



# ANNEX 1

## Inventory of *C. difficile* Zoonotic Types

Version 1  
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## Zoonotic *C. difficile* types by source of isolation

### 1. Animals food chain

*Ribotypes found in pigs:* 002, 005, 010, 011, 013, 014, 015, 020, 023, 029, 033, 035, 038, 045, 049, 046, 050, 062, 066, 078, 081, 120, 126, 150, 193, 237, 413, 598, 620

*Ribotypes in cattle:* 002, 003, 005, 010, 012, 014, 015, 023, 029, 033, 045, 049, 056, 064, 077, 078, 081, 087, 103, 106, 126, 127, 137, 446, 596, 598, 620, 652, 660

*Ribotypes in poultry:* 001, 003, 010, 014, 020, 023, 025, 031, 039, 053, 056, 070, 084, 087, 103, 120, 205, 207, 220, 228, 446

### 2. Wild animals

*Ribotypes:* 002, 005, 009, 010, 012, 013, 014/020, 033, 045, 056, 078, 081, 106, 126, 204, 258, 295

### 3. Environment

*Ribotypes:* 001, 002, 003, 005, 008, 009, 010, 011, 012, 014, 015, 017, 018, 020, 021, 023, 026, 027, 029, 031, 033, 034, 035, 039, 040, 041, 042, 045, 046, 049, 051, 052, 053, 056, 066, 067, 070, 072, 078, 081, 101, 103, 106, 117, 126, 150, 151, 153, 205, 220, 244, 251, 297

### 4. Food

*Ribotypes in meat:* 001, 003, 012, 014, 027, 045, 053, 071, 077, 078, 087

*Ribotypes in vegetables:* 001, 001/072, 002, 009, 010, 012, 011/049, 014/020/077, 015, 023, 032, 033, 051, 053, 056, 064, 070, 078, 101, 126, 137, 150, 237, 244, 394, 584



## References

- Abdel-Glil MY, Thomas P, Schmoock G, Abou-El-Azm K, Wieler LH, Neubauer H, Seyboldt C. Presence of *Clostridium difficile* in poultry and poultry meat in Egypt. *Anaerobe*. 2018 Jun;51:21-25. doi: 10.1016/j.anaerobe.2018.03.009. Epub 2018 Mar 20. PMID: 29571666.
- Agnoletti F, Arcangeli G, Barbanti F, Barco L, Brunetta R, Cocchi M, Conedera G, D'Este L, Drigo I, Spigaglia P, Mazzolini E. Survey, characterization and antimicrobial susceptibility of *Clostridium difficile* from marine bivalve shellfish of North Adriatic Sea. *Int J Food Microbiol*. 2019 Jun 2;298:74-80. doi: 10.1016/j.ijfoodmicro.2019.03.003. Epub 2019 Mar 10. PMID: 30927688.
- Al-Saif NM, O'Neill GL, Magee JT et al (1998) PCR-ribotyping and pyrolysis mass spectrometry fingerprinting of environmental and hospital isolates of *Clostridium difficile*. *J Med Microbiol* 47:117–1121
- Andrés-Lasheras, S., Bolea, R., Mainar-Jaime, R., Kuijper, E., Sevilla, E., Martín-Burriel, I. and Chirino-Trejo, M. (2017), Presence of *Clostridium difficile* in pig faecal samples and wild animal species associated with pig farms. *J Appl Microbiol*, 122: 462-472. <https://doi.org/10.1111/jam.13343>
- Avbersek J, et al. Diversity of *Clostridium difficile* in pigs and other animals in Slovenia. *Anaerobe* 2009; 15:252–255
- Avbersček J, Pirsč T, Pate M et al (2014) *Clostridium difficile* in goats and sheep in Slovenia: characterisation of strains and evidence of age-related shedding. *Anaerobe* 28:163–167
- Bakri M. Prevalence of *Clostridium difficile* in raw cow, sheep, and goat meat in Jazan, Saudi Arabia. *Saudi J Biol Sci*. 2018 May;25(4):783-785. doi: 10.1016/j.sjbs.2016.07.002. Epub 2016 Aug 4. PMID: 29740244; PMCID: PMC5936870.
- Bandelj, P., Blagus, R., Briski, F. et al. Identification of risk factors influencing *Clostridium difficile* prevalence in middle-size dairy farms. *Vet Res* 47, 41 (2016).
- Berger FK, Mellmann A, Bischoff M, von Müller L, Becker SL, Simango C, Gärtner B. Molecular epidemiology and antimicrobial resistance of *Clostridioides difficile* detected in chicken, soil and human samples from Zimbabwe. *Int J Infect Dis*. 2020 Jul;96:82-87. doi: 10.1016/j.ijid.2020.04.026. Epub 2020 Apr 18. PMID: 32311450.
- Bouttier S, Barc MC, Felix B et al (2010) *Clostridium difficile* in ground meat, France. *Emerg Infect Dis* 16:733–735
- Burt SA, Meijer K, Burggraaff P, Kamerich WS, Harmanus C. Wild mice in and around the city of Utrecht, the Netherlands, are carriers of *Clostridium difficile* but not ESBL-producing Enterobacteriaceae, Salmonella spp. or MRSA. *Lett Appl Microbiol*. 2018 Nov;67(5):513-519. doi: 10.1111/lam.13066. Epub 2018 Sep 19. PMID: 30144118.



Burt SA, Siemeling L, Kuijper EJ, Lipman LJ. Vermin on pig farms are vectors for *Clostridium difficile* PCR ribotypes 078 and 045. *Vet Microbiol*. 2012 Nov 9;160(1-2):256-8. doi: 10.1016/j.vetmic.2012.05.014. Epub 2012 May 18. PMID: 22682200.

Costa MC, Sta<sup>o</sup>mpfli HR, Arroyo LG (2011) Epidemiology of *Clostridium difficile* on a veal farm: prevalence, molecular characterization and tetracycline resistance. *Vet Microbiol* 152:379–384

Costa MC, Reid-Smith R, Gow S et al (2012) Prevalence and molecular characterization of *Clostridium difficile* isolated from feedlot beef cattle upon

Curry SR, Marsh JW, Schlackman JL et al (2012) Prevalence of *Clostridium difficile* in uncooked ground meat products from Pittsburgh, P

de Boer E, Zwartkruis-Nahuis A, Heuvelink AE et al (2011) Prevalence of *Clostridium difficile* in retailed meat in the Netherlands. *Int J Food Microbiol* 144:561–564

Eckert C, Burghoffer B, Barbut F (2013) Contamination of ready-to-eat raw vegetables with *Clostridium difficile* in France. *J Med Microbiol* 62:1435–1438

Hampikyan H, Bingol EB, 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 May; 139:120-124. doi: 10.1016/j.meatsci.2018.01.020. Epub 2018 Feb 3. PMID: 29413671.

Hargreaves KR, Colvin HV, Patel KV et al (2013) Genetically diverse *Clostridium difficile* strains harboring abundant prophages in an estuarine environment. *Appl Environ Microbiol* 79:6236–6243

Harvey RB, Norman KN, Andrews K et al (2011b) *Clostridium difficile* in retail meat and processing plants in Texas. *J Vet Diagn Invest* 23:807–811

Hawken P, Weese JS, Friendship R (2013) Longitudinal Study of *Clostridium difficile* and MethicillinResistant *Staphylococcus* Associated with Pigs from Weaning through to the End of Processing. *J Food Prot* 76:624–630

Hoffer E, Haechler H, Frei R et al (2010) Low occurrence of *Clostridium difficile* in fecal samples of healthy calves and pigs at slaughter and in minced meat in Switzerland. *J Food Prot* 73:973–975

Hopman NEM, Oorburg D, Sanders I et al (2011b) High occurrence of various *Clostridium difficile* PCR ribotypes in pigs arriving at the slaughterhouse.

Hussain I, Borah P, Sharma RK, Rajkhowa S, Rupnik M, Saikia DP, Hasin D, Hussain I, Deka NK, Barkalita LM, Nishikawa Y, Ramamurthy T. Molecular characteristics of *Clostridium difficile* isolates from human and animals in the North Eastern region of India. *Mol Cell Probes*. 2016 Oct;30(5):306-311. doi: 10.1016/j.mcp.2016.08.010. Epub 2016 Aug 26. PMID: 27569848.

Indra A, Lassnig H, Baliko N et al (2009) *Clostridium difficile*: a new zoonotic agent? *Wien Klin Wochensr* 121:91–95

Janezic S, Potocnik M, Zidaric V et al (2016) Highly divergent *Clostridium difficile* strains isolated from the environment. *PLoS One* 11:e0167101

Jo<sup>o</sup>bstl M, Heuberger S, Indra A et al (2010) *Clostridium difficile* in raw products of animal origin. *Int J Food Microbiol* 138:172–175



Keessen EC, Donswijk CJ, Hol SP et al (2011a) Aerial dissemination of *Clostridium difficile* on a pig farm and its environment. *Environ Res* 111:1027–1032

Kim HY, et al. High prevalence of *Clostridium difficile* PCR ribotype 078 in pigs in Korea. *Anaerobe* 2018; 51:42–46

Knight DR, Riley TV (2013) Prevalence of gastrointestinal *Clostridium difficile* carriage in Australian sheep and lambs. *Appl Environ Microbiol* 79:5689–5692

Koene MGJ, Mevius D, Wagenaar JA et al (2012) *Clostridium difficile* in Dutch animals: their presence, characteristics and similarities with human isolates. *Clin Microbiol Infect* 18:778–784

Kotila SM, Pitkänen T, Brazier J et al (2013) *Clostridium difficile* contamination of public tap water distribution system during a waterborne outbreak in Finland. *Scand J Public Health* 41:541–545

Knight DR, Thean S, Putsathit P et al (2013) Cross-sectional study reveals high prevalence of *Clostridium difficile* non-PCR ribotype 078 strains in Australian veal calves at slaughter. *Appl Environ Microbiol* 79:2630–2635

Knight DR, Squire MM, Riley TV (2015b) Nationwide surveillance study of *Clostridium difficile* in Australian neonatal pigs shows high prevalence and heterogeneity of PCR ribotypes. *Appl Environ Microbiol* 81:119–123

Krutova M, et al. 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

Le Maréchal C, Gateau C, Poezevara T, Couturier J, Rouxel S, Syed Zaidi R, Houard E, Pourcher AM, Denis M, Barbut F. Characterization of *Clostridioides difficile* strains isolated from manure and digestate in five agricultural biogas plants. *Anaerobe*. 2020 Apr;62:102180. doi: 10.1016/j.anaerobe.2020.102180. Epub 2020 Feb 21. PMID: 32092414.

Lim SC, Foster NF, Elliott B, Riley TV. High prevalence of *Clostridium difficile* on retail root vegetables, Western Australia. *J Appl Microbiol*. 2018 Feb;124(2):585-590. doi: 10.1111/jam.13653. PMID: 29193458.

Lim SC, Knight DR, Moono P, Foster NF, Riley TV. *Clostridium difficile* in soil conditioners, mulches and garden mixes with evidence of a clonal relationship with historical food and clinical isolates. *Environ Microbiol Rep*. 2020 Sep 25. doi: 10.1111/1758-2229.12889. Epub ahead of print. PMID: 32975368.

Metcalf D, Costa MC, Dew WMV et al (2010b) *Clostridium difficile* in vegetables, Canada. *Lett Appl Microbiol* 51:600–602

Metcalf D, Avery BP, Janecko N et al (2011) *Clostridium difficile* in seafood and fish. *Anaerobe* 17:85–86

Muratoğlu, Karlo & Akkaya, Esra & Hampikyan, Hamparsun & Bingol, Enver Baris & Çetin, Ömer & Colak, Hilal. (2020). Detection, Characterization and Antibiotic Susceptibility of *Clostridioides (Clostridium) difficile* in Meat Products. *Food Science of Animal Resources*. 40. 10.5851/kosfa.2020.e34.

Nore´n T, Johansson K, Unemo M (2014) *Clostridium difficile* PCR ribotype 046 is common among neonatal pigs and humans in Sweden. *Clin Microbiol Infect* 20: O2–O6



Norman KN, Scott HM, Harvey RB et al (2011) Prevalence and genotypic characteristics of *Clostridium difficile* in a closed and integrated human and swine population. *Appl Environ Microbiol* 77:5755–5760

Norman KN, Harvey RB, Andrews K et al (2014) Survey of *Clostridium difficile* in retail seafood in College Station, Texas. *Food Addit Contam Part A Chem Anal Control Exp Risk Assess* 31:1127–1129

Orden C, Neila C, Blanco JL et al (2017b) Recreational sandboxes for children and dogs can be a source of Non-human *C. difficile* Reservoirs and Sources: Animals, Food, Environment 241 epidemic ribotype

Pasquale V, Romano VJ, Rupnik M et al (2011) Isolation and characterization of *Clostridium difficile* from shellfish and marine environments. *Folia Microbiol* 56:431–437

Pasquale V, Romano V, Rupnik M et al (2012) Occurrence of toxigenic *Clostridium difficile* in edible bivalve molluscs. *Food Microbiol* 31:309–312

Pirs T, Ocepek M, Rupnik M (2008) Isolation of *Clostridium difficile* from food animals in Slovenia. *J Med Microbiol* 57:790–792

Putsathit P, Neela VK, Joseph NMS, Ooi PT, Ngamwongsatit B, Knight DR, Riley TV. Molecular epidemiology of *Clostridium difficile* isolated from piglets. *Vet Microbiol*. 2019 Oct;237:108408. doi: 10.1016/j.vetmic.2019.108408. Epub 2019 Sep 4. PMID: 31585650.

Rahimi E, Jalali M, Weese JS (2014) Prevalence of *Clostridium difficile* in raw beef, cow, sheep, goat, camel and buffalo meat in Iran. *BMC Public Health* 14:119

Ramos CP, Santana JA, Morcatti Coura F, Xavier RGC, Leal CAG, Oliveira Junior CA, Heinemann MB, Lage AP, Lobato FCF, Silva ROS. Identification and Characterization of *Escherichia coli*, *Salmonella* Spp., *Clostridium perfringens*, and *C. difficile* Isolates from Reptiles in Brazil. *Biomed Res Int*. 2019 May 27;2019:9530732. doi: 10.1155/2019/9530732. PMID: 31263711; PMCID: PMC6556801.

Rivas L, Dupont PY, Gilpin BJ, Cornelius AJ. Isolation and characterization of *Clostridium difficile* from a small survey of wastewater, food and animals in New Zealand. *Lett Appl Microbiol*. 2020 Jan;70(1):29-35. doi: 10.1111/lam.13238. Epub 2019 Nov 29. PMID: 31631350.

Rodriguez C, Taminiau B, Van Broeck J et al (2012) *Clostridium difficile* in young farm animals and slaughter animals in Belgium. *Anaerobe* 18:621–625

Rodriguez C, Avesani V, Van Broeck J et al (2013) Presence of *Clostridium difficile* in pigs and cattle intestinal contents and carcass contamination at the slaughterhouse in Belgium. *Int J Food Microbiol* 166:256–262

Rodriguez C, Bouchafa L, Soumillion K, Ngyuvula E, Taminiau B, Van Broeck J, Delmée M, Daube G. Seasonality of *Clostridium difficile* in the natural environment. *Transbound Emerg Dis*. 2019 Nov;66(6):2440-2449. doi: 10.1111/tbed.13301. Epub 2019 Aug 7. PMID: 31338965.

Rodriguez C, Taminiau B, Avesani V et al (2014b) Multilocus sequence typing analysis and antibiotic resistance of *Clostridium difficile* strains isolated from retail meat and humans in Belgium. *Food Microbiol* 42:166–171



Rodriguez-Palacios A, Koochmarai M, LeJeune JT (2011a) Prevalence, enumeration, and antimicrobial agent resistance of *Clostridium difficile* in cattle at harvest in the United States. *J Food Prot* 74:1618–1624

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

Rodriguez-Palacios A, Staˆmpfli HR, Duffield T et al (2007a) *Clostridium difficile* in retail ground meat, Canada. *Emerg Infect Dis* 13:485–487

Romano V, Albanese F, Dumontet S, Krovacek K et al (2012) Prevalence and genotypic characterization of *Clostridium difficile* from ruminants in Switzerland. *Zoonoses Public Health* 59:545–548

Romano V, Pasquale V, Lemee L, El Meouche I, Pestel-Caron M, Capuano F, Buono P, Dumontet S. *Clostridioides difficile* in the environment, food, animals and humans in southern Italy: Occurrence and genetic relatedness. *Comp Immunol Microbiol Infect Dis*. 2018 Aug;59:41-46. doi: 10.1016/j.cimid.2018.08.006. Epub 2018 Sep 11. PMID: 30290886.

Schneeberg A, Neubauer H, Schomock G et al (2013a) *Clostridium difficile* genotypes in piglet populations in Germany. *J Clin Microbiol* 51:3796–3803

Schneeberg A, Neubauer H, Schomock G et al (2013b) Presence of *Clostridium difficile* PCR ribotype clusters related to 033, 078 and 045 in diarrhoeic calves in Germany. *J Med Microbiol* 62:1190–1198~

Songer JG, Trinh HT, Killgore GE et al (2009) *Clostridium difficile* in retail meat products, USA, 2007. *Emerg Infect Dis* 15:819–821

Squire MM, Carter GP, Mackin KE et al (2013) Novel Molecular Type of *Clostridium difficile* in Neonatal Pigs, Western Australia. *Emerg Infect Dis* 19:790–792

Stein K, et al. PCR-ribotype distribution of *Clostridium difficile* in Irish pigs. *Anaerobe* 2017;48:237–241

Steyer A, Gutierrez-Aguirre I, Racˆki N et al (2015) The detection rate of enteric viruses and *Clostridium* 242 C. Rodriguez Diaz et al. *difficile* in a waste water treatment plant effluent. *Food Environ Virol* 7:164–172

Tkalec V, Janezic S, Skok B, Simonic T, Mesaric S, Vrabic T, Rupnik M. High *Clostridium difficile* contamination rates of domestic and imported potatoes compared to some other vegetables in Slovenia. *Food Microbiol*. 2019 Apr;78:194-200. doi: 10.1016/j.fm.2018.10.017. Epub 2018 Oct 30. PMID: 30497603.

Varshney JB, Very KJ, Williams JL et al (2014) Characterization of *Clostridium difficile* isolates from human fecal samples and retail meat from Pennsylvania. *Foodborne Pathog Dis* 11:822–829

Weese JS, Reid-Smith RJ, Avery BP et al (2010b) Detection and characterization of *Clostridium difficile* in retail chicken. *Lett Appl Microbiol* 50:362–365

Weese JS, Wakeford T, Reid-Smith R et al (2010c) Longitudinal investigation of *Clostridium difficile* shedding in piglets. *Anaerobe* 16:501–504



Weese JS, Rousseau J, Deckert A et al (2011) Clostridium difficile and methicillin-resistant Staphylococcus aureus shedding by slaughter-age pigs. BMC Vet Res 7:41

Zidaric V, Beigot S, Lapajne S et al (2010) The occurrence and high diversity of Clostridium difficile genotypes in rivers. Anaerobe 16(4):371–375

Zidaric V, Pardon B, Dos Vultos T et al (2012) Different antibiotic resistance and sporulation properties within multiclinal Clostridium difficile PCR ribotypes 078, 126, and 033 in a single calf farm. Appl Environ Microbiol 78:8515–8522