmetazole (1)	2 (HUS)
Ohnishi, 2012[41]	6	9	>16	levofloxacin (6)	2–6 days after illness onset (mean ± SD, 3.3 ± 1.5 days).		1 HUS in a patient not receiving any antibiotics	
Menne, 2012[70]	52	246	47.7 ± 18.4	meropenem and ciprofloxacin and additionally rifaximin in patients in ICU after a diagnosis of HUS	After diagnosis of HUS			
Wong, 2012[8]	25	234	<10	TMP/SMX (9), metronidazole (3), b-lactams (9), azithromycin (4)	Within 7 days after the onset of diarrhoea		36 HUS. Antibiotics: 9/25. No antibiotics: 27/234. TMP/SMX (4), b-lactams (2), metronidazole (2), azithromycin (1).	
Smith, 2012[38]	19 Cases / 25 controls		< 20		Within 3 days after the onset of diarrhoea		19 HUS. Bactericidal antibiotic: 12 cases / 6 controls, b-lactams 9 cases / 1 control. Bacteriostatic antibiotic: 7 cases / 19 controls	
	27 Cases / 38 controls		< 20		Within 7 days after the onset of diarrhoea		27 HUS. Bacteriosidal antibiotic 17 cases/10 controls, b-lactams 14 cases/5 control. Bacteriostatic antibiotic 10 cases/28 controls	
Geerdes-Fenge, 2013[60]	7	17	36 ± 25 (range 4–81)	before onset of illness: metronidazole (5), cefotaxime (1), pip/tazo (1), amoxicillin (1), ciprofloxacin (5)	metronidazole before day 3 in 3/4 and ciprofloxacin + metronidazole on day 8 in 1/4 of patients who developed HUS		19 HUS, n1: 4, n2: 15 metronidazole (4), ciprofloxacin (2), cefotaxime (1), pip/tazo (1)	

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Table 3 (*continued*)

Study (first author, year)	n1: pts on antibiotic	n2: pts not on antibiotic	Age (years)	Administered antibiotics (number of pts who received them)	Onset (and duration) of antibiotic administration	Duration of illness/ diarrhoea/bloody diarrhoea	Total HUS/TTP, and HUS after antibiotic administration (antibiotic and number of pts)	Number of deaths
Delmas, 2014[69]	4	5					4 HUS	
Tajiri, 2015[66]	83	35	≤ 14	fosfomycin (50), norfloxacin (4), erythromycin (2), clarithromycin (1), cefteram (1), flomoxef (10), cefmetazole (2), cefotiam (2), cefpirome (1), ceftriaxone (5), cefotaxime (3), ampicillin (2)	Within 5 days of onset of diarrhoea		64 HUS. Fosfomycin (20), norfloxacin (2), erythromycin (1), clarithromycin (1), cefteram (1), flomoxef (3), cefmetazole (1), cefotiam (1), cefpirome (1), ceftriaxone (5), cefotaxime (2), ampicillin (2)	
Launders, 2015[39]	394	2929		Unknown (184), quinolones (81), metronidazole (47), b-lactams (42), macrolides (41)			172 HUS. B-lactams (7, 6 of which were penicillin derivatives)	
Freedman, 2017[40]	20	58	< 19	Unknown			10 HUS. n1: 5, n2: 5	

Table 4
Authors' assessment of the effect of antibiotic treatment on patients with STEC infection.

	Number of pts	Effect of antibiotics			Comment
		Beneficial	Detrimental	Neither	
Riley, 1983[44]	47			+	
Remis, 1984[45]	28			+	
Ryan, 1986[46]	22			+	
Carter, 1987[54]	68		+		Antibiotic administration was associated with risk of death. However, the study does not specify which of the patients that received antibiotics developed HUS.
MacDonald, 1988[47]	25			+	
Griffin, 1988[48]	320			+	
Ostroff, 1989[49]	93		+	+	An increased predisposition for HUS/TTP was noted in patients receiving cotrimoxazole or gentamicin. Although the two patients who developed TTP had received gentamicin, both of them were immunocompromised.
Pavia, 1990[55]	14		+		
Martin, 1990[58]	117	+			Antibiotic administration was significantly less common in patients with poor outcome or severe disease. However, the study does not assess whether antibiotic administration is associated to the development of HUS
Cimolai, 1990[59]	101	+			Less HUS with antibiotics but similar duration of diarrhoea.
Proulx, 1992[33]	96			+	
Bell, 1997[50]	278			+	
Slutsker, 1998[51]	93		+	+	Detrimental for children <13 years old for risk of HUS
Takeda, 1998[62]	329	+			
Ikeda, 1999[64]	292	+			
Hashimoto, 1999[53]	126	-			Strain produced Stx1 only. No cases of HUS occurred.
Fukushima, 1999[63]	425	+			All patients received antibiotics
Shiomi, 1999[61]	42	+			
Wong, 2000[57]	71		+		
Dundas, 2001[52]	120		+	+	Detrimental, when administered within 4 weeks before infection.
Cadwgan, 2002[43]	32			+	
Tserenpuntsag, 2005[42]	238			+	
Lynn, 2005[36]	395			+	
Matsel, 2009[68]	22	-			Provides no opinion – description of 22 HUS cases
Rivero, 2010[56]	44		+		
Piercefield, 2010[37]	72			+	
Matano, 2011[67]	4	+			
Ohnishi, 2012[41]	15	+		+	No significant difference among groups. Authors suggest that fluoroquinolone therapy is effective in preventing HUS if administered early.
Menne, 2012[70]	298	+			Antibiotics after diagnosis of HUS. Patients with established HUS seemed to benefit from antibiotic treatment.
Wong, 2012[8]	259		+		
Smith, 2012[38]	109		+	+	Bactericidal antibiotics, particularly b-lactams, were associated with HUS. No difference was observed with the use of bacteriostatic agents.
Geerdes-Fenge, 2013[60]	24	+			
Delmas, 2014[69]	24	-			Provides no opinion
Tajiri, 2015[66]	118	+			
Launders, 2015[39]	3323		+	+	The use of b-lactams was associated with the development of HUS. No association was observed with the use of non b-lactam antibiotics
Freedman, 2017[40]	78			+	Difference between groups not statistically significant, however warns against the use of antibiotics.

who received gentamycin or TMP/SMX were more likely to develop HUS. Nevertheless, both patients that developed TTP after gentamycin administration were immunocompromised, suggesting that gentamycin may not have been the cause of the manifestation of TTP.[2]

Freedman et al.[40] conducted a retrospective case series of 78 children with STEC in Canada. Antibiotics were administered in 50% (5/10) of patients that developed HUS compared with 22% (15/68) of patients that did not. However, this difference was not statistically significant ($P = 0.11$).

Two other studies found that antibiotic administration increased the risk of development of HUS, but only under specific conditions. Slutsker et al.[51] found that antibiotic administration increased the risk of developing HUS in children under 13 years of age, while Dundas et al.[52] found that the administration of antibiotics within 4 weeks before infection was a risk factor for the development of HUS.

In addition, two studies found that only specific classes of antibiotics increased the risk of developing HUS. Smith et al.[38] concluded that the use of bactericidal agents, particularly b-lactams, conferred an increased risk of HUS. The use of bacteriostatic agents, such as sulfonamides, did not. Similarly, Launders et al.,[39] in a retrospective cohort of 3323 symptomatic cases of STEC O157 in the UK, observed that the use of b-lactams was associated with the development of HUS, while no association was evident with the use of non b-lactam antibiotics, such as fluoroquinolones.

Finally, Hashimoto et al.[53] found no association between antibiotics and HUS. The results of their report strongly reflect that the type of toxin produced by the infecting STEC strain is a significant determinant for the development of HUS. The report presents an

outbreak in a Japanese school by *E. coli* O118:H2, a STEC strain that produces only Stx1. Despite affecting 126 people, no cases of HUS occurred. The administration of antibiotics did not exacerbate the condition of the patients. It did however prolong pathogen excretion in stool and may have increased the number of carriers.

Studies that associate the administration antibiotics with an increase in the risk of HUS in STEC infections

Ten studies, including five of those already mentioned, concluded that antibiotics increase the risk of HUS.[8,38,39,49,51,52,54–57] Carter et al.[54] reported an outbreak of *E. coli* O157:H7 colitis in a nursing home. Of the 12 patients that developed HUS only 1 survived. Antibiotic administration after the onset of symptoms was associated with an increased risk of death and with an increased risk of acquiring the infection at the secondary stage of the epidemic. However, the study does not specify which of the patients that received antibiotics developed HUS. The authors speculated that the association between antibiotic administration and risk of death might be a reflection of the severity of illness, as more severely ill patients were more likely to receive antibiotics.

Another study investigating an institutional outbreak was conducted by Pavia et al.[55]. They found that the administration of TMP/SMX within the first 72 h of the onset of diarrhoea was associated both with the development of HUS and with a longer duration of diarrhoea and bloody diarrhoea. Of the 8 patients that developed HUS, 5 were treated with TMP/SMX and one had received sulfasalazine. Likewise, the interim results of the study by Wong et al. found that antibiotic administration increased the risk of developing HUS, especially in children treated within 72 h of illness onset. The antibiotics associated with HUS were TMP/SMX and b-lactams.[57] In their completed report, it was reported that higher rates of HUS were also observed in children that received metronidazole and, to a lesser extent, azithromycin.[8]

A study involving children with diarrhoea conducted in Argentina,[56] the country with the highest incidence of HUS, found a positive association between the development of HUS and the administration of antibiotic therapy. These results were based on the observation that antibiotics were administered in 7 of the 16 cases of STEC infected children that developed HUS.

Studies that associate the administration antibiotics with a reduction in the risk of HUS in STEC infections

In contrast to the results mentioned above, other investigators have found the administration of antibiotics beneficial for STEC infections. The first study to find merit in antibiotic administration was published by Martin et al.[58] which the authors conducted a retrospective study of HUS cases in Minnesota. They showed that in patients that had developed HUS, the administration of antibiotics during the prodromal illness was associated with a less severe course. However, the study does not assess whether antibiotic administration is associated to the subsequent development of HUS.

Cimolai et al.[59] found that, while they had no effect on the duration of diarrhoea or bloody diarrhoea, the administration of TMP/SMX or ampicillin for more than 24 h in patients with *E. coli* O157:H7 infection prevented the progression to HUS.

In a small study conducted in Germany during the 2011 O104:H4 epidemic, Geerdes-Fenge et al.,[60] demonstrated that administration of ciprofloxacin reduced the occurrence of HUS in STEC infected patients. In total, 24 STEC infected patients were included, 7 of which received antibiotics. Of the 5 patients that received ciprofloxacin, only 2 (40%) developed HUS, whereas out of 19 patients that did not receive ciprofloxacin, 17 (89%) developed HUS, a difference found statistically significant ($p = 0.043$).

Most of the studies demonstrating the benefit of antibiotics in STEC infections hail from Japan. Many of these were conducted during the 1996 epidemic in Sakai,[61–64] during which 6561 cases were reported.[65] In all reports patients were treated with antibiotics, as the recommendations of the Japanese Ministry of Health and Welfare advocated their use.[2] In the majority of reports, the most commonly prescribed antibiotic was fosfomycin.[61–64] In the largest of these, all 425 children examined at Sakai City Hospital were administered bactericidal antibiotics (not specified otherwise), fosfomycin and lactobacilli at presentation. Twelve cases of HUS were reported, 11 of which had received antibiotics within the first 3 days of illness. All 12 children recovered with no significant sequelae.[63]

Takeda[62] conducted a retrospective survey of the outbreak. Questionnaires completed by chief physicians of 1682 hospitals from all over Japan revealed that more than 90% of the 1271 patients reported had received antibiotics. Further analysis of 329 cases with confirmed *E. coli* O157:H7 infection showed that 310 had received antibiotics, with fosfomycin being the drug of choice in 92% of patients. Of the 329 patients, 68 developed HUS. There was a significant decrease of the incidence of HUS in the group that received antibiotics. Within the group, the administration of antibiotics within the first 3 days of symptom onset, further reduced the incidence of HUS, significantly. Nevertheless, patients who received antibiotics after the third day of illness still exhibited a significantly lower risk of progressing to HUS than the group that did not receive any antimicrobial treatment. A strategy proposed by the author, aiming to prevent the progression of STEC infection to HUS, consisted of diagnosis of STEC induced hemorrhagic colitis within 3 days, administration of oral fosfomycin and the use of two treatment adjuvants, namely Synsorb Pk, an Stx binding agent in the gut and anti-Stx monoclonal antibody.

Ikeda et al.[64] evaluated the effects of antibiotics and timing of administration on preventing HUS, by comparing the administration of fosfomycin within the first 2 days of illness with the administration of other antibiotics in the same time frame and with the administration of fosfomycin after the first 2 days of illness. Fosfomycin administration within 2 days of illness was found superior to other antibiotics in preventing HUS. However, if fosfomycin was given after the second day of illness, it did not prevent HUS. The control group not receiving antibiotics in this study consisted only of 2 patients,[2] therefore limiting the conclusions that could be extrapolated from this study.

Prevention of HUS by fosfomycin alone or in combination with cefotaxime was compared against fluoroquinolones by Shiomi et al.[61]. None of the 15 patients treated with oral fluoroquinolones (sparfloxacin or norfloxacin) developed HUS, whereas 3 of 15 patients treated with i.v. fosfomycin and in 2 of 12 patients treated with i.v. cefotaxime and oral fosfomycin went on to develop HUS. All treatment regiments were administered within 3 days of illness. Oral fluoroquinolones eliminated the fecal excretion of STEC and Stx, although this effect was not studied for the other antibiotics. The limitations of this study were that the 3 different treatment modalities described were each administered at different hospitals and that the majority of patients in the oral fluoroquinolone group had received other antibiotics prior to admission. In addition, the study did not include a control group.

The value of oral fosfomycin was also examined by Tajiri et al.,[66] who conducted a retrospective case control study of HUS in STEC infected children in Japan, between 1997 and 2013, not including any patients who contracted the infection during the 1996 outbreak in Sakai. Factors associated with the development of HUS were young age (<3 years old) and initial CRP value $\geq$ 1.2 mg/dl. The use of oral fosfomycin was found to significantly reduce occurrence of HUS. Specifically, the odds ratio of developing HUS between the group of patients who took oral fosfomycin within the first 5 days of illness and those who did not was 0.15 (95% confidence interval 0.05–0.45). Nevertheless, in a case series consisting

of 4 patients with STEC infection during an outbreak Toyama, Japan in 2011,[67] fosfomycin did not prevent the development of HUS in any of the patients, all 4 of which developed encephalopathy and 2 died.

Limited information could be gathered from other reports. In a report concerning the outbreak in Walkerton, Canada in 2000, Matsel and White[68] state that of the 22 children with HUS they included, only 3 had taken antibiotics during the stage of colitis, while of the 9 patients with HUS reported by Delmas et al.[69] in France, 4 had received antibiotics during the prodromal illness. Neither report clarified about the timing of administration or the agents administered.

Finally, Menne et al.[70] assessed the role of various treatment strategies in patients with already developed HUS. The study was conducted during the 2011 outbreak of O104:H4 strain in Germany. In one of the centres involved in the study, patients with HUS received, among other treatments, meropenem and ciprofloxacin and additionally rifaximin if they were admitted to the intensive care unit (ICU). Compared with patients at other centres, who did not receive antibiotics, these patients had fewer seizures, did not require abdominal surgery and excreted *E. coli* for a shorter time-period. The authors concluded that patients with established HUS may benefit from antibiotic treatment.

Discussion

Introduction

As HUS remains an untreatable condition, the prevention of its development is the prime goal in STEC infected patients. The aim of this article is to present an up to date review of the current literature, regarding the administration of antibiotics in STEC infections. It is evident that some classes do indeed increase the incidence of HUS and should therefore be avoided. However, that does not seem to apply to all antibiotics, as some classes have been administered safely and successfully in STEC infected patients, reducing the incidence of HUS.

This review provides new insight on the association of HUS with antibiotics, as well as to designate safe antibiotic treatment options for STEC infected patients, possibly leading to a reduction of the incidence of HUS.

Strain specific response to antibiotics

One of the major determinants of HUS development is the infecting STEC strain and the type of toxin produced by it. This is evident by the reports of both clinical[53] and epidemiologic[14,71] studies, which state that HUS rarely, if ever, develops in patients infected with solely Stx1 producing STEC.

The induction of toxin production by antibiotics also depends on the type of toxin. In strains producing both toxins, production of Stx2, but not Stx1, increases after exposure to certain antibiotics.[10–12] For example, ciprofloxacin has been found to induce Stx2 production and inhibit Stx1 production, in strains producing both or only one of the toxins.[12] This could be explained in part by the fact that only the regulation of Stx2 has been linked to the SOS response, whereas Stx1 production appears to be affected by other factors, such as iron concentrations.[4]

The type of toxin produced by the STEC strain can be rapidly identified using the direct detection of Stx1 and Stx2 in stool samples by real-time PCR for the genes of the toxins or by rapid immunoassays.[72] Furthermore, in an outbreak setting, the type of Stx produced by the responsible STEC strain can be identified with the utilisation of whole genome sequencing (WGS).[73] WGS has been utilised in the 2011 O104:H4 STEC outbreak in Germany, and was able to completely sequence the responsible STEC strain within 62 h.[74] Sequencing data are uploaded to a website such as the one of the Center's for Genomic Epidemiology (www.genomicepidemiology.org), and data about the serotype, virulence and resistance profile of the STEC isolate can become available within hours from WGS uploading.[73] As was the case for the outbreak in Germany, WGS provides such information early in the course of the outbreak. As more data is compiled regarding the effects of specific antibiotics on Stx production by individual STEC strains, we propose that these supplementary data can be linked to the individual STEC strain and incorporated to already existing web-based tools, the same way virulence or resistance genes are.[72] A rapid characterisation of the responsible STEC strain and its response to specific antibiotics, could enable antibiotics to be administered safely.[20] An example of this would be the isolation of an Stx1 producing strain, in which case the administration of antibiotic treatment is unlikely to lead to the development of HUS.

The role of specific antibiotics

Following the infecting STEC strain, the second most important aspect determining the progression to HUS following antibiotic administration, is the type of antibiotic used. Many studies provide contradicting data about the same antibiotic, so an attempt has been made to provide commentary only on antibiotics that data appear to be more uniform. In general, the results of in vitro studies show that the responses of STEC strains to fosfomycin,[20] fluoroquinolones,[11,12,15,16,19,20,22–24] TMP/SMX,[11,15] B-lactams[10,12,15,18,26] and rifampicin[10,11,27] are highly strain specific. Adequate clinical data were available for fosfomycin, fluoroquinolones, TMP/SMX and b-lactams, whereas the commentary on gentamycin, rifamycins and colistin is based more on data from in vitro and in vivo studies.

Fosfomycin

Clinical studies in Japan highlight the value of fosfomycin.[61–64,66] In Japan, as it is evident from these studies, the vast majority of patients with STEC infections receive antibiotics and, in most cases, a clear benefit is noted from fosfomycin administration.[62–64,66] Most in vitro experiments regarding fosfomycin show that the antibiotic does not affect[12,17,19,23] or actually decreases[16,20] Stx release from STEC. Nonetheless, fosfomycin appears to increase the release of Stx[16,20,26] in some studies.

A large amount of clinical data[62–64,66] suggest that fosfomycin can be a promising agent in STEC infections, especially in sporadic cases, where the strain and its responses to antibiotic administration are unknown. However, the beneficial effects of fosfomycin, as shown in the Japanese clinical studies, could represent a localised phenomenon, as it is possible that the STEC strains endemic to Japan do not increase Stx release after exposure to fosfomycin. Therefore, it is possible that fosfomycin could induce HUS in the setting that the infecting STEC strain responds to the antibiotic in a way that increases the release of Stx. This possibility notwithstanding, fosfomycin should be examined as a potential agent for STEC infections. As fosfomycin has been found to be active against other bacteria causing dysentery, such as *Campylobacter jejuni* and *S. dysenteriae*,[75–77] fosfomycin use should be further investigated as to whether it could be utilised as part of an initial empiric regimen in patients presenting with dysentery.

Fluoroquinolones

In vitro studies generally demonstrate mostly unfavourable results after fluoroquinolone administration. Ciprofloxacin has been found to induce the production of Stx in many studies,[11,12,15,16,19,20,22–24] while only few have found that Stx production is inhibited by it.[16,20,22] Most of these studies have used

O157:H7 STEC strains. Two studies focusing on the effects of antibiotics on STEC O104:H4 toxin production have contradicting results regarding the effects of ciprofloxacin, as in one study toxin production is induced by ciprofloxacin[19] while in the other it is inhibited by the antibiotic.[20] Regarding the clinical relevance of these results, the conclusions that can be drawn from these studies are limited, as the responses of STEC strains to fluoroquinolones are heavily specific to the strain examined and the toxin produced by it. Similarly, the results of in vivo studies regarding fluoroquinolones are quite varied as some have found benefit in survival of STEC infected animals after the administration of fluoroquinolones[17,29,30] whereas others found that they caused HUS in the animals that received them, with survival being no different than control.[12,23,32]

On the contrary, the results of clinical studies have shown surprisingly positive results regarding the use of fluoroquinolones. A retrospective cohort of 3323 symptomatic O157 STEC infections in the UK found no association between the use of fluoroquinolones and the development of HUS.[39] Ciprofloxacin has been shown to reduce the risk of HUS development in a small clinical study from Germany during the 2011 O104:H4 STEC outbreak,[60] while another report regarding the same epidemic found that the combination of meropenem and ciprofloxacin reduced the rate of complications in patients who had already developed HUS.[70] In studies conducted in Japan, fluoroquinolones were either found to not affect[41] or even reduce the incidence of HUS.[61]

Finally, a recommendation exists that warns against the administration of fluoroquinolones. However, this was based only on in vitro and in vivo data from a single study, while no clinical studies regarding to the use of fluoroquinolones were included.[78] Given that results from in vivo studies are rather mixed and that the use of fluoroquinolones in clinical studies has been shown to improve outcomes, the use of fluoroquinolones in STEC infections needs to be reevaluated.

TMP/SMX

Information gathered from in vitro and in vivo experiments regarding the use of TMP/SMX in STEC infections is rather limited. In vivo studies have demonstrated that, while some benefit may exist from the early administration of TMP/SMX, it is soon lost, with HUS ensuing if the drug is administered three days after the inoculation of bacteria.[30]

Nevertheless, quite a few clinical studies concerning TMP/SMX are available, the majority of which report that its use increases the incidence of HUS[8,49,55,57] and even more so if administered during the first 72 h after the onset of symptoms.[57] Only one study reported that the administration of TMP/SMX for more than 24 h in patients with STEC O157:H7 infection prevented its progression to HUS,[59] while two others concluded that administration of sulfonamides[38] or TMP/SMX[33] had no effect in the occurrence of HUS. However, antibiotics in the latter study[33] were administered rather late in the course of illness[6] and it's likely that they had little if any effect on its course. Based on the available clinical evidence, the risks of TMP/SMX administration appear to outweigh the benefits and it is recommended that this agent should be avoided in STEC infections.

B-lactams

Ampicillin was found effective in treating STEC and preventing HUS in STEC infected mice, regardless of the timing of administration. In humans, only 1 study has shown that ampicillin may be able to prevent HUS, whereas numerous others demonstrate that the administration of b-lactams can have detrimental effects, by increasing the risk of HUS,[38,39,57] especially if administered within 72 h of symptom onset.[57] Given that the majority of clinical studies demonstrate that b-lactam administration is associated with the development of HUS, it is recommended that these agents should not be administered in STEC infected patients.

Gentamycin

The only clinical study that includes gentamycin[49] reports that it increases the risk of TTP. However, as both patients that developed TTP after gentamycin administration were immunocompromised, it is probable that gentamycin may not have been the cause.[2] Its behaviour as demonstrated by in vitro experiments shows promising results. Gentamycin does not induce an SOS response,[10] decreases STEC cytotoxicity to Vero cells[18] and does not induce phage production.[19] Its effects on Stx production are controversial, as most studies report that it decreases the production of Stx1 & Stx2,[11,12,18,28] whereas one study reports that, while gentamycin does not increase the gene expression of Stx2, it increases Stx2 release from STEC culture supernatants, likely representing the release of preformed toxin.[10] Administration of gentamycin alone failed to treat STEC infected mice. Combined with rifampicin however, gentamycin was found to increase their survival and further decreases Stx production by STEC.[28]

In conclusion, while the overall in vitro profile of gentamycin appears promising, the data from in vivo and clinical studies are not. As the evidence regarding gentamycin are inconclusive at best, its role should be further examined before conclusions are drawn. Nonetheless, it may be of use as a combination therapy with rifampicin,[28] but that remains to be seen.

Rifamycins

The response of STEC to both rifampicin and rifaximin has been evaluated by various studies. Rifaximin has been used as an adjuvant in patients admitted to the ICU with established HUS already receiving meropenem and ciprofloxacin, in a study that concluded that antibiotic treatment in general reduced the rate of complications in patients with established HUS.[70] In addition, rifaximin does not induce or possibly decreases phage replication or STEC cell lysis and does not increase the release Stx1 or Stx2.[19,24] Rifampicin has been found to decrease the mortality of STEC infected mice,[27] an effect further increased by its combination with gentamycin.[28]

Rifamycins demonstrate preferential inhibition of promoters associated with Stx transcription than of promoters associated with heat shock proteins,[24] proteins that protect the cell wall from lysis.[25] Combined with the fact that rifampicin does not induce an SOS response in STEC, this could mean that rifamycins may inhibit Stx production while at the same time do not induce the release of preformed toxin, which has been suggested as the mechanism behind the increased release of Stx by STEC following exposure to gentamycin.[10]

In conclusion, the use of rifamycins (and especially rifaximin) in STEC infections merits further study, perhaps more than any other class of antibiotics, as their mechanism of action as well as their in vitro profile suggest that they are unlikely to lead to a significant increase in Stx production and release.

Colistin

Data from in vitro experiments suggest that colistin can probably be of use in STEC infections. Colistin targets the cell wall and, as it is not involved in any DNA related processes, it does not induce an SOS response. It also binds endotoxin molecules, thereby limiting their toxicity. It has been demonstrated that co-incubation of STEC with colistin leads to a reduction of Stx release and diminishes the concentration of endotoxins, resulting in the increased survival of Vero cells. As colistin has a very limited bioavailability, the risk of nephrotoxicity in humans can be bypassed by per os administration of the drug.[21] Recent experiments in pigs infected with the ETEC strain *E. coli* O149:F4 have shown promising results.

Colistin was not detectable in the blood of infected pigs after oral administration, while it was barely detectable but too low to be quantified in blood samples from non-infected pigs. In addition, the degradation of colistin by simulated gastric fluids (composed of 3.2 g/L pepsin and 2 g/L NaCl at a pH of 1.2) lead to an increase of its antimicrobial activity against the ETEC strain used.[79] As colistin appears to be poorly absorbed, the oral administration of colistin may have the added benefit of achieving higher luminal concentrations. Further studies are required to evaluate its value in human STEC infections.

The importance of timing in antibiotic administration

In addition to the administered antibiotic, the timing of its administration appears to play a role in progression of the infection to HUS development, as early antibiotic treatment may prevent the manifestation of HUS. This is evident from clinical studies conducted in Japan, during the Sakai outbreak in 1996. One study reported that fosfomycin decreased the incidence of HUS, but only if administered within the first two days of illness.[64] Another study reported that, while antibiotic administration (mostly fosfomycin) diminished the incidence of HUS regardless of timing, administration of antibiotics within the first 3 days of illness further decreased the incidence of the complication. Delays in antibiotic administration lead to a progressive increase of the incidence, in accordance to the day of initiation of antibiotic treatment.[62] In contrast to these results, it has been reported that while the administration of TMP/SMX and b-lactams to STEC infected children increased the risk of HUS, the risk further increased if the antibiotics were administered within 72 h of the onset of illness.[57]

Unfortunately, no other clinical studies evaluated the impact of the timing of antibiotic treatment on the development of HUS. However, data from studies of STEC infected mice support its role as a factor. It appears that the effects of timing depend largely on the antibiotic administered. Whereas early or late administration did not appear to affect the effectiveness of a 2-day course of either norfloxacin, fosfomycin, kanamycin, ampicillin or clarithromycin, it had a significant impact on TMP/SMX treated mice. Early administration of TMP/SMX lead to a 100% survival rate, in contrast to late administration, which lead to a similar rate of complications and a worse survival rate than controls.[30]

Additional studies showed that a 5-day course of fosfomycin[29] as well as a single dose of azithromycin[23] were effective in preventing death in STEC infected mice, provided they were administered within 6 h.[23,29] However, whether these results have clinical relevance is a matter of discussion, as in both experiments treatment was found to be effective if administered within 6 h after the inoculation of bacteria. The extent of infection at 6 h or earlier may not yet be significant enough to cause illness, therefore these results may not represent a cure of infected mice, but rather the sterilisation of their gastric contents, before the illness had even begun.

In conclusion, it appears that early treatment with antibiotics may be able to prevent the manifestation of HUS, provided an appropriate agent is selected. Agents such as b-lactams and TMP/SMX are best avoided in STEC infections, regardless of the timing of administration. Given that only fosfomycin has been evaluated successfully in a clinical setting,[62,64] additional studies are required to evaluate whether the timing of administration of antibiotics other than fosfomycin has any effect in preventing the development of HUS.

Treatment adjuvants and new insights: Synsorb Pk, monoclonal antibodies and zinc

Takeda[62] recommended the use of Synsorb Pk, an Stx binding agent, as part of the treatment of children with STEC infection, aiming to prevent the progression to HUS. Unfortunately, a randomised, double-blind, placebo-controlled clinical trial, including 145 children with diarrhoea-associated HUS, conducted between 1997 and 2001 in the United States and Canada, failed to show that Synsorb Pk diminishes the severity of HUS.[80] However, Takeda[62] stated that Synsorb Pk was used in combination with fosfomycin at the early stage of illness, in cases with high fecal excretion of Stx and *E. coli* O157, resulting in recovery from illness. This suggests that the agent was used before the development of HUS, although this is not directly stated. In the aforementioned trial,[80] Synsorb Pk was used in children with already developed HUS, aiming to lessen the severity of illness. It seems prudent that the agent should be reexamined in patients with STEC colitis instead (without HUS manifestations), with the endpoint being the prevention of HUS.

Takeda[62] also recommended the use of monoclonal antibodies for the same purpose. The role of monoclonal antibodies in STEC infections remains to be elucidated. An Stx2 binding antibody, termed caStx2, has shown to be effective in preventing illness and death in STEC infected mice. A phase I trial of caStx2 has shown that the antibody was well tolerated by healthy volunteers and that it has a small volume of distribution, hinting that it is not absorbed by tissues and thus remains in the intravascular compartment.[81] A phase II trial of caStx2 is yet to be conducted.

A recent study evaluating another anti-Stx2 monoclonal antibody, Urtoxazumab, has demonstrated promising results. Urtoxazumab was able to significantly increase survival and weight gain and significantly reduce the incidence of brain lesions in neonatal gnotobiotic piglets infected with an Stx1 and Stx2 producing STEC strain. If clinical trials prove its efficacy and safety, this agent may have a significant effect on the incidence of HUS following infection with STEC.[82]

Zinc could provide another important treatment adjuvant in STEC infections. Trials in developing countries have demonstrated that zinc can reduce the duration and severity of acute diarrhoea in children. In vitro, zinc has been shown to inhibit the adherence of STEC strains to HeLa cells and inhibits the expression of Stx at both the protein and the RNA level, in strains expressing either or both Stx toxins. Zinc, at concentrations of 0.4 mM, achieves an over 90% inhibition of Stx production both at the basal level and after STEC treatment with ciprofloxacin or trimethoprim.[83] It also reduces the peroxide-induced translocation of Stx across human colon cells from the apical to basolateral side and inhibits the STEC SOS response.[84] These effects are independent of the zinc nutritional status of the host. In vivo experiments using rabbits demonstrated that zinc protected their intestine from STEC induced damage, reduced the adherence of STEC to the epithelium and reduced the amount of Stx in luminal fluids.[83]

The concentration of zinc in the colon under the circumstances of STEC infection cannot be reliably predicted, as it can either be decreased from dilution of zinc from the increased presence of fluids due to the diarrhoea or it can be increased, due to the malabsorptive state brought on by the diarrhoea.[83] Therefore, the appropriate dose cannot easily be calculated. Nevertheless, zinc should be formally evaluated in humans with STEC infection, as its mechanism of action targets the pathophysiologic basis of HUS, by reducing both Stx production and absorption and ameliorates the SOS response, reducing the production of Stx, even after exposure to ciprofloxacin and trimethoprim, two of the most vilified antibiotics in this setting.

Conclusions

In conclusion, it appears that the effect of antibiotics on the induction or inhibition of Stx production by STEC is highly strain specific. Therefore, as it has been suggested by other researchers,[20]

that in the case of a future outbreak, prompt isolation and characterisation of the responsible STEC strain regarding its response to specific antibiotics, can prove vital, by permitting antibiotic administration. In outbreak settings, rapid strain identification is possible through WGS, which provides information regarding the serotype, virulence and resistance profile of the STEC isolate, following in silico analysis of the sequence identified.[73] The addition of information regarding the response of each strain to specific antibiotics on the already existing web-based tools (www.genomicepidemiology.org), may permit antibiotic administration, by predicting the responses of the isolate to antibiotics and enabling the selection of an antibiotic that does not lead to an increase of the release of Stx by that particular strain.

While this can provide a guide to antibiotic administration in epidemic settings, such information is not available in sporadic cases. Identification of the type of Stx produced by direct detection in stool samples, either by real-time PCR or by rapid immunoassays[72] may permit antibiotic administration in the case that only Stx1 is detected. Furthermore, fosfomycin appears to be a relatively safe and effective choice, as it is probably the antibiotic with the most positive results so far, as demonstrated in studies of STEC epidemics in Japan. Nevertheless, it is unknown whether these results are applicable outside of Japan, as it is possible that STEC strains in other locations may increase the release of Stx following exposure to fosfomycin. In contrast, accumulating clinical evidence suggest that the use of b-lactams and TMP/SMX can be detrimental and these agents should therefore be avoided. The opposite appears to be true for fluoroquinolones, as a number of clinical studies contradict the results of in vitro experiments, which warn against the use of these antibiotics. Their role, as well as the role of colistin, gentamycin, rifamycins and treatment adjuvants such as zinc, monoclonal antibodies and Synsorb Pk or other intestinal Stx binding agents, merits further study.

Conflict of Interest

All authors declare that they have no conflict of interest.

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