MDPI

Perspective

The Environment, Farm Animals and Foods as Sources of _Clostridioides difficile_ Infection in Humans

Declan Bolton *[ID] and Pilar Marcos [ID]

Teagasc Food Research Centre, Ashtown, D15 DY05 Dublin, Ireland
* Correspondence: declan.bolton@teagasc.ie; Tel.: +353-1-8059539

Abstract: The recent discovery of the same _Clostridioides difficile_ ribotypes associated with human infection in a broad range of environments, animals and foods, coupled with an ever-increasing rate of community-acquired infections, suggests this pathogen may be foodborne. The objective of this review was to examine the evidence supporting this hypothesis. A review of the literature found that forty-three different ribotypes, including six hypervirulent strains, have been detected in meat and vegetable food products, all of which carry the genes encoding pathogenesis. Of these, nine ribotypes (002, 003, 012, 014, 027, 029, 070, 078 and 126) have been isolated from patients with confirmed community-associated _C. difficile_ infection (CDI). A meta-analysis of this data suggested there is a higher risk of exposure to all ribotypes when consuming shellfish or pork, with the latter being the main foodborne route for ribotypes 027 and 078, the hypervirulent strains that cause most human illnesses. Managing the risk of foodborne CDI is difficult as there are multiple routes of transmission from the farming and processing environment to humans. Moreover, the endospores are resistant to most physical and chemical treatments. The most effective current strategy is, therefore, to limit the use of broad-spectrum antibiotics while advising potentially vulnerable patients to avoid high-risk foods such as shellfish and pork.

Keywords: _Clostridioides difficile_; ribotypes; environment; food; epidemiology

1. Introduction

Clostridioides difficile is a Gram-positive, endospore-forming anaerobic bacterium often carried asymptomatically in the human gastrointestinal tract [1–5]. However, when conditions are favourable, the endospores germinate in the colon, vegetative cells multiply, and toxins are produced [6], resulting in watery, non-bloody diarrhoea with abdominal pain, toxic megacolon and/or pseudomembranous colitis, which may be fatal [7–9].

The most common risk factor associated with CDI is the use/misuse of broad-spectrum antibiotics. _C. difficile_ is often resistant to a wide range of antibiotics [10], and the administration of antibiotics like clindamycin, cephalosporins, penicillins and fluoroquinolones eliminate competitive bacteria in the colon and promote _C. difficile_ outgrowth [11]. The elderly, infants, other immune compromised, and patients on antibiotic therapies are therefore most at risk [1,2,4], although the incidence of CDI in pregnant women, children and patients with inflammatory bowel disease (IBD) has also increased [12].

The generally accepted route for human CDI is transmission from the healthcare environment [13]. However, in recent years the proportion of community-acquired CDI, where the patient has no association with a healthcare facility, has increased [14]. At the same time, non-human reservoirs, including the natural environment (soil, rivers and lakes) [15] and animals, including domestic pets [16,17], food animals [18–20] and wild fauna [21] have been reported. Moreover, food may be contaminated [22,23].

The link between _C. difficile_ and animals has been known for at least 60 years. In 1960, McBee [24] isolated this bacterium from the large intestine of a seal in Antarctica. By 1974 _C. difficile_ had also been detected in animal faeces (donkeys, horses, cows and camels) and in

the environment (hay, soil, sand and mud) [25]. In the early 1980s, *C. difficile* reservoirs were reported in healthy pigs and cattle [26,27] and in asymptomatic domestic pets, such as dogs, cats and birds, which had a prevalence of 21%, 30% and 33%, respectively [28]. Thus it was suggested that animals could be a vehicle of transmission to humans [29]. Interestingly, a common human pathogenic *C. difficile* ribotype (ribotype 078) was also isolated from pigs, cattle, and horses later, providing additional evidence of zoonotic transmission of *C. difficile* between animals and humans [30–33]. In more recent years, several studies have reported *C. difficile* in animals, on carcasses [21,34], in food processing facilities and in both raw and cooked foods [35–41].

Despite the increase in community-acquired CDI and data on *C. difficile* in the food chain, it is difficult to prove the source of infection in a given patient or outbreak as the same ribotypes and strains are common to both healthcare and food chain sources. Moreover, the patient may have acquired *C. difficile* sometime before the conditions in the colon changed to promote outgrowth. The objective of this review was to examine the evidence (CDI, virulence, ribotypes, environment, food animal and food sources and the current epidemiology of CDI in humans) supporting the hypothesis that *C. difficile* may be foodborne.

2. *C. difficile* Infection (CDI) in Humans

Elderly people are especially vulnerable to CDI, and cases are more likely to result in severe outcomes [42], possibly due to a decreased immune response or changes in the intestinal microbiota with age [43,44]. An underlying condition, chemotherapy or gastrointestinal surgery can increase susceptibility to CDI [45], which may become recurrent, leading to increased morbidity and mortality [46,47]. Broad-spectrum antibiotics significantly reduce the gut microflora diversity and alter the bile composition in the colon, facilitating CDI and recurrent infection in humans [48]. Treatment with acid suppression medication to prevent ulcers or treat acid-related diseases is also a risk factor for recurrence [49–51].

Metronidazole is used to treat mild to moderate CDI, while vancomycin is used in more severe cases, although the combination of both may be used when there are complications [52]. When these are ineffective, fidaxomicin has been proposed as an alternative to vancomycin [53,54] and has proven effective in preventing recurrent infection [55].

3. Virulence

Within the host, *C. difficile* endospores germinate into vegetative cells, colonise the intestinal tract and produce toxins resulting in disease [56,57], which causes intestinal inflammation, perforation, toxic megacolon and pseudomembranous colitis [58,59]. Mortality rates range from less than 2% to 17% [60,61]. The main virulence factors in *C. difficile* are toxin A and toxin B, encoded by the *tcdA* (308 kDa) and *tcdB* (270 kDa) genes located on a pathogenicity locus (PaLoc) (Figure 1 and Table 1). Both are large clostridial glycosylation toxins and are activated in response to environmental signals during the late log and stationary phases. In addition to the toxins, two regulatory proteins (TcdR and TcdC) and a protein whose function remains unclear (TcdE) complete the PaLoc [62,63]. TcdR (also referred to as TcdD) is a positive regulator activated in stationary phase growth, while TcdC is a negative regulator produced during the exponential phase. Mutations, such as deletions in the *tcdC* gene, may cause increased production of toxins A and B [62,64].
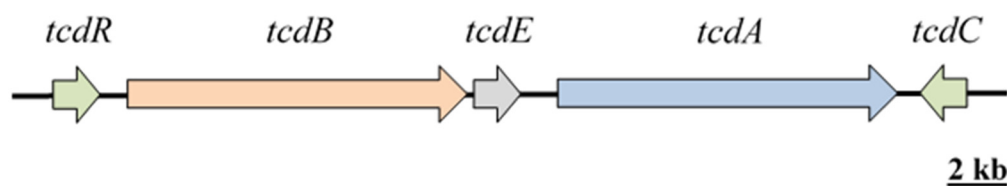

Figure 1. Illustration of the *C. difficile* Pathogenicity locus (PaLoc). Adapted from [65].

TcdA and TcdB possess the same biological activities, among which is the disruption of the cytoskeleton that leads to cytopathic effects in cultured cells. They also possess proinflammatory activity and can stimulate intestinal epithelial cells and immune cells to produce cytokines and chemokines [66,67]. Even low doses of toxins A and B damage the tight junctions of the gut epithelial barrier, facilitating the translocation of commensal bacteria, inflammation and cell apotheosis [66–68]. Sequence variations, deletions, and duplications within the pathogenicity locus account for different toxinotypes of *C. difficile*, with 27 currently identified. Certain strains can present only one of the toxins genes (A^-B^+ or A^+B^-), however, they reportedly still cause severe disease in humans [62]. In addition, the cytotoxicitybetween toxins that belong to different toxinotypes may vary, making the relation between strain type and CDI severity even more complex [59]. Strains lacking toxin A are more frequently reported due to deletions in the receptor-binding repetitive regions of TcdA caused by the recombination between short repetitive sequences highly conserved in this toxin gene [63]. Donta et al. [66] reported TcdB to be 4 to 200-fold more cytotoxic than TcdA in a mouse model. Therefore, strains producing toxin B have a higher severity in humans.

Up to a third of *C. difficile* isolates also produce the transferase *C. difficile* binary toxin (CDT) [69,70]. CDT, composed of CDTa (biological activity) and CDTb (binding), inhibits the protein actin, damaging the cytoskeleton of the gastrointestinal tract (GIT) cells [71]. The presence of the full-length CDT locus implies the potential expression of the binary toxin, and although some strains contain portions of the CDT locus, these are predicted as non-binary toxin-producing strains [68,70]. CDT-producing strains have been previously associated with a higher production of toxins A and B, leading to an increased disease severity [71,72]. However, CDT is not always present in severe cases [73,74]. In addition, CDT can also be produced by only B^+ and non-toxigenic strains (A^-B^-) [72]. Although CDT production is commonly associated with higher severity of *C. difficile* infection, the role of this toxin during infection and its mechanism of secretion is still not well understood.

Table 1. The virulence factors in *C. difficile* and their function.

Virulence Factor	Encoding Genes	Role in CDI	References
Toxin A	*tcdA*	Multiple cytopathic and cytotoxic effects on the targeted cells include disruption of Rho, Rac and Cdc42-dependent signalling, the actin cytoskeleton and the tight adherence junctions, increasing epithelial permeability, allowing commensal bacterial translocation, inflammation, diarrhoea and sometimes death.	[66–68,75]
Toxin B	*tcdB*		
TcdR	*tcdR*	TcdR is a positive regulator (produced in response environmental conditions) that triggers the induction of transcription of the toxin genes (*tcdA* and *tcdB*).	[76,77]
TcdC	*tcdC*	TcdC is a negative regulator that inhibits the expression of *tcdA* and *tcdB*. Mutations may cause increased production of toxins A and B.	[62,64]
TcdE	*tcdE*	TcdE may function as a lytic protein to facilitate the release of toxins A and B to the extracellular environment by a phage-like system, as these toxins lack signal peptides.	[78,79]
CDT	*cdtA & cdtB*	*C. difficile* binary toxin (CDT) is a transferase that disrupts the normal cytoskeletal function of cells by inhibiting the protein actin. The altered actin cytoskeleton causes an imbalance between actin and microtubules.	[69–71]

4. Ribotypes

There are in excess of 800 *C. difficile* ribotypes (RT), some of which are associated with increased virulence [6,80,81], including RT027 and RT078 [82,83]. These ribotypes are also more prevalent in human cases. RT027 (toxinotype III) has a mutation in *tcdC*, resulting in significantly increased production of toxins A and B while also carrying the genes encoding CDT production and fluoroquinolone resistance [84,85]. Although prevalence has decreased in Europe in recent years, RT027 is associated with a higher mortality and morbidity rate than other ribotypes [86]. The fluoroquinolone resistance, which emerged in two genetically distinct epidemiological lineages (FQR1 and FQR2), was a key driver in the rapid emergence of RT027 [57]. Moreover, this is essential to the increased severity of this ribotype, as this strain typically infects elderly hospital patients on fluoroquinolone treatment [5].

RT078 carries a 39 bp deletion in the *tcdC* gene and therefore overproduces toxins A and B in addition to the binary toxin CDT. In contrast to RT027, which is mostly hospital-acquired, RT078 is more prevalent in younger people and is generally associated with the community [87]. RT078 strains are resistant to fluoroquinolones and erythromycin, which has contributed to their higher prevalence in CDI [88]. Ribotype 126 has the same mutation in its *tcdC* gene found in RT078, is resistant to moxifloxacin and tetracycline and is also considered hypervirulent [89–91]. Other significant ribotypes from a public health perspective include RT017 and RT018. Although the former only produces toxin B, it is resistant to fluoroquinolones and rifampicin and has been associated with numerous outbreaks [92–94]. RT018 has high toxin production capacity, increased cell adhesion, is multidrug-resistant (erythromycin, clindamycin and moxifloxacin) and has become endemic in several countries, including Italy, Spain, Austria and Slovenia [95–97].

5. *C. difficile* in the Environment, Farm Animals and Food

5.1. Water

Toxigenic *C. difficile* has been isolated from a variety of aquatic environments, including drinking water, rivers, sewage effluent and swimming pools [98,99]. Coastal beaches and river sediments are also contaminated [98,99], in some cases by runoff from fields or effluents from wastewater treatment plants [100]. Indeed, *C. difficile* is often detected in water from treatment plants [101], and contamination of drinking water was the source of at least one *C. difficile* outbreak in Finland [102]. Thus, *C. difficile* survives in water and through the effluent treatment process [100].

5.2. Soil, Manure and Silage

C. difficile is commonly found in soil on farms as well as in forests, recreational parks, residential gardens, etc. [103–107]. These authors reported the highest prevalence in urban settings (57%), followed by farms (31%) and forests (28%). Shivaperumal et al. [108] found prevalence rates of 62%, 13% and 15% in garden soil, manure and compost, respectively, while Fröschle et al. [109] reported *C. difficile* to be the most prevalent *Clostridium* spp. in grass silage and cattle manure.

5.3. Farm Environment and Animals

Marcos et al. [110] reported that *C. difficile* were widespread in soil, water and faeces on beef, sheep and broiler farms, with the prevalence ranging from 7% to 83% and counts from 2.9 to 8.4 $\log_{10}$ cfu/g or /mL, depending on the animal species and sample type being tested. Other studies also found *C. difficile* in the faeces of a range of farm animals, including cattle, sheep, poultry and pigs [111–116]. Of these, pigs are the most important source of *C. difficile* [113,116], with the relative prevalence by age being 45%, 3% and 1% in suckling piglets, post-weaning piglets and finishing pigs, respectively [114]. Although these animals may show symptoms (diarrhoea), most are asymptomatic [114]. Other similar studies have reported a prevalence of 37% [115] and 78% [111] in piglets and 4% [115], 62% [117] and 9% [16] in mature pigs.

C. difficile are also found in cattle, especially younger animals. Rodriguez et al. [113] reported a prevalence of 11% in calves and 6% in adult cattle. Other studies have found these bacteria in 11% [118], 14% [117] and 22% of calves [111] and 7% of mature animals [16]. Sheep, including lambs, are also potential carriers, with 0.6 to 2% in the former and 7% reported in the latter [16,119].

Toxigenic *C. difficile* strains have also been reported in poultry faeces in several countries, including the USA (2.3%) [120], the Netherlands (5.8%) [107], Egypt (11.5%) [121], India (14%) [122], Zimbabwe (29%) [123] and Slovenia (62.3%) [124].

5.4. C. difficile at the Animal Slaughter Stage

Pathogenic bacteria in faeces on the hide/fleece or in the gastrointestinal tract are readily transferred to the carcass during slaughter and dressing [125]. *C. difficile* was found in 1%, 3% and 28% of porcine gut contents at slaughter in Belgium [126], Austria [18] and the Netherlands [127], respectively. Reported carcass contamination rates include 7% in Belgium [126], 15% in Canada [128] and 23% in Taiwan [129]. The prevalence of bovine carcass contamination ranges from 7–8% [111,126] but may be as high as 34% [130]. Ovine carcass contamination rates of 15% and 25% have been reported in Iran and Turkey, respectively [130,131]. While poultry carcass data is lacking, Candel-Pérez et al. [132] found *C. difficile* in 28% of gizzard and 6% of liver samples collected in a poultry processing plant in Spain. In Ireland, beef, sheep and broiler carcass contamination rates ranged from 40% to 100%, 40% to 60% and 10% to 40%, respectively, depending on the sampling stage during carcass processing [16].

Ribotypes 002, 005, 013, 014, 015, 019, 035, 062, 081, 087 and 126 have been identified in porcine faeces and rectal swabs at slaughter plants in Europe [18,111,126,127,133]. The *C. difficile* ribotypes isolated from other animal carcasses include 027 from cattle and IR46 from ovine carcasses [131]. Poultry slaughter data is lacking, although Koene et al. [16] found toxigenic ribotypes 056, 014 and 003 in faecal samples from poultry in Dutch slaughter plants.

5.5. C. difficile in Retail Foods

C. difficile has been reported in a range of foods at the retail stage. Thus, the consumption of contaminated retail foods, especially ready-to-eat (RTE) foods, is a risk factor for human infection [134]. Marcos et al. tested meat, dairy and vegetable retail foods and detected *C. difficile* in 9 out of the 240 samples tested [110]. These include corned beef (1), spinach leaves (2), iceberg and little gem lettuce (1 sample each), wild rocket, coleslaw, whole milk yoghurt and cottage cheese (also 1 sample each). Of these samples, direct counts were obtained for the spinach leaves (5.8 $\log_{10}$ cfu/g), coleslaw (4.3 $\log_{10}$ cfu/g) and cottage cheese (6.8 $\log_{10}$ cfu/g).

5.6. C. difficile in Meat and Seafood

Both raw and RTE meat and seafood are frequently contaminated with *C. difficile* [35,118], and the prevalence, including toxin gene profiles and ribotypes, is summarised in Table 2. The reported contamination rates include 41% [35] and 20% [135] for raw pork meat, 12% for ground pork meat [36] and up to 29% for pork sausages and RTE pork products [135]. A beef contamination rate of 42% was reported by Rodriguez-Palacios et al. [118], while ground beef rates include 2% [37], 12% [36], 20% [118] and 50% [35]. In one study, de Boer et al. [38] detected *C. difficile* in 6% of raw lamb samples. Reported poultry contamination rates include 1% [38], 3% [39], 8% [136,137], 13% [36,120] and 44% [35]. *C. difficile* has also been detected in shellfish and fish in several countries, with prevalence ranging from 4% to 49% [138–141].

Table 2. Meat and seafood retail foods contaminated with *C. difficile*, including toxin gene profiles (toxins A, B and CDT) and ribotypes.

Product	Raw or RTE	Total No. (%) Positive	Toxin Gene Profile	Ribotype(s)	Reference
Ground pork	Raw	3/7 (41.3%)	$A^+ B^+ CDT^+$	027 078	[35]
Ground pork	Raw	14/115 (12%)	$A^+ B^+ CDT^+$	027 078	[36]
Ground pork	Raw	2/66 (3.0%)	$A^+ B^+ CDT^-$	029	[39]
Pork meat	Raw	35/303 (11.5%)	$A^+ B^+ CDT^+$	078	[136]
Pork sausages	RTE	10/16 (62.5%)	$A^+ B^+ CDT^+$	027 078	[35]
Ground beef	Raw	13/26 (42.4%)	$A^+ B^+ CDT^+$	027 078	[35]
Ground beef	Raw	11/53 (20.8%)	$A^+ B^+ CDT^+$	M31	[118]
			$A^+ B^+ CDT^-$	014 077	
Ground beef	Raw	14/115 (12%)	$A^+ B^+ CDT^+$	027 078	[36]
Ground beef	Raw	2/105 (1.9%)	$A^+ B^+ CDT^{ND}$	012	[37]
Ground beef	Raw	21/303 (6.9%)	$A^+ B^+ CDT^+$	PA22	[136]
Beef	Raw	1/67 (1.5%)	$A^+ B^+ CDT^-$	029	[39]
Beef sausages	RTE	1/7 (14.3%)	$A^+ B^+ CDT^+$	027	[35]
Corned beef	RTE	1/10 (10%)	$A^{ND} B^+ CDT^{ND}$	ND [1]	[110]
Ground veal	Raw	1/7 (14.3%)	$A^+ B^+ CDT^+$	M31	[118]
Turkey	Raw	44/303 (14.5%)	$A^+ B^+ CDT^+$	PA01 PA05 PA16	[136]
Ground turkey	Raw	4/9 (44.4%)	$A^+ B^+ CDT^+$	078	[35]
Lamb	Raw	1/16 (6.3%)	$A^+ B^+ CDT^+$	045	[38]
Chicken	Raw	7/257 (2.7%)	$A^+ B^+ CDT^-$	001 003 071 087	[38]
Chicken	Raw	1/67 (1.5%)	$A^+ B^+ CDT^-$	029	[39]
Chicken	Raw	25/310 (8.0%)	$A^+ B^+ CDT^-$	ND [1]	[137]
Chicken	Raw	26/203 (12.8%)	$A^+ B^+ CDT^+$	078	[23]
Chicken	Raw	24/303 (7.8%)	$A^+ B^+ CDT^+$	PA05 PA16	[136]
Chicken	Raw	4/32 (12.5%)	$A^+ B^+ CDT^+$	078	[110]
Chicken	RTE	1/130 (0.8%)	$A^+ B^+ CDT^-$	014 020	[41]
Shellfish	Raw	118/702 (16.8%)	$A^+ B^+ CDT^+$	126 475	[141]

Table 2. *Cont.*

Product	Raw or RTE	Total No. (%) Positive	Toxin Gene Profile	Ribotype(s)	Reference
Bivalve molluscs	Raw	26/53 (49%)	$A^+ B^+ CDT^+$	078	[139]
			$A^+ B^+ CDT^-$	002 012 014/020 018 001	
Bivalve molluscs	Raw	36/925 (3.9%)	$A^+ B^+ CDT^+$	078 126 010	[140]
			$A^- B^+ CDT^-$	017 001	

+: Positive; −: Negative; [1] ND: Not determined.

5.7. C. difficile in Vegetables

The information on *C. difficile* isolated from vegetables is summarised in Table 3, with overall prevalence rates of 2% to 5% [22,103,142]. Lim et al. detected *C. difficile* in 56% of organic and 50% of non-organic potatoes, 22% of organic beetroots, 56% of organic onions and 53% of organic carrots [143]. Tkalec et al. found this pathogen in 9% of leaf vegetables, 7% of ginger, 26% and 60% of potatoes, and 14.3% of homegrown leaf vegetables [144]. RTE salads contamination rates included 2% [41], 3% [142], 3.3% [145] and 8% (153).

Table 3. Vegetable retail foods contaminated with *C. difficile,* including toxin gene profiles (toxins A, B and CDT) and ribotypes.

Product	Raw or RTE	Total No. (%) Positive	Toxin Gene Profile	Ribotype(s)	Reference
Root vegetables (potatoes, beetroots, onions and carrots)	Raw	30/100 (30%)	$A^+ B^+ CDT^+$	QX 274	[143]
			$A^+ B^+ CDT^-$	002 137 QX519 QX049 101	
			$A^- B^+ CDT^+$	070 237 584	
			$A^- B^- CDT^+$	033	
Root vegetables (potatoes, ginger) and leaf vegetables	Raw and RTE	28/154 (18.2%)	$A^+ B^+ CDT^-$	001/072 011/049 014/020 012 070 150 394 SLO129 SLO187 SLO279	[144]
			$A^+B^+CDT^+$	027 244 126 023	
Lettuce	RTE	1/54 (1.9%)	$A^+ B^+ CDT^+$	126	[41]

Table 3. *Cont.*

Product	Raw or RTE	Total No. (%) Positive	Toxin Gene Profile	Ribotype(s)	Reference
Vegetables (potato, onion, mushroom, carrot, radish and cucumber)	Raw	7/300 (2.4%)	A^+ B^{ND} CDT ND	ND [1]	[103]
Salad (lettuce, lamb's lettuce) and vegetable (pea sprouts)	RTE	3/104 (2.8%)	A^+ B^+ CDT$^-$	014/020 001 015	[142]
Vegetables (carrots, potatoes, garlic, ginger, beets, mushrooms, lettuce, green onions, radishes, etc.)	Raw and RTE	5/111 (4.5%)	A^+ B^+ CDT$^+$	078	[22]
			A^+ B^+ CDT$^-$	ND [1]	
Salad (baby leaf spinach)	RTE	2/60 (3.3%)	A^+ B^+ CDT$^+$	078 126	[145]
Salad (baby leaf spinach, organic mixed leaf salad, organic lettuce)	RTE	3/40 (7.5%)	A^+ B^+ CDT ND	001	[146]
			A^- B^+ CDT ND	017	
Spinach leaves	RTE	2/10 (20%)	A^-B^+ CDT$^-$	ND [1]	[110]
Iceberg lettuce leaves	RTE	1/10 (10%)	A^-B^+ CDT$^-$	ND [1]	[110]
Little Gem lettuce leaves	RTE	1/10 (10%)	A^-B^+ CDT$^-$	ND [1]	[110]
Wild rocket leaves	RTE	1/10 (10%)	A^-B^+ CDT$^+$	ND [1]	[110]
Coleslaw	RTE	1/10 (10%)	A^-B^+ CDT$^-$	ND [1]	[110]

+: Positive; −: Negative; [1] ND: Not determined.

All of these ribotypes have toxin genes associated with illness in humans. Many have been isolated directly from patients with CDI (Table 4), including 001, 002, 003, 010, 011, 012, 014, 015, 017, 018, 020, 023, 027, 029, 070, 071, 072, 077, 078, 087, 101, 126, 137 and 150. Of these, 002, 003, 012, 014, 027, 029, 070, 078 and 126 have been reported in confirmed community-acquired CDI, while 001, 017, 027, 072, 078 and 126 are hypervirulent.

Table 4. Further characterisation (pathogenicity, hypervirulence and association with community-acquired CDI) of the ribotypes isolated from foods (Tables 2 and 3).

Ribotype	Pathogenic			Hypervirulent			CA CDI [1]	Reference(s)
	yes	no	unk [2]	yes	no	unk		
001	✓			✓				[81,99,147–150]
002	✓				✓		✓	[81,99,148,149,151,152]
003	✓				✓		✓	[81,99]
010	✓				✓			[150]
011	✓				✓			[148]
012	✓				✓		✓	[81,148–150,153]
014	✓				✓		✓	[81,99,148,151,153]
015	✓				✓			[148,149,151]
017	✓			✓				[145,148,149,154]
018	✓				✓			[148,149]
020	✓				✓			[148,149,151]

Table 4. *Cont.*

Ribotype	Pathogenic	Hypervirulent	CA CDI [1]	Reference(s)
023	✓	✓		[148,149,151]
027	✓	✓	✓	[72,73,147–149,153,155]
029	✓	✓	✓	[99,153]
070	✓	✓	✓	[81,149]
071	✓	✓		[149]
072	✓	✓		[99,148,149,156]
077	✓	✓		[149]
078	✓	✓	✓	[72,73,148,149,153–155]
087	✓	✓		[148,149]
101	✓	✓		[149]
126	✓	✓	✓	[6,80,81,99,149,153]
137	✓	✓		[149]
150	✓	✓		[149]
033, 045, 049, 237, 244, 394, 475, 584, M31, PA01, PA05, PA16, PA22, QX049, QX274, QX519, SLO129, SLO187, SLO279		No information		

[1] CA CDI = community acquired *C. difficile* infection; [2] unk = unknown.

5.8. Meta-Analysis

The data presented in Tables 2 and 3 were analysed using Graphpad Prism version 9.3.1. The odds ratios (OR) (the odds of consuming a contaminated product) were calculated for each food type. Briefly, the OR was calculated as the number of positive over negative samples reported for each study. Turkey (with only two studies) was combined with the chicken data (poultry category), while the single lamb study was omitted. The medians and 95% confidence intervals were obtained and were then used to prepare the forest plots. In these Figures, the vertical line is set at an OR = 1 (50:50 chance of the food being contaminated). When all ribotypes are considered, shellfish and pork present a higher risk to the consumer (Figure 2). However, when the analysis is repeated, focusing exclusively on ribotypes 027 and 078 (the 2 hypervirulent strains most commonly associated with human infection), the increased risk is only associated with the consumption of pork (Figure 3).
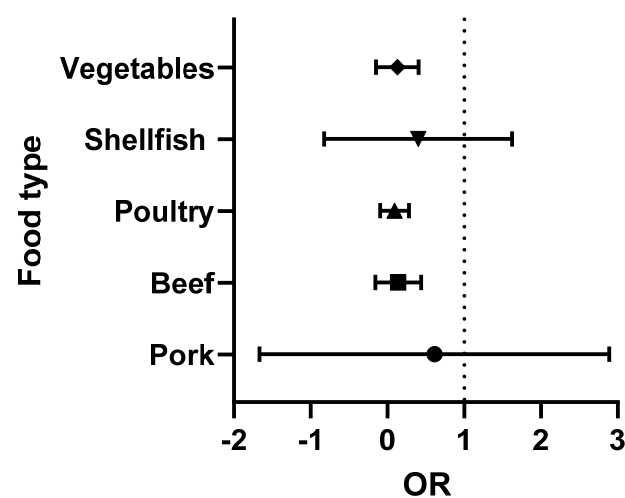

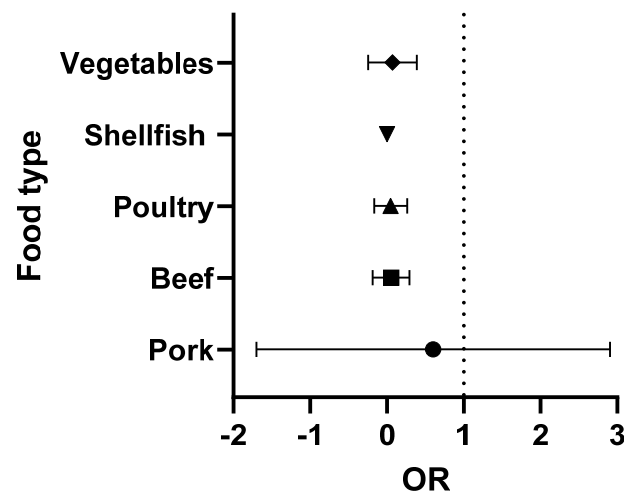**Figure 2.** Forest plot of the OR of *C. difficile* (all ribotypes) in each food type.

Figure 3. Forest plot of the OR of *C. difficile* 027 and 078 in each food type.

6. The Epidemiology of Foodborne Infection

In 1978, *C. difficile* was recognised as the causative agent of pseudomembranous colitis and diarrhoea in patients on antimicrobial therapy and it was a hospital-associated disease [157]. In the 1980s and 1990s, the incidence of CDI increased significantly, driven by the use of broad-spectrum third-generation cephalosporins (to which *C. difficile* is intrinsically resistant), but the disease was rarely fatal [158,159]. There was a further increase in CDI in the first 10 years of this century driven by the emergence and epidemic spread of the hypervirulent strain, ribotype 027 [160]. The epidemiology of CDI also changed in terms of clinical presentation, response to treatment, and disease outcome. Community-acquired CDI, defined as cases with symptom onset in the community with no history of hospitalisation in the previous 12 weeks or symptom onset within 48 h of hospital admission [161], also emerged. Since then, the incidence of CDI has remained high in developed countries [159,162], and rates of community acquired CDI have increased, accounting for 41%, 30% and 14% of total CDI in the USA, Australia and the EU, respectively [96,159,163]. Furthermore, community acquired CDI patients are generally younger, healthy, often female and lack the traditional risk factors of CDI, including a history of antimicrobial usage [164].

The natural habitat of *C. difficile* is the mammalian gastrointestinal tract (GIT). These bacteria colonise the neonatal GIT, proliferate and are excreted in the faeces to which other newborn animals are exposed, and the cycle recommences. As mammals develop, other bacterial species colonise the GIT, and the prevalence of *C. difficile* decreases [165]. The GIT microbiota inhibit germination, vegetative growth and toxin production, thus protecting against *C. difficile* [48]. However, in the 1990s, this protection was removed when cephalosporins were used in animal husbandry, and food animals became a major reservoir and amplification host for *C. difficile* [119,166], resulting in the contamination of the environment and a range of foods [100,119,166].

Once the environment is contaminated, there are multiple direct and indirect routes to humans, including via food (as illustrated in Figure 4). It is all but impossible to provide incontrovertible proof of foodborne transmission because of the ubiquitous nature of *C. difficile*, delayed onset of symptoms, ability to persist for extended periods as an endospore, etc. However, it has been shown that *C. difficile* endospores in animal waste, wastewater treatment sludge, soil, manure and compost may survive for extended periods of time, facilitating direct contamination of vegetables and fruit or meat via cross-contamination of carcasses during slaughter and processing [108,147]. Water also frequently contains *C. difficile* endospores [99,100,148], and food production may also be contaminated via water used for irrigation or food processing [100,144]. Moreover, the presence of endospores in rivers may contaminate fish and seafood [100,138,139,141]. Transfer from food and wild animals and from domestic pets has also been described [116,149].
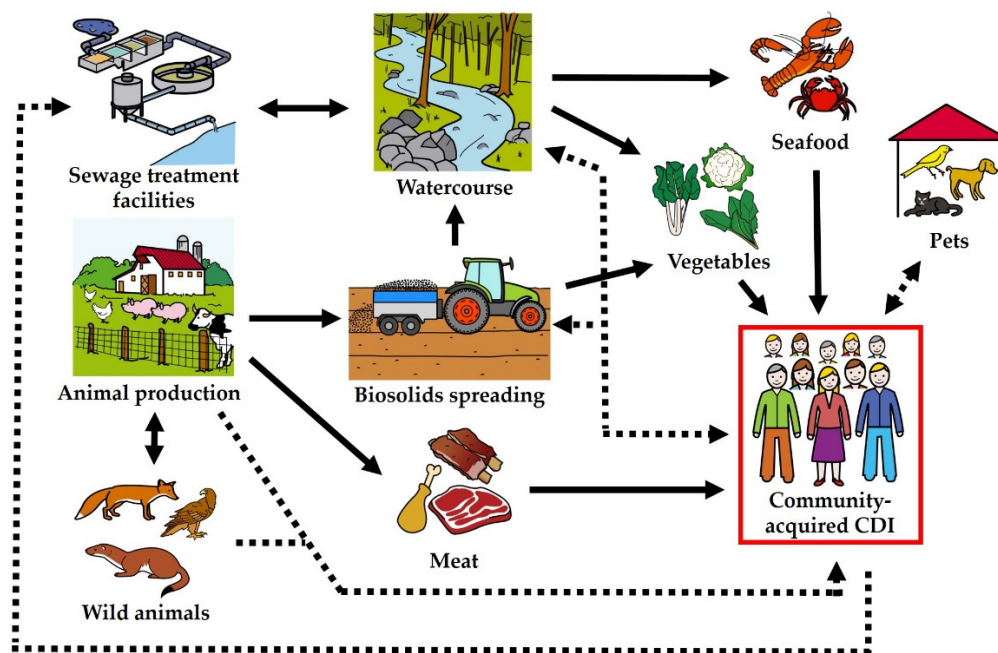
Figure 4. The cycle of community-associated CDI infections from zoonotic, environmental or foodborne sources. Adapted from [100] using ARASAAC pictograms.

Of particular interest, from the public health perspective, is the detection of similar *C. difficile* isolates in farm animals and in humans suffering from CDI, suggesting this pathogen may be zoonotic [150]. Whole genome sequencing (WGS) analysis has shown that ribotypes 078, 126 and 066, commonly found in pigs and/or cattle, are genetically identical to those in humans [151–155]. Although ribotype data for sheep is limited, ribotypes 014, 010 and 045 are common to both humans and ovine sources [119,156,167], while human-related ribotypes 001, 014 and 039 are also found in broilers [120–122,168].

7. Control Strategies

CDI can be controlled in hospitals using deep environmental cleaning, appropriate hand hygiene, stringent infection control and antimicrobial stewardship [169]. However, the same strategies cannot be used in agriculture and food processing [15]. Reduced usage of antibiotics in food animal production would reduce *C. difficile* amplification but is unlikely as increasing global food demand is driving increased antimicrobial usage in animal husbandry, which is projected to rise by 67% by 2030 [15,140]. In 2006 the EU banned the use of antibiotics as growth promoters, followed by the USA in 2017, but other major food-producing countries still allow this practice [170].

Preventing the recycling and dissemination of *C. difficile* endospores in animal slurries applied to land as organic fertilisers would also facilitate reduced environmental contamination and animal carriage. However, research is required to develop effective treatments [171]. Vaccination of food-producing animals is another possible control strategy, but an effective vaccine has not been developed yet [15]. Controlling *C. difficile* in food is dependent on reducing or eliminating the endospores, which are resistant to chilled (4 °C) and freezing (−18 °C and −80 °C) temperatures [172,173]. Although the endospores are resistant at 80 °C [172–174] and will survive the recommended cooking time temperature combinations recommended for meat [174], they are eliminated at 98 °C for 2 min [175]. The same authors suggested microwave irradiation (800 W/60 s) also achieved complete inactivation by denaturing the outer coat.

C. difficile endospores are also resistant to desiccation, hydrostatic pressure [37,176–179] and a range of food preservatives, including sodium nitrite, sodium nitrate and sodium metabisulfite, at permitted concentrations [180]. In contrast, nisin [181], black seed oil,

myrrh water [182], garlic juice, peppermint oil, trans-cinnamaldehyde, allicin, menthol and zingerone [183] have a potential application, but validation studies are required before they can be used in controlling *C. difficile* in food.

8. Conclusions

Based on the information provided, it was concluded that *C. difficile* is widespread in the environment and along the food chain. Many food isolates carry the virulence factors required for human infection, and there is no conceivable reason why food is not a source of these pathogens. This conclusion is further supported by the presence of the same ribotypes in food and humans suffering from community-acquired CDI. Based on our analysis, potentially vulnerable consumers should be advised not to handle or consume shellfish or pork.

References

1. Heinlen, L.; Ballard, J.D. Clostridium difficile infection. *Am. J. Med. Sci.* **2010**, *340*, 247–252. [CrossRef] [PubMed]
2. Rousseau, C.; Poilane, I.; De Pontual, L.; Maherault, A.C.; Le Monnier, A.; Collignon, A. *Clostridium difficile* carriage in healthy infants in the community: A potential reservoir for pathogenic strains. *Clin. Inf. Dis.* **2012**, *55*, 1209–1215. [CrossRef] [PubMed]
3. Galdys, A.L.; Curry, S.R.; Harrison, L.H. Asymptomatic *Clostridium difficile* colonization as a reservoir for *Clostridium difficile* infection. *Expert Rev. Anti-Infect. Ther.* **2014**, *12*, 967–980. [CrossRef]
4. Martin, J.S.; Monaghan, T.M.; Wilcox, M.H. *Clostridium difficile* infection: Epidemiology, diagnosis and understanding transmission. *Nat. Rev. Gastroenterol. Hepatol.* **2016**, *13*, 206–216. [CrossRef]
5. Czepiel, J.; Dróżdż, M.; Pituch, H.; Kuijper, E.J.; Perucki, W.; Mielimonka, A.; Goldman, S.; Wultańska, D.; Garlicki, A.; Biesiada, G. *Clostridium difficile* infection: Review. *Eur. J. Clin. Microbiol. Infect. Dis.* **2019**, *38*, 1211–1221. [CrossRef]
6. Vedantam, G.; Clark, A.; Chu, M.; McQuade, R.; Mallozzi, M.; Viswanathan, V.K. *Clostridium difficile* infection: Toxins and non-toxin virulence factors, and their contributions to disease establishment and host response. *Gut Microbes* **2012**, *3*, 121–134. [CrossRef]
7. Manabe, Y.C.; Vinetz, J.M.; Moore, R.D.; Merz, C.; Charache, P.; Bartlett, J.G. *Clostridium difficile* colitis: An efficient clinical approach to diagnosis. *Ann. Intern. Med.* **1995**, *123*, 835–840. [CrossRef]
8. Borriello, S.P. Pathogenesis of Clostridium difficile infection. *J. Antimicrobial. Chemo.* **1998**, *41*, 13–19. [CrossRef]
9. Brazier, J.S. Clostridium difficile: From obscurity to superbug. *Brit. J. Biomed. Sci.* **2008**, *65*, 39–44. [CrossRef]
10. Peng, Z.; Jin, D.; Kim, H.B.; Stratton, C.W.; Wu, B.; Tang, Y.-W.; Sun, X. Update on Antimicrobial Resistance in *Clostridium difficile*. *J. Clin. Microbiol.* **2017**, *55*, 1998–2008. [CrossRef]
11. Owen, R.C.; Donskey, C.J.; Gaynes, R.P.; Loo, V.G.; Muto, C.A. Antimicrobial-associated risk factors for *Clostridium difficile* infection. *Clin. Infect. Dis.* **2008**, *46*, 19–31. [CrossRef]
12. Ananthakrishnan, A. *Clostridium difficile* infection: Epidemiology, risk factors and management. *Nat. Rev. Gastroenterol. Hepatol.* **2011**, *8*, 17–26. [CrossRef] [PubMed]
13. Enoch, D.A.; Aliyu, S.H. Is *Clostridium difficile* infection still a problem for hospitals? *Can. Med. Assoc. J.* **2012**, *184*, 17–18. [CrossRef] [PubMed]
14. Kim, G.; Zhu, N.A. Community-acquired *Clostridium difficile* infection. *Can Fam Physician.* **2017**, *63*, 131–132.
15. Lim, S.C.; Knight, D.R.; Riley, T.V. *Clostridium difficile* and One Health. *Clin. Microbiol. Infect.* **2020**, *26*, 857–863. [CrossRef]

16. Koene, M.; Mevius, D.; Wagenaar, J.; Harmanus, C.; Hensgens, M.; Meetsma, A.; Putirulan, F.; van Bergen, M.; Kuijper, E. *Clostridium difficile* in Dutch animals: Their presence, characteristics and similarities with human isolates. *Clin. Microbiol. Infect.* **2012**, *18*, 778–784. [CrossRef]

17. Schneeberg, A.; Rupnik, M.; Neubauer, H.; Seyboldt, C. Prevalence and distribution of *Clostridium difficile* PCR ribotypes in cats and dogs from animal shelters in Thuringia, Germany. *Anaerobe* **2012**, *18*, 484–488. [CrossRef]

18. Indra, A.; Lassnig, H.; Baliko, N.; Much, P.; Fiedler, A.; Huhulescu, S. *Clostridium difficile*: A new zoonotic agent? *Wien. Klin. Wochenschr.* **2009**, *121*, 91–95. [CrossRef]

19. Schmid, A.; Messelhäusser, U.; Hörmansdorfer, S.; Sauter-Louis, C.; Mansfeld, R. Occurrence of zoonotic Clostridia and Yersinia in healthy cattle. *J. Food Protect.* **2013**, *76*, 1697–1703. [CrossRef]

20. Noren, T.; Johansson, K.; Unemo, M. *Clostridium difficile* PCR-ribotype 046 is common among neonatal pigs and humans in Sweden. *Clin. Microbiol. Infect.* **2014**, *20*, O2–O6. [CrossRef]

21. Rodriguez-Palacios, A.; Borgmann, S.; Kline, T.R.; LeJeune, J.T. *Clostridium difficile* in foods and animals: History and measures to reduce exposure. *Animal Health Res. Rev.* **2013**, *14*, 11–29. [CrossRef]

22. Metcalf, D.; Costa, M.C.; Dew, W.M.; Weese, J.S. *Clostridium difficile* in vegetables, Canada. *Lett. Appl. Microbiol.* **2010**, *51*, 600–602. [CrossRef] [PubMed]

23. Weese, J. *Clostridium difficile* in food—Innocent bystander or serious threat? *Clin. Microbiol. Infect.* **2010**, *16*, 3–10. [CrossRef] [PubMed]

24. McBee, R.H. Intestinal flora of some antarctic birds and mammals. *J. Bacteriol.* **1960**, *79*, 311–312. [CrossRef] [PubMed]

25. Hafiz, S. *Clostridium difficile* and Its Toxins. Ph.D. Thesis, Department of Microbiology, University of Leeds, Leeds, UK, 1974.

26. Princewell, T.J.T.; Agba, M.I. Examination of bovine faeces for the isolation and identification of *Clostridium* species. *J. Appl. Bacteriol.* **1982**, *52*, 97–102. [CrossRef]

27. Nagy, J.; Bilkei, G. Neonatal piglet losses associated with *Escherichia coli* and *Clostridium difficile* infection in a Slovakian outdoor production unit. *Vet. J.* **2003**, *166*, 98–100. [CrossRef]

28. Borriello, S.P.; Honour, P.; Turner, T.; Barclay, F. Household pets as a potential reservoir for *Clostridium difficile* infection. *J. Clin. Pathol.* **1983**, *36*, 84–87. [CrossRef]

29. Weber, A.; Kroth, P.; Heil, G. Domestic animals as excreters of *Clostridium difficile*. *Dtsch. Med. Wochenschr.* **1988**, *113*, 1617–1618.

30. Goorhuis, A.; Debast, S.B.; van Leengoed, L.A.; Harmanus, C.; Notermans, D.W.; Bergwerff, A.A.; Kuijper, E.J. *Clostridium difficile* PCR ribotype 078: An emerging strain in humans and in pigs? *J. Clin. Microbiol.* **2008**, *46*, 1157. [CrossRef]

31. Jhung, M.A.; Thompson, A.D.; Killgore, G.E.; Zukowski, W.E.; Songer, G.; Warny, M.; Johnson, S.; Gerding, D.N.; McDonald, L.C.; Limbago, B.M. Toxinotype V *Clostridium difficile* in humans and food animals. *Emer. Infect. Dis.* **2008**, *14*, 1039–1045. [CrossRef]

32. Rupnik, M.; Widmer, A.; Zimmermann, O.; Eckert, C.; Barbut, F. *Clostridium difficile* toxinotype V, ribotype 078, in animals and humans. *J. Clin. Microbiol.* **2008**, *46*, 2146. [CrossRef] [PubMed]

33. Debast, S.B.; van Leengoed, L.A.; Goorhuis, A.; Harmanus, C.; Kuijper, E.J.; Bergwerff, A.A. *Clostridium difficile* PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from affected humans. *Environ. Microbiol.* **2009**, *11*, 505–511. [CrossRef] [PubMed]

34. Susick, E.K.; Putnam, M.; Bermudez, D.M.; Thakur, S. Longitudinal study comparing the dynamics of *Clostridium difficile* in conventional and antimicrobial free pigs at farm and slaughter. *Vet. Microbiol.* **2012**, *157*, 172–178. [CrossRef]

35. Songer, J.G.; Trinh, H.T.; Killgore, G.E.; Thompson, A.D.; McDonald, L.C.; Limbago, B.M. *Clostridium difficile* in Retail Meat Products, USA, 2007. *Emer. Infect. Dis.* **2009**, *15*, 819–821. [CrossRef]

36. Weese, J.; Avery, B.P.; Rousseau, J.; Reid-Smith, J. Detection and Enumeration of *Clostridium difficile* Spores in Retail Beef and Pork. *Appl. Environ. Microbiol.* **2009**, *15*, 5009–5011. [CrossRef]

37. Bouttier, S.; Barc, M.C.; Felix, B.; Lambert, S.; Collignon, A.; Barbut, F. *Clostridium difficile* in ground meat, France. *Emer. Infect. Dis.* **2010**, *16*, 733–735. [CrossRef]

38. de Boer, E.; Zwartkruis-Nahuis, A.; Heuvelink, A.E.; Harmanus, C.; Kuijper, E.J. Prevalence of *Clostridium difficile* in retailed meat in The Netherlands. *Int. J. Food Microbiol.* **2011**, *144*, 561–564. [CrossRef] [PubMed]

39. Quesada-Gomez, C.; Mulvey, M.R.; Vargas, P.; Gamboa-Coronado Mde, l.M.; Rodriguez, C.; Rodriguez-Cavillini, E. Isolation of a toxigenic and clinical 25 genotype of *Clostridium difficile* in retail meats in Costa Rica. *J. Food Pro.* **2013**, *76*, 348–351. [CrossRef]

40. Kouassi, K.A.; Dadie, A.T.; N'Guessan, K.F.; Dje, K.M.; Loukou, Y.G. *Clostridium perfringens* and *Clostridium difficile* in cooked beef sold in Côte d'Ivoire and their antimicrobial susceptibility. *Anaerobe* **2014**, *28*, 90–94. [CrossRef]

41. Primavilla, S.; Farneti, S.; Petruzzelli, A.; Drigo, I.; Scuota, S. Contamination of hospital food with *Clostridium difficile* in Central Italy. *Anaerobe* **2019**, *55*, 8–10. [CrossRef]

42. Miller, M.; Gravel, D.; Mulvey, M.; Taylor, G.; Boyd, D.; Simor, A.; Gardam, M.; McGeer, A.; Hutchinson, J.; Moore, D.; et al. Health Care-Associated *Clostridium difficile* Infection in Canada: Patient Age and Infecting Strain Type Are Highly Predictive of Severe Outcome and Mortality. *Clin. Infect. Dis* **2010**, *50*, 194–201. [CrossRef] [PubMed]

43. Kelly, C.P. Immune response to Clostridium difficile infection. *Eur. J. Gastroenterol. Hepatol* **1996**, *8*, 1048–1053. [CrossRef] [PubMed]

44. Ogra, P.L. Ageing and its possible impact on mucosal immune responses. *Ageing Res. Rev.* **2010**, *9*, 101–106. [CrossRef] [PubMed]

45. Cole, S.A.; Stahl, T.J. Persistent and Recurrent *Clostridium difficile* Colitis. *Clin. Colon Rectal Sur.* **2015**, *28*, 65–69. [CrossRef] [PubMed]

46. Dharbhamulla, N.; Abdelhady, A.; Domadia, M.; Patel, S.; Gaughan, J.; Roy, S. Risk Factors Associated with Recurrent *Clostridium difficile* Infection. *J. Clin. Med. Res.* **2019**, *11*, 1–6. [CrossRef]

47. Song, J.H.; Kim, Y.S. Recurrent *Clostridium difficile* Infection: Risk Factors, Treatment, and Prevention. *Gut Liver* **2019**, *13*, 16–24. [CrossRef]

48. Britton, R.A.; Young, V.B. Role of the intestinal microbiota in resistance to colonization by *Clostridium difficile*. *Gastroenterology* **2014**, *146*, 1547–1553. [CrossRef]

49. Min, J.H.; Kim, Y.S. Proton pump inhibitors should be used with caution in critically Ill patients to prevent the risk of *Clostridium difficile* infection. *Gut Liver* **2016**, *10*, 493–494. [CrossRef]

50. Tariq, R.; Singh, S.; Gupta, A.; Pardi, D.S.; Khanna, S. Association of gastric acid suppression with recurrent *Clostridium difficile* infection: A systematic review and meta-analysis. *JAMA Internal Med.* **2017**, *177*, 784–791. [CrossRef]

51. Mehta, P.; Nahass, R.G.; Brunetti, L. Acid suppression medications during hospitalization as a risk factor for recurrence of *Clostridioides difficile* infection: Systematic review and meta-analysis. *Clin. Infect. Dis.* **2021**, *545*, 1–7. [CrossRef]

52. Surawicz, C.M.; Brandt, L.J.; Binion, D.G.; Ananthakrishnan, A.N.; Curry, S.R.; Gilligan, P.H.; McFarland, L.V.; Mellow, M.; Zuckerbraun, B.S. Guidelines for diagnosis, treatment, and prevention of *Clostridium difficile* infections. *Am. J. Gastroenterol.* **2013**, *108*, 478–498. [CrossRef] [PubMed]

53. Louie, T.J.; Miller, M.A.; Mullane, K.M.; Weiss, K.; Lentnek, A.; Golan, Y.; Gorbach, S.; Sears, P.; Shue, Y.-K. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. *N. Eng. J. Med.* **2011**, *364*, 422–431. [CrossRef] [PubMed]

54. Chaparro-Rojas, F.; Mullane, K.M. Emerging therapies for *Clostridium difficile* infection—Focus on fidaxomicin. Infect. *Drug Res.* **2013**, *6*, 41–53.

55. Iv, E.C.O.; Iii, E.C.O.; Johnson, D.A. Clinical update for the diagnosis and treatment of *Clostridium difficile* infection. *World J. Gastro. Pharmacol. Ther.* **2014**, *5*, 1–26. [CrossRef] [PubMed]

56. Dyer, C.; Hutt, L.P.; Burky, R.; Joshi, L.T. Biocide Resistance and Transmission of *Clostridium difficile* Spores Spiked onto Clinical Surfaces from an American Health Care Facility. *Appl. Environ. Microbiol.* **2019**, *85*, e01090-19. [CrossRef] [PubMed]

57. Depestel, D.D.; Aronoff, D.M. Epidemiology of *Clostridium difficile* infection. *J. Pharm. Pract.* **2013**, *26*, 464–475. [CrossRef] [PubMed]

58. Barbut, F.; Petit, J.C. Epidemiology of *Clostridium difficile*-associated infections. *Clin. Microbiol. Infect.* **2001**, *7*, 405–410. [CrossRef]

59. Akerlund, T.; Svenungsson, B.; Lagergren, A.; Burman, L.G. Correlation of disease severity with fecal toxin levels in patients with *Clostridium difficile*-associated diarrhea and distribution of PCR ribotypes and toxin yields in vitro of corresponding isolates. *J. Clin. Microbiol.* **2006**, *44*, 353–358. [CrossRef]

60. Evans, C.T.; Safdar, N. Current trends in the epidemiology and outcomes of *Clostridium difficile* infection. *Clin Infect Dis.* **2015**, *60* (Suppl. S2), S66–S71. [CrossRef]

61. Czepiel, J.; Krutova, M.; Mizrahi, A.; Khanafer, N.; Enoch, D.A.; Patyi, M.; Deptuła, A.; Agodi, A.; Nuvials, X.; Pituch, H.; et al. Mortality Following *Clostridioides difficile* Infection in Europe: A Retrospective Multicenter Case-Control Study. *Antibiotic* **2021**, *10*, 299. [CrossRef]

62. Voth, D.; Ballard, J.D. *Clostridium difficile* Toxins: Mechanism of Action and Role in Disease. *Clin. Microbiol. Rev.* **2005**, *2*, 247–263. [CrossRef] [PubMed]

63. Rupnik, M.; Janezic, S. An update on *Clostridium difficile* toxinotyping. *J. Clin. Microbiol.* **2016**, *54*, 13–18. [CrossRef] [PubMed]

64. Hundsberger, T.; Braun, V.; Weidmann, M.; Leuke, P.; Sauerborn, M.; von Eichel-Streiber, C. Transcription analysis of the genes *tcdA-E* of the pathogenicity locus of *Clostridium difficile*. *Eur. J. Biochem.* **1997**, *244*, 735–742. [CrossRef] [PubMed]

65. Matamouros, S.; England, P.; Dupuy, B. *Clostridium difficile* toxin expression is inhibited by the novel regulator TcdC. *Mol. Microbiol.* **2007**, *64*, 1274–1288. [CrossRef] [PubMed]

66. Donta, S.T.; Sullivan, N.; Wilkins, T.D. Differential effects of *Clostridium difficile* toxins on tissue-cultured cells. *J. Clin. Microbiol.* **1982**, *15*, 1157–1158. [CrossRef]

67. Feltis, B.A.; Wiesner, S.M.; Kim, A.S.; Erlandsen, S.L.; Lyerly, D.L.; Wilkins, T.D.; Wells, C.L. *Clostridium difficile* toxins A and B can alter epithelial permeability and promote bacterial paracellular migration through HT-29 enterocytes. *Shock* **2000**, *14*, 629–634. [CrossRef]

68. Riggs, M.M.; Sethi, A.K.; Zabarsky, T.F.; Eckstein, E.C.; Jump, R.L.P.; Donskey, C.J. Asymptomatic carriers are a potential source for transmission of epidemic and nonepidemic *Clostridium difficile* strains among long-term care facility residents. *Clin. Infect. Dis.* **2007**, *45*, 992–998. [CrossRef]

69. Stiles, B.G.; Wigelsworth, D.J.; Popoff, M.R.; Barth, H. Clostridial binary toxins: Iota and c2 family portraits. *Front. Cell. Infect. Microbiol.* **2011**, *1*, 11. [CrossRef]

70. Gerding, D.; Johnson, S.; Rupnik, M.; Aktories, K. *Clostridium difficile* binary toxin CDT: Mechanism, epidemiology, and potential clinical importance. *Gut Microbes* **2014**, *5*, 15–27. [CrossRef]

71. Aktories, K.; Papatheodorou, P.; Schwan, C. Binary *Clostridium difficile* toxin (CDT)—A virulence factor disturbing the cytoskeleton. *Anaerobe* **2018**, *53*, 21–29. [CrossRef]

72. Kuehne, S.A.; Collery, M.M.; Kelly, M.L.; Cartman, S.T.; Cockayne, A.; Minton, N.P. Importance of toxin A, toxin B, and CDT in virulence of an epidemic *Clostridium difficile* strain. *J. Infect. Dis.* **2014**, *209*, 83–86. [CrossRef] [PubMed]

73. Wilson, V.; Cheek, L.; Satta, G.; Walker-Bone, K.; Cubbon, M.; Citron, D. Predictors of death after *Clostridium difficile* infection: A report on 128 strain-typed cases from a teaching hospital in the United Kingdom. *Clin. Infect. Dis.* **2010**, *50*, 77–81. [CrossRef] [PubMed]

74. Goldenberg, S.D.; French, G.L. Lack of association of *tcdC* type and binary toxin status with disease severity and outcome in toxigenic *Clostridium Difficile*. *J. Infect.* **2011**, *62*, 355–362. [CrossRef] [PubMed]

75. Di Bella, S.; Ascenzi, P.; Siarakas, S.; Petrosillo, N.; di Masi, A. *Clostridium difficile* Toxins A and B: Insights into Pathogenic Properties and Extraintestinal Effects. *Toxins* **2016**, *8*, 134. [CrossRef] [PubMed]

76. Mizuno, T.; Kaibuchi, K.; Yamamoto, T.; Kawamura, M.; Sakoda, T.; Fujioka, H.; Matsuura, Y.; Takai, Y. A stimulatory GDP/GTP exchange protein for smg p21 is active on the post-translationally processed form of c-Ki-ras p21 and rhoA p21. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 6442–6446. [CrossRef] [PubMed]

77. Mani, N.; Dupuy, B. Regulation of toxin synthesis in *Clostridium difficile* by an alternative RNA polymerase sigma factor. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 5844–5849. [CrossRef]

78. Tan, K.S.; Wee, B.Y.; Song, K.P. Evidence for holin function of tcdE gene in the pathogenicity of *Clostridium difficile*. *J. Med. Microbiol.* **2001**, *50*, 613–619. [CrossRef]

79. Govind, R.; Dupuy, B. Secretion of *Clostridium difficile* toxins A and B requires the holin-like protein TcdE. *PLoS Pathog.* **2012**, *8*, e1002727. [CrossRef]

80. Dayananda, P.; Wilcox, M.H. A Review of Mixed Strain *Clostridium difficile* colonization and infection. *Front. Microbiol.* **2019**, *10*, 692. [CrossRef]

81. Azimirad, M.; Krutova, M.; Yadegar, A.; Shahrokh, S.; Olfatifar, M.; Aghdaei, H.A.; Fawley, W.N.; Wilcox, M.H.; Zali, M.R. *Clostridioides difficile* ribotypes 001 and 126 were predominant in Tehran healthcare settings from 2004 to 2018: A 14-year-long cross-sectional study. *Emerg. Microbes Infect.* **2020**, *1*, 1432–1443. [CrossRef]

82. Neely, F.; Lambert, M.L.; Van Broeck, J.; Delmée, M. Clinical and laboratory features of the most common *Clostridium difficile* ribotypes isolated in Belgium. *J. Hosp. Infect.* **2017**, *4*, 394–399. [CrossRef] [PubMed]

83. Stein, K.; Egan, S.; Lynch, H.; Harmanus, C.; Kyne, L.; Herra, C.; McDermott, S.; Kuijper, E.; Fitzpatrick, F.; FitzGerald, S.; et al. PCR-ribotype distribution of *Clostridium difficile* in Irish pigs. *Anaerobe* **2017**, *48*, 237–241. [CrossRef]

84. Kuijper, E.J.; Coignard, B.; Tüll, P. Emergence of *Clostridium difficile*-associated disease in North America and Europe. Clinical microbiology and infection: The official publication of the European Society of Clinical Microbiology and Infectious Diseases. *Clin. Microbiol. Infect.* **2006**, *6*, 2–18. [CrossRef] [PubMed]

85. Hookman, P.; Barkin, J.S. *Clostridium difficile* associated infection, diarrhea and colitis. *World J. Gastroenterol.* **2009**, *15*, 1554–1580. [CrossRef] [PubMed]

86. Janezic, S.; Garneau, J.R.; Monot, M. Comparative Genomics of *Clostridium difficile*. *Adv. Exp. Med. Biol.* **2018**, *1050*, 59–75. [PubMed]

87. Jones, A.M.; Kuijper, E.J.; Wilcox, M.H. *Clostridium difficile*: A European perspective. *J. Infect.* **2013**, *66*, 115–128. [CrossRef]

88. Baldan, R.; Trovato, A.; Bianchini, V.; Biancardi, A.; Cichero, P.; Mazzotti, M.; Nizzero, P.; Moro, M.; Ossi, C.; Scarpellini, P.; et al. *Clostridium difficile* PCR ribotype 018, a successful epidemic genotype. *J. Clin. Microbiol.* **2015**, *53*, 2575–2580. [CrossRef]

89. Knetsch, C.W.; Hensgens, M.P.M.; Harmanus, C.; van der Bijl, M.W.; Savelkoul, P.H.M.; Kuijper, E.J.; Corver, J.; van Leeuwen, H.C. Genetic markers for *Clostridium difficile* lineages linked to hypervirulence. *Microbiology* **2011**, *157*, 3113–3123. [CrossRef]

90. Schneeberg, A.; Neubauer, H.; Schmoock, G.; Baier, S.; Harlizius, J.; Nienhoff, H.; Brase, K.; Zimmermann, S.; Seyboldt, C. *Clostridium difficile* genotypes in piglet populations in Germany. *J. Clin. Microbiol.* **2013**, *51*, 3796–3803. [CrossRef]

91. Álvarez-Pérez, S.; Blanco, J.L.; Harmanus, C.; Kuijper, E.; Garcia, M.E. Subtyping and antimicrobial susceptibility of *Clostridium difficile* PCR ribotype 078/126 isolates of human and animal origin. *Vet. Microbiol.* **2017**, *199*, 15–22. [CrossRef]

92. Cairns, M.D.; Stabler, R.A.; Shetty, N.; Wren, B.W. The continually evolving *Clostridium difficile* species. *Future Microbiol.* **2012**, *7*, 945–957. [CrossRef] [PubMed]

93. Cairns, M.D.; Preston, M.D.; Lawley, T.D.; Clark, T.G.; Stabler, R.A.; Wren, B.W. Genomic epidemiology of a protracted hospital outbreak caused by a toxin A-negative *Clostridium difficile* sublineage PCR ribotype 017 strain in London, England. *J. Clin. Microbiol.* **2015**, *53*, 3141–3147. [CrossRef] [PubMed]

94. Collins, D.A.; Hawkey, P.M.; Riley, T.V. Epidemiology of *Clostridium difficile* infection in Asia. *Antimicrob. Resist. Infect. Control.* **2013**, *2*, 21. [CrossRef] [PubMed]

95. Spigaglia, P.; Barbanti, F.; Dionisi, A.M.; Mastrantonio, P. *Clostridium difficile* isolates resistant to fluoroquinolones in Italy: Emergence of PCR ribotype 018. *J. Clin. Microbiol.* **2010**, *48*, 2892–2896. [CrossRef]

96. Bauer, M.P.; Notermans, D.W.; van Benthem, B.H.; Brazier, J.S.; Wilcox, M.H.; Rupnik, M.; Monnet, D.L.; van Dissel, J.T.; Kuijper, E.J. *Clostridium difficile* infection in Europe: A hospital-based survey. *Lancet* **2011**, *377*, 63–73. [CrossRef]

97. Barbanti, F.; Spigaglia, P. Characterization of *Clostridium difficile* PCR-ribotype 018: A problematic emerging type. *Anaerobe* **2016**, *42*, 123–129. [CrossRef]

98. Zidaric, V.; Beigot, S.; Lapajne, S.; Rupnik, M. The occurrence and high diversity of *Clostridium difficile* genotypes in rivers. *Anaerobe* **2010**, *16*, 371–375. [CrossRef]

99. Bazaid, F. *Distribution and Sources of Clostridium difficile Present in Water Sources*; MSc University of Guelph: Guelph, Canada, 2012.

100. Warriner, K.; Xu, C.; Habash, M.; Sultan, S.; Weese, S.J. Dissemination of *Clostridium difficile* in food and the environment: Significant sources of *C. difficile* community acquired infection? *J. Appl. Microbiol.* **2016**, *122*, 542–555. [CrossRef]

101. Moradigaravand, D.; Gouliouris, T.; Ludden, C.; Reuter, S.; Jamrozy, D.; Blane, B.; Naydenova, P.; Judge, K.H.; Aliyu, S.F.; Hadjirin, N.A.; et al. Genomic survey of *Clostridium difficile* reservoirs in the East of England implicates environmental contamination of wastewater treatment plants by clinical lineages. *Microb. Genom.* **2018**, *4*, e000162. [CrossRef]

102. Kotila, S.M.; Pitkänen, T.; Brazier, J.; Eerola, E.; Jalava, J.; Kuusi, M.; Könönen, E.; Laine, J.; Miettinen, I.T.; Vuento, R.; et al. *Clostridium difficile* contamination of public tap water distribution system during a waterborne outbreak in Finland. *Scand. J. Public Health* **2013**, *41*, 541–545. [CrossRef]

103. Al Saif, N.; Brazier, J.S. The distribution of *Clostridium difficile* in the environment of South Wales. *J. Med. Microbiol.* **1996**, *45*, 133–137. [CrossRef] [PubMed]

104. Baverud, V.; Gustafsson, A.; Franklin, A.; Aspan, A.; Gunnarsson, A. *Clostridium difficile*: Prevalence in horses and environment, and antimicrobial susceptibility. *Equine Vet. J.* **2003**, *35*, 465–471. [CrossRef] [PubMed]

105. Higazi, T.B.; AL-Saghir, M.; Burkett, M.; Pusok, R. PCR detection of *Clostridium difficile* and its toxigenic strains in public places in Southeast Ohio. *Int. J. Microbiol. Res.* **2011**, *2*, 105–111.

106. Rodriguez, C.; Bouchafa, L.; Soumillion, K.; Ngyuvula, E.; Taminiau, B.; VanBroeck, J.; Delmée, M.; Daube, G. Seasonality of *Clostridium difficile* in the natural environment. *Transbound. Emerg. Dis.* **2019**, *66*, 2440–2449. [CrossRef] [PubMed]

107. Dharmasena, M.; Jiang, X. Isolation of toxigenic *Clostridium difficile* from animal manure and composts being used as biological soil amendments. *Appl. Environ. Microbiol.* **2018**, *84*, e00738-18. [CrossRef]

108. Shivaperumal, N.; Chang, B.J.; Riley, T.V. High Prevalence of *Clostridium difficile* in Home Gardens in Western Australia. *Appl. Environ. Microbiol. J.* **2020**, *87*, e01572-20. [CrossRef]

109. Fröschle, B.; Messelhäusser, U.; Höller, C.; Lebuhn, M. Fate of *Clostridium botulinum* and incidence of pathogenic clostridia in biogas processes. *J. Appl. Microbiol.* **2015**, *119*, 936–947. [CrossRef]

110. Marcos, P.; Whyte, P.; Rogers, T.; McElroy, M.; Fanning, S.; Frias, J.; Bolton, D. The prevalence of *Clostridioides difficile* on farms, in abattoirs and in retail foods in Ireland. *Food Microbiol.* **2021**, *98*, 103781. [CrossRef]

111. Rodriguez, C.; Taminiau, B.; Van Broeck, J.; Avesani, V.; Delmee, M.; Daube, G. *Clostridium difficile* in young farm animals and slaughter animals in Belgium. *Anaerobe* **2012**, *18*, 621–625. [CrossRef]

112. Wu, Y.C.; Lee, J.J.; Tsai, B.Y.; Liu, Y.F.; Chen, C.M.; Tien, N.; Tsai, P.J.; Chen, T.H. Potentially hypervirulent *Clostridium difficile* PCR ribotype 078 lineage isolates in pigs and possible implications for humans in Taiwan. *Int. J. Med. Microbiol.* **2016**, *306*, 115–122. [CrossRef]

113. Rodriguez, C.; Hakimi, D.E.; Vanleyssem, R.; Taminiau, B.; Van Broeck, J.; Delmee, M.; Korsak, N.; Daube, G. *Clostridium difficile* in beef cattle farms, farmers and their environment: Assessing the spread of the bacterium. *Vet. Microbiol.* **2017**, *210*, 183–187. [CrossRef] [PubMed]

114. Kim, H.Y.; Cho, A.; Kim, J.W.; Kim, H.; Kim, B. High prevalence of *Clostridium difficile* PCR ribotype 078 in pigs in Korea. *Anaerobe* **2018**, *51*, 42–46. [CrossRef]

115. Krutova, M.; Zouharova, M.; Matejkova, J.; Tkadlec, J.; Krejci, J.; Faldyna, M.; Nyc, O.; Bernardy, J. The emergence of *Clostridium difficile* PCR ribotype 078 in piglets in the Czech Republic clusters with *Clostridium difficile* PCR ribotype 078 isolates from Germany, Japan and Taiwan. *Int. J. Med. Microbiol.* **2018**, *308*, 770–775. [CrossRef] [PubMed]

116. Rodriguez, C.; Taminiau, B.; van Broeck, J.; Daube, G. *Clostridium difficile* in Food and Animals: A Comprehensive Review. *Adv. Exp. Med. Biol.* **2016**, *932*, 65–92. [PubMed]

117. Keel, K.; Brazier, J.S.; Post, K.W.; Weese, S.; Songer, J.G. Prevalence of PCR Ribotypes among *Clostridium difficile* Isolates from Pigs, Calves, and Other Species. *J. Clin. Microbiol.* **2007**, *6*, 1963–1964. [CrossRef]

118. Rodriguez-Palacios, A.; Staempfli, H.R.; Duffield, T.; Weese, J.S. *Clostridium difficile* in Retail Ground Meat, Canada. *Emerg. Infect. Dis.* **2007**, *3*, 485–487. [CrossRef]

119. Knight, D.R.; Riley, T.V. Prevalence of Gastrointestinal *Clostridium difficile* Carriage in Australian Sheep and Lambs. *Appl. Environ. Microbiol.* **2013**, *79*, 5689–5692. [CrossRef]

120. Harvey, R.; Norman, K.; Andrews, K.; Hume, M.; Scanlan, C.; Callaway, T. *Clostridium difficile* in poultry and poultry meat. *Foodborne Path. Dis.* **2011**, *8*, 1321–1323. [CrossRef]

121. Abdel-Glil, M.Y.; Thomas, P.; Schmoock, G.; Abou-El-Azm, K.; Wieler, L.H.; Neubauer, H.; Seyboldt, C. Presence of *Clostridium difficile* in poultry and poultry meat in Egypt. *Anaerobe* **2018**, *51*, 21–25. [CrossRef]

122. Hussain, I.; Borah, P.; Sharma, R.K.; Rajkhowa, S.; Rupnik, M.; Saikia, D.P. Molecular characteristics of *Clostridium difficile* isolates from human and animals in the North Eastern region of India. *Mol. Cell Probes* **2016**, *30*, 306–311. [CrossRef]

123. Simango, C.; Mwakurudza, S. *Clostridium difficile* in broiler chickens sold at market places in Zimbabwe and their antimicrobial susceptibility. *Int. J. Food Microbiol.* **2008**, *124*, 268–270. [CrossRef] [PubMed]

124. Zidaric, V.; Zemljic, M.; Janezic, S.; Kocuvan, A.; Rupnik, M. High diversity of *Clostridium difficile* genotypes isolated from a single poultry farm producing replacement laying hens. *Anaerobe* **2008**, *14*, 325–327. [CrossRef] [PubMed]

125. European Food Safety Authority (EFSA). Scientific Opinion on the public health hazards to be covered by inspection of meat (bovine animals). *Eur. Food Saf. Aut. J.* **2013**, *11*, 1–261.

126. Rodriguez, C.; Avesani, V.; Van Broeck, J.; Taminiau, B.; Delmée, M.; Daube, G. Presence of *Clostridium difficile* in pigs and cattle intestinal contents and carcass contamination at the slaughterhouse in Belgium. *Int. J. Food Microbiol.* **2013**, *166*, 256–262. [CrossRef]

127. Hopman, N.E.; Oorburg, D.; Sanders, I.; Kuijper, E.J.; Lipman, L.J. High occurrence of various *Clostridium difficile* PCR ribotypes in pigs arriving at the slaughterhouse. *Vet. Quart.* **2011**, *31*, 179–181. [CrossRef]

128. Hawken, P.; Weese, J.S.; Friendship, R.; Warriner, K. Carriage and dissemination of *Clostridium difficile* and methicillin resistant *Staphylococcus aureus* in pork processing. *Food Control* **2013**, *31*, 433–437. [CrossRef]

129. Wu, Y.C.; Chen, C.M.; Kuo, C.J.; Lee, J.J.; Chen, P.C.; Chang, Y.C. Prevalence and molecular characterization of *Clostridium difficile* isolates from a pig slaughterhouse, pork, and humans in Taiwan. *Int. J. Food Microbiol.* **2013**, *242*, 37–44. [CrossRef]

130. Hampikyan, H.; Bingol, E.B.; Muratoglu, K.; Akkaya, E.; Cetin, O.; Colak, H. The prevalence of *Clostridium difficile* in cattle and sheep carcasses and the antibiotic susceptibility of isolates. *Meat Sci.* **2018**, *139*, 120–124. [CrossRef]

131. Esfandiari, Z.; Weese, J.S.; Ezzatpanah, H.; Chamani, M.; Shoaei, P.; Yaran, M.; Ataei, B.; Maracy, M.R.; Ansariyan, A.; Ebrahimi, F.; et al. Isolation and characterization of *Clostridium difficile* in farm animals from slaughterhouse to retail stage in Isfahan, Iran. *Food. Path. Dis.* **2015**, *10*, 864–866. [CrossRef]

132. Candel-Pérez, C.; Santaella-Pascual, J.; Ros-Berruezo, G.; Martínez-Gracia, C. Occurrence of *Clostridioides* [*Clostridium*] *difficile* in poultry giblets at slaughter and retail pork and poultry meat in southeastern Spain. *J. Food Prot.* **2021**, *84*, 310–314. [CrossRef]

133. Keessen, E.C.; van den Berkt, A.J.; Haasjes, N.H.; Hermanus, C.; Kuijper, E.J.; Lipman, L.J. The relation between farm specific factors and prevalence of *Clostridium difficile* in slaughter pigs. *Vet. Microbiol.* **2011**, *54*, 130–134. [CrossRef] [PubMed]

134. Lund, B.M.; Peck, M.W. A possible route for foodborne transmission of *Clostridium difficile*? *Food. Path. Dis.* **2015**, *12*, 177–182. [CrossRef] [PubMed]

135. Harvey, R.; Norman, K.; Andrews, K.; Norby, B.; Hume, M.; Scanlan, C.; Hardin, M.; Scott, H. *Clostridium difficile* in retail meat and processing plants in Texas. *J. Vet. Diag. Investig.* **2011**, *23*, 807–811. [CrossRef]

136. Varshney, J.B.; Very, K.J.; Williams, J.L.; Hegarty, J.P.; Stewart, D.B.; Lumadue, J. Characterization of *Clostridium difficile* isolates from human fecal samples and retail meat from Pennsylvania. *Food. Path. Dis.* **2014**, *11*, 822–829. [CrossRef]

137. Guran, H.S.; Ilhak, O.I. *Clostridium difficile* in retail chicken meat parts and liver in the Eastern Region of Turkey. *J. Con. Prot. Food Saf.* **2015**, *10*, 359–364. [CrossRef]

138. Metcalf, D.; Avery, B.P.; Janecko, N.; Matic, N.; Reid-Smith, R.; Weese, J.S. *Clostridium difficile* in seafood and fish. *Anaerobe* **2011**, *17*, 85–86. [CrossRef] [PubMed]

139. Pasquale, V.; Romano, V.; Rupnik, M.; Capuano, F.; Bove, D.; Aliberti, F.; Krovacek, K.; Dumontet, S. Occurrence of toxigenic *Clostridium difficile* in edible bivalve molluscs. *Food Microbiol.* **2012**, *31*, 309–312. [CrossRef]

140. Troiano, T.; Harmanus, C.; Sanders, I.; Pasquale, V.; Dumontet, S.; Capuano, F.; Romano, V.; Kuijper, E.J. Toxigenic *Clostridium difficile* PCR ribotypes in ediblemarine bivalve molluscs in Italy. *Int. J. Food Microbiol.* **2015**, *208*, 30–34. [CrossRef]

141. Agnoletti, F.; Arcangelia, G.; Barbantib, F.; Barcoa, L.; Brunettaa, R.; Cocchia, M.; Conederaa, G.; D'Estea, L.; Drigoa, I.; Spigagliab, P.; et al. Survey, characterization and antimicrobial susceptibility of *Clostridium difficile* from marine bivalve shellfish of North Adriatic Sea. *Int. J. Food Microbiol.* **2019**, *298*, 74–80. [CrossRef]

142. Eckert, C.; Burghoffer, B.; Barbut, F. Contamination of ready-to-eat raw vegetables with *Clostridium difficile*, France. *J. Med. Microbiol.* **2013**, *62*, 1435–1438. [CrossRef]

143. Lim, S.C.; Foster, N.F.; Elliott, B.; Riley, T.V. High prevalence of *Clostridium difficile* on retail root vegetables, Western Australia. *J. Appl. Microbiol.* **2018**, *124*, 585–590. [CrossRef]

144. Tkalec, V.; Janezica, S.; Skoka, B.; Simonica, T.; Mesarica, S.; Vrabica, T.; Rupnik, M. High *Clostridium difficile* contamination rates of domestic and imported potatoes compared to some other vegetables in Slovenia. *Food Microbiol.* **2019**, *78*, 194–200. [CrossRef]

145. Marcos, P.; Whyte, P.; Burgess, C.; Ekhlas, D.; Bolton, D. Detection and Genomic Characterisation of *Clostridioides difficile* from Spinach Fields. *Pathogens* **2022**, *11*, 1310. [CrossRef]

146. Bakri, M.M.; Brown, D.J.; Butcher, J.P.; Sutherland, A.D. *Clostridium difficile* in ready-to-eat salads, Scotland. *Emerg. Infect. Dis.* **2009**, *15*, 817–818. [CrossRef] [PubMed]

147. Dharmasena, M.; Wang, H.; Wei, T.; Bridges, W.C., Jr.; Jiang, X. Survival of *Clostridioides difficile* in finished dairy compost under controlled conditions. *J. Appl. Microbiol.* **2021**, *131*, 996–1006. [CrossRef] [PubMed]

148. Xu, C.; Weese, J.S.; Flemming, C.; Odumeru, J.; Warriner, K. Fate of *Clostridium difficile* during wastewater treatment and incidence in Southern Ontario watersheds. *J. Appl. Microbiol.* **2014**, *117*, 891–904. [CrossRef] [PubMed]

149. Wei, Y.; Sun, M.; Zhang, Y.; Gao, J.; Kong, F.; Liu, D.; Yu, H.; Du, J.; Tang, R. Prevalence, genotype and antimicrobial resistance of *Clostridium difficile* isolates from healthy pets in Eastern China. *BMC Infect. Dis.* **2019**, *19*, 46. [CrossRef]

150. Weese, J.S. Clostridium (Clostridioides) difficile in animals. *J. Vet. Diagn.Investig. Off. Publ. Am. Assoc. Vet. Lab. Diag.* **2020**, *32*, 213–221. [CrossRef]

151. Hammitt, M.C.; Bueschel, D.M.; Keel, M.K.; Glock, R.D.; Cuneo, P.; DeYoung, D.W.; Reggiardo, C.; Trinh, H.T.; Songer, J.G. A possible role for *Clostridium difficile* in the etiology of calf enteritis. *Vet. Microbiol.* **2008**, *127*, 343–352. [CrossRef]

152. Costa, M.C.; Stämpfli, H.R.; Arroyo, L.G.; Pearl, D.L.; Weese, J.S. Epidemiology of *Clostridium difficile* on a veal farm: Prevalence, molecular characterization and tetracycline resistance. *Vet. Microbiol.* **2011**, *152*, 379–384. [CrossRef]

153. Costa, M.C.; Reid-Smith, R.; Gow, S.; Hannon, S.J.; Booker, C.; Rousseau, J.; Benedict, K.M.; Morley, P.S.; Weese, J.S. Prevalence and molecular characterization of *Clostridium difficile* isolated from feedlot beef cattle upon arrival and mid-feeding period. *BMC Vet. Res.* **2012**, *8*, 38. [CrossRef] [PubMed]

154. Keessen, E.C.; Harmanus, C.; Dohmen, W.; Kuijper, E.J.; Lipman, L.J. *Clostridium difficile* infection associated with pig farms. *Emerg. Infect. Dis.* **2013**, *19*, 1032–1034. [CrossRef] [PubMed]

155. Magistrali, C.F.; Maresca, C.; Cucco, L.; Bano, L.; Drigo, I.; Filippini, G.; Dettori, A.; Broccatelli, S.; Pezzotti, G. Prevalence and risk factors associated with *Clostridium difficile* shedding in veal calves in Italy. *Anaerobe* **2015**, *33*, 42–47. [CrossRef] [PubMed]

156. Romano, V.; Albanese, F.; Dumontet, S.; Krovacek, K.; Petrini, O.; Pasquale, V. Prevalence and genotypic characterization of *Clostridium difficile* from ruminants in Switzerland. *Zoo. Pub. Health* **2012**, *59*, 545–548. [CrossRef] [PubMed]

157. Larson, H.E.; Price, A.B.; Honour, P.; Borriello, S.P. *Clostridium difficile* and the aetiology of pseudomembranous colitis. *Lancet* **1978**, *1*, 1063–1066. [CrossRef] [PubMed]

158. Olson, M.M.; Shanholtzer, C.J.; Lee, J.T., Jr.; Gerding, D.N. Ten years of prospective *Clostridium difficile*-associated disease surveillance and treatment at the Minneapolis VA Medical Center, 1982–1991. *Infect. Control Hosp. Epidemiol.* **1994**, *15*, 371–381. [CrossRef]

159. Slimings, C.; Riley, T. Antibiotics and hospital-acquired *Clostridium difficile* infection: Update of systematic review and meta-analysis. *J. Antimicrob. Chemother.* **2014**, *69*, 881–891. [CrossRef]

160. Freeman, J.; Vernon, J.; Morris, K.; Nicholson, S.; Todhunter, S.; Longshaw, C.; Wilcox, M.H. Pan-European Longitudinal Surveillance of Antibiotic Resistance among Prevalent *Clostridium difficile* Ribotypes' Study Group. *Clin. Microbiol. Infect.* **2015**, *21*, 248.e9–248.e16. [CrossRef]

161. McDonald, L.C.; Coignard, B.; Dubberke, E.; Song, X.; Horan, T.; Kutty, P.K.; Ad Hoc *Clostridium difficile* Surveillance Working Group. Recommendations for surveillance of *Clostridium difficile*-associated disease. *Infect. Control. Hosp. Epidemiol.* **2007**, *28*, 140–145. [CrossRef]

162. Lessa, F.C.; Mu, Y.; Bamberg, W.M.; Beldavs, Z.G.; Dumyati, G.K.; Dunn, J.R.; Farley, M.M.; Holzbauer, S.M.; Meek, J.I.; Phipps, E.C.; et al. Burden of *Clostridium difficile* infection in the United States. *N. Engl. J. Med.* **2015**, *372*, 825–834. [CrossRef]

163. Khanna, S. Microbiota restoration for recurrent *Clostridioides difficile*: Getting one step closer every day! *J. Intern. Med.* **2021**, *290*, 294–309. [CrossRef] [PubMed]

164. Kuntz, J.L.; Chrischilles, E.A.; Pendergast, J.F.; Herwaldt, L.A.; Polgreen, P.M. Incidence of and risk factors for community-associated Clostridium difficile infection: A nested case-control study. *BMC Infect. Dis.* **2011**, *11*, 194. [CrossRef] [PubMed]

165. Moono, P.; Lim, S.C.; Riley, T.V. High prevalence of toxigenic *Clostridium difficile* in public space lawns in Western Australia. *Sci. Rep.* **2017**, *7*, 41196. [CrossRef]

166. Rodriguez Diaz, C.; Seyboldt, C.; Rupnik, M. Non-human *C. difficile* reservoirs and sources: Animals, food, environment. *Adv. Exp. Med. Biol.* **2018**, *1050*, 227–243.

167. Avberšek, J.; Pirš, T.; Pate, M.; Rupnik, M.; Ocepek, M. *Clostridium difficile* in goats and sheep in Slovenia: Characterisation of strains and evidence of age-related shedding. *Anaerobe* **2014**, *28*, 163–167. [CrossRef] [PubMed]

168. Rodriguez-Palacios, A.; Barman, T.; LeJeune, J.T. Three-week summer period prevalence of *Clostridium difficile* in farm animals in a temperate region of the United States (Ohio). *Can. Vet. J.* **2014**, *55*, 786–789. [PubMed]

169. Cataldo, M.A.; Granata, G.; Petrosillo, N. *Clostridium difficile* infection: New approaches to prevention, non-antimicrobial treatment, and stewardship. *Expert Rev. Anti-Infect. Ther.* **2017**, *15*, 1027–1040. [CrossRef]

170. Maron, D.F.; Smith, T.J.; Nachman, K.E. Restrictions on antimicrobial use in food animal production: An international regulatory and economic survey. *Glob. Health* **2013**, *9*, 48. [CrossRef]

171. Squire, M.M.; Riley, T.V. *Clostridium difficile* infection in humans and piglets: A 'One Health' opportunity. *Curr. Top. Microbiol. Immunol.* **2013**, *365*, 299–314.

172. Deng, K.; Plaza-Garrido, A.; Torres, J.A.; Paredes-Sabja, D. Survival of *Clostridium difficile* spores at low temperatures. *Food Microbiol.* **2015**, *46*, 218–221. [CrossRef]

173. Flock, G.; Chen, C.-H.; Yin, H.-B.; Fancher, S.; Mooyottu, S.; Venkitanarayanan, K. Effect of chilling, freezing and cooking on survivability of *Clostridium difficile* spores in ground beef. *Meat Sci.* **2016**, *112*, 161. [CrossRef]

174. Rodriguez-Palacios, A.; Reid-Smith, R.J.; Staempfli, H.R.; Weese, J.S. *Clostridium difficile* survives minimal temperature recommended for cooking ground meats. *Anaerobe* **2010**, *16*, 540–542. [CrossRef] [PubMed]

175. Ojha, S.C.; Chankhamhaengdecha, S.; Singhakaew, S.; Ounjai, P.; Janvilisri, T. Inactivation of *Clostridium difficile* spores by microwave irradiation. *Anaerobe* **2016**, *38*, 14–20. [CrossRef] [PubMed]

176. Doona, C.J.; Feeherry, F.E.; Setlow, B.; Wang, S.; Li, W.; Nichols, F.C. Effects of high-pressure treatment on spores of *Clostridium* species. *Appl. Environ. Microbiol.* **2016**, *82*, 5287–5297. [CrossRef]

177. Deng, K.; Talukdar, P.K.; Sarker, M.R.; Paredes-Sabja, D.; Torres, J.A. Survival of *Clostridium difficile* spores at low water activity. *Food Microbiol.* **2017**, *65*, 274–278. [CrossRef]

178. Broda, D.M.; Delacy, K.M.; Bell, R.G.; Terry, J.; Cook, R.L. Psychrotrophic *Clostridium* spp. associated with 'blown pack' spoilage of chilled vacuum-packed red meats and dog rolls in gas-impermeable plastic casings. *Int. J. Food Microbiol.* **1996**, *29*, 335–352. [CrossRef]

179. Atasoy, F.; Gücükoğlu, A. Detection of *Clostridium difficile* and toxin genes in samples of modified atmosphere packaged (MAP) minced and cubed beef meat. *Ank. ÜNiversitesi Vet. Fakültesi Derg.* **2017**, *64*, 165–170.

180. Lim, S.C.; Foster, N.F.; Riley, T.V. Susceptibility of *Clostridium difficile* to the food preservatives sodium nitrite, sodium nitrate and sodium metabisulphite. *Anaerobe* **2016**, *37*, 67–71. [CrossRef]

181. Le Lay, C.; Dridi, L.; Bergeron, M.G.; Ouellette, M.; Fliss, I. Nisin is an effective inhibitor of *Clostridium difficile* vegetative cells and spore germination. *J. Med. Microbiol.* **2016**, *65*, 169–175. [CrossRef]

182. Aljarallah, K.M. Inhibition of *Clostridium difficile* by natural herbal extracts. *J. Taibah Univ. Med. Sci.* **2016**, *11*, 427–431. [CrossRef]

183. Roshan, N.; Riley, T.V.; Hammer, K.A. Antimicrobial activity of natural products against *Clostridium difficile* in vitro. *J. Appl. Microbiol.* **2017**, *123*, 92–103. [CrossRef] [PubMed]