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Clostridium difficile — from *Bacillus difficilis* to *Clostridioides difficile*, global molecular epidemiology and possible implications for the Russian Federation

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Summary

Clostridium difficile, recently renamed to *Clostridioides difficile* is the main cause of nosocomial diarrhea in developed nations. In recent years the appearance of so called "hypervirulent" strains like ribotype 027 (RT027) originating from North America has shaped the epidemiology in many parts of the world posing a huge burden on the healthcare system. These hypervirulent strains among others (e.g. RT017) are associated with resistance towards several antibiotics (e.g. fluoroquinolones) favoring their selection. In Europe, Israel and the American continent RT027 seems to have become the most prevalent strain, while in other parts of the world other RTs dominate. In Far East Asia, RT017 is the predominant strain, while in Australia RT014/020 and RT002 are mostly prevalent. However, for most parts of the world the *C. difficile* world map is rather incomplete, such as in most African countries, Middle East Asia, South Asia but also Eastern Europe including the Russian Federation. Multi-center studies are therefore needed to assess the impact of this pathogen including its molecular epidemiology and corresponding resistance.

keywords: genotyping; splAST; MLST; surveillance; Russia; BI/NAP; whole genome sequencing (WGS); cgMLST

Introduction

Clostridium difficile is a gram positive rod shaped spore forming bacterium which is the main causative agent for nosocomial diarrhea posing a huge burden for the healthcare system leading to high morbidity and mortality [1, 2].

The majority of strains produce two toxins; toxin A and B with the corresponding genes *tcdA* and *tcdB* [3] while a third one, binary toxin (*cdtAB*) is preferentially detected in more virulent isolates [4]. Toxin gene negative strains can be considered apathogenic and may protect the host organism from being colonized with toxigenic strains [5].

The pathogen is ubiquitously found and can be isolated from environmental sources (e.g. food, soil and water) and from a broad variety of different animal species [6–9].

Antibiotic use is the main risk factor for disease development disruption of the normal gut microbiome, which may lead to colonization and proliferation of *C. difficile* [10]. Alongside mild cases of diarrhea more severe courses of disease like pseudomembranous colitis and toxic megacolon are possible [11]. The carrier rate of *C. difficile* in healthy adults may vary ranging from 0–15% [12] while in infants this prevalence can be much higher and sometimes exceeding 80% [13]. Interestingly up to the age of 2 years there is usually no clinical significance since disease development is rare [14].

The pathogen was first described by Hall and O'Toole in 1938 after its isolation from the stool of infants as "*Bacillus difficilis*" [15]. The term "difficile" derives from the Latin word "difficult" and points out to the challenge to isolate this infectious agent in the microbiological laboratory. In the 1970s taxonomy changed to *Clostridium difficile* [16] with the term "*Clostridium*" deriving from Prazmowski in 1880 of the morphologically similar bacterium "*Clostridium butyricum*", which has been used for a broad variety of gram-positive bacteria in following years [17]. In 1978 the association between *C. difficile* and antibiotic induced diarrhea (pseudomembranous colitis) has been described [18]. Since the development of discriminatory genotypic methods, nomenclature is more in flux than being formerly based mainly on morphology and biochemical traits. *C. difficile* was therefore renamed in 2013 to *Peptoclostridium difficile* as proposed by Yutin and Galperin [19]In 2016 however, a further reclassification occurred being currently classified as *Clostridioides difficile* [15].

The incidence of *C. difficile* infections (CDI) can be estimated for Europe to be as high as 4.1/10.000 patient days (PD) per hospital [20] (2008) with similar rates are reported for the US [5.4/10.000 PD [21]] (2012). However, a large study conducted in 2012/2013 in Europe mean incidence was 7/10.000 PD with large differences

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depending on the investigated country ranging from 0.7–28.7/10.000 PD [22]. From a meta-analysis including 37.663 Asian patients CDI incidence was estimated to be 5.3/10.000 PD [23] and therefore similar when compared to rates encountered in Europe and the US.

Since studies concerning the impact of *C. difficile* infections (CDI) on the healthcare system have been conducted especially for industrialized nations and lack in particular in low and middle income countries this fact emphasizes the need for intensified research [24].

Molecular typing of *C. difficile* and antimicrobial resistance

C. difficile can be distinguished genetically by a broad variety of methods. The most common assays are PCR ribotyping [25], multilocus sequence typing (MLST) [26], surface layer protein A gene sequence typing (*spl*AST) [27] and pulse field gel electrophoresis [28]. One typing method that might gain more attention in the future possibly replacing the latter assays when it becomes more affordable is whole genome sequencing [29]. A nomenclature of a core genome MLST (cgMLST) scheme has been proposed recently [29].

Of these genotyping methods, ribotyping is currently the most commonly utilized assay for genotypic characterization of *C. difficile* in Europe according to a questionnaire being available in 16/32 European countries in the year 2014 [26].

The molecular epidemiology is always in flux and may change within a few years. Recently the introduction of "hypervirulent" strains such as ribotype 027 (RT027) originating from North America especially to Europe but also other parts of the world (e.g. South America) has contributed to a higher CDI incidence in the affected areas [30]. Resistances toward other antimicrobials, which are frequently used in human medicine (e.g. macrolides and fluoroquinolones), seem to be a factor for selection especially for more virulent strains such as RT027 [31]. Of note high rates of rifampicin resistance have also been noted for the RT027 strains in Europe [31, 32]. Furthermore, an association between lack of strain diversity and high levels of antimicrobial resistance has been described for Europe, which corroborates with an epidemic RT027 setting [31, 33]. Additionally to RT027, other RTs are also associated with antibiotic resistance. RT17 being highly prevalent in the Far East is also linked with resistance towards a broad variety of antimicrobials including fluoroquinolones, macrolides [34], which might favor its selection as well.

Although the impact of animal and environmental sources of *C. difficile* is yet unclear, some strains such as RT078 and RT126 seem to be live-stock associated and *C. difficile* can be detected on several food sources like vegetables and meat along with a broad variety of other RTs that can be frequently found in the human population [35–45].

Some RTs seem to be distributed across several world regions (e.g. RT001 and RT002) while others remain restricted to certain areas (e.g. RT176 to Europe, Table 1). It could also be shown that strains such as RT001 and RT027 are frequently hospital associated and can be driven back by the application of antibiotic stewardship including the reduction of antibiotic use and avoidance of CDI high risk antimicrobials (clindamycin, fluoroquinolones, cephalosporins aminopenicillins with betalactamase inhibitors) [46]. Although resistance towards antibiotics used for *C. difficile* therapy (mainly metronidazole, vancomycin and fidaxomicin) is still rare in Europe [31], other world regions encounter resistance rates of metronidazole of up to 18% (e.g. China and Israel) [47], while reduced susceptibility towards vancomycin [except in Israel [48]] and fidaxomicin are fortunately still rare [47]. Due to the fact that the vancomycin concentration in human feces is usually >1.000 mg/L, the clinical significance of elevated MICs (>4mg/L) remains still unclear [49].

C. difficile epidemiology is permanently changing emphasizing the need for ongoing surveillance procedures including molecular typing to detect emerging virulent strains and rising rates of antimicrobial resistance [50, 51].

Epidemiology in Europe

In 2008, according to a multi-center study conducted in 34 European countries including 106 laboratories, RT014/020 was the most prevalent genotype (16%), followed by RT001 (9%) and RT078 (8%), while RT027 isolates were rarely detected (5%) at that date [50].

However, in a point prevalence study carried out a few years later in 2012/2013, RT027 isolates were most prevalent reaching 19% followed by RT001/072 (11%), RT014/020 (10%), while RT078 was detected in 3% of all isolates respectively [51]. Major differences may also be evident on the regional level (e.g. between neighboring countries). In the Czech Republic, RT176, which is closely related to RT027 and RT001 (29% and 24% respectively), are the most common RTs [52]. RT176 is also distributed in Poland and dominates in addition to RT027 (14% and 62% respectively) [53].

In Italy, RT018 is the dominant strain (≥40%) being associated with high grade fluoroquinolone resistance [54, 55]. In Finland, RT027 is the most common RT (19%) followed by RT001 (13%), RT014/020 (14%), RT023 (6%), RT002 (5%) and RT078 (5%) [56]. Of note RT017 isolates have been detected in Southern Europe [e.g. Portugal [57]], which represent the most common RTs in the Eastern parts of Asia. The most prevalent Portuguese RTs were RT001, RT017, RT014/020 (26%, 17% and 6% respectively) [57]. For vast parts of Eastern Europe (e.g. Ukraine, Belarus and the Russian Federation respectively), however, data are scarce.

Epidemiology in Asia

Only few studies have been conducted in Asia concerning the molecular *C. difficile* epidemiology. Additionally these studies often use genotyping methods like *spl*AST thereby limiting a comparison to the ribotyping nomenclature. In contrast to Europe, up to this date the strain distribution is not heavily influenced by RT027 for most regions despite its occasional appearance in several Asian countries [e.g. in China and Singapore [58–60]]. The epidemiologically most important strains are RT017, RT018, RT014, RT002 and RT001 [61]. RT017 seems to be most prevalent e.g. in the mainland of China RT017 with a rate as high as 37% [62–64], while RT002 seems to play an important role in Hong Kong reaching 9% [65]. RT017 is furthermore associated with multidrug resistance [33], which might also favor the selection of this strain as seen for RT027. Interestingly phylogenetic studies suggest a North American origin of this strain [66]. In Korea, RT017 is also highly prevalent (up to 26%) [67, 68]. Of note RT027 has also emerged although being still rather uncommon in Korea [69]. For Japan representative genotyping data are scarce but the most common RTs seem to be RT001, RT002, RT014, RT052, RT369 and the genotype smz/018 (corresponds to RT018 in *spl*AST nomenclature) [70]. Interestingly, RT017 seems to be only of minor epidemiological importance compared to other East Asian countries. RT027 has been detected in the past but has not yet been established in Japan [71]. In Thailand, RT017 is also one of the most common RTs (11%) together with RT014/020 (16%), RT010 (non-toxigenic, 11%), RT039 (9%) and RT009 (6%) [72]. In Indonesia in a small multi-center study the most prevalent strain was RT017 (24%), while RT014/020 and RT002 represented 3% and 1% of all samples, respectively [73]. A huge strain diversity was present and 49% of all isolates were non-toxigenic [73]. In a recently published study from Malaysia, RT017 was also most prevalent (20%) followed by RT043 (10%) with a high percentage of non-toxigenic isolates [74]. In Taiwan among all strains RT017 was also most prominent with 31% but also RTs which belong to the RT078 group (RT078/RT126/RT127) are prevalent to some extend (6%) [75]. In 50 of 170 isolates non-toxigenic strains were present in this Taiwanese study [75]. For India only one study is available in which the most prevalent RTs were RT001, RT017 and RT106 (37%, 34% and 13% respectively) [76]. In the Near and Middle East however, the epidemiology seems to differ markedly. In Israel, RT027 is the most common RT reaching 32% resembling the situation in Europe and America where RT027 has become epidemic [48]. Due the fact that this study has used *spl*AST, a comparison to the ribotyping nomenclature remains unfortunately restricted for other genotypes (e.g. cr-02; 18%). This is in sharp contrast to other Middle Eastern countries. In Kuwait RT001 (14%), RT002 (16%), RT003 (8%), RT014 (6%), RT126 (10%) and RT139 (8%) were of the most numerous strains in a study including isolated from hospital and community associated diarrhea [77]. In another study focusing on community-acquired infections in Kuwait, RT139 dominated with a 29% prevalence rate [78]. This strain make-up is quite similar to the situation seen in Lebanon, where all these strains were also present except RT003 and RT139 [79]. Most prevalent RTs were RT002 (9%), RT014 (17%), RT020 (5%), RT070 (7%), RT106 (8%) [79]. For an Iran pediatric ward, 12% were RT027 strains [80] while in another study RT078 was prevalent in 21% of 19 cultural samples [81]. However, for healthy adults no molecular data are available.

World Region	Europe	Far East Asia	North and South America	Australia	Near and Middle East Asia
Study site, number of isolates	n=1196 [51]	Hong Kong (HK) n=345 [65] Mainland China (MC) n=110–411 [62–64] Korea (KR) n=140–408 [67, 68] Taiwan (TW) n=170 [75] Thailand (THA) n=105 [72] India (IND) n=121 [76]	USA n=350 [83] Chile n=719 [85]	n=440 [95]	Israel n=208 [48] Kuwait n=146 [77] Lebanon n=107 [79]
Ribotype distribution					
RT001/(072)	11%	<1% (HK); 12–15% (MC); ≤14% (KR); 37% (IND)	3% (USA)	-	14% (Kuwait) 3% (Lebanon)
RT002	4%	9% (HK); ≤4% (KR)	5% (USA)	16%	16% (Kuwait) 9% (Lebanon)
RT014/(020)	10%	1% (HK); ≤5% (KR); 16% (THA)	2% (USA)	34%	6% (Kuwait) 22% (Lebanon)
RT017	<1%	<1% (HK); 16–26% (KR); 14–37% (MC); 31% (TW); 11% (THA); 34% (IND)	4% (USA)	2%	-
RT018	3%	≤26% (KR)	-	-	-
RT027	19%	≤2% (KR)	26% (USA), 79% (Chile)	-	32% (Israel)
RT078	3%	≤3% (KR); <1% (MC), <1% (TW)	4% (USA)	1%	1% (Lebanon)
RT106	-	13% (IND)	5% (USA)	-	8% (Lebanon)
RT126	-	4% (TW)	-	-	10% (Kuwait) 1% (Lebanon)
RT176	2%	-	-	-	-

Table 1.
Ribotype (RT) distribution according to different world regions; only studies with a representative number of isolates were included (≥100) being published after the global introduction of RT027. For Europe and the US one large multi-center study was cited. Studies may also include non-toxigenic strains.

Epidemiology in North, Central and South America

The epidemiology on the American continent has been largely influenced by the appearance and dissemination of the RT027 strain. After its first description as being causative for numerous outbreaks in Canada [82] this so called "hypervirulent" strain soon spread to Europe and South America.

In the US the strain make-up is therefore similar to the situation as seen in Europe with RT027 as the most prevalent strain (26%) [83]. Other strains of certain epidemiological importance in the US were RT001/072, RT002, RT014/020, RT017, RT078 and RT106 (3%, 5%, 2%, 4%, 4% and 5% respectively) [83]. In Mexico around 51% of all *C. difficile* isolates are RT027 according to a larger multi-center study [84]. In Chile the impact of RT027 can even be considered higher than in the US and Mexico in which RT027 being detected within 79% of all human *C. difficile* isolates [85].

According to small monocentric study conducted in Costa Rica RT001, RT002 and RT017 seem to play a role in this country [86]. In the past, outbreaks with RT027 and RT012 have been also described in Costa Rican hospitals as far back as 2009 [87]. In Brazil RT014 could be isolated in a single center [88] and RT133 and RT233 were most prevalent (50% and 25% respectively) with a small sample set at a Brazilian University hospital [89]. However, no RT27 or RT078 have been reported for the largest South American country so far [90]. Additionally in Panama RT027 has been reported but no representative data are available [91]. For other American countries data about molecular epidemiology are even scarcer.

Epidemiology in Australia

In Australia RT244 has been described as a potential "hypervirulent" strain in Victoria being related to the RT027 strain [92]. RT027 has not been established in Australia with the first patient having a travelling history to the US and up to this date only RT027 strains have been detected in the Melbourne region [93, 94]. However, according to a large multi-center study carried out in 2013–2014 most prevalent RTs were RT014 and RT002 (30% and 16% respectively) [95]. RT244 was only of minor importance and RT027 was not detected in this study [95]. Of note RT244 strains have also been reported to cause outbreaks in New Zealand [96].

Epidemiology in Africa

For Africa almost no data about molecular *C. difficile* epidemiology is available.

In Algeria one study has been published so far including only 11 isolates of which four were non-toxigenic (RT084) and the other seven strains included mainly RT014 and RT020 [97]. In South Africa for instance in a small study including 32 isolates RT017 was most prevalent (50%) while 16% of all isolates were RT001 [98]. A small study from Ghana revealed only three toxigenic isolates out of a total of fifteen [99]. The most frequent (non-toxigenic) RT was RT084 as seen in the Algerian study [97]. In another study conducted in Tanzania only seven isolates could be retrieved including three RT038, two RT045 and two unknown RTs [100].

Implications for the Russian Federation

Little is known about the impact of *C. difficile* on the Russian Federation's healthcare system.

Overall antibiotic consumption was as high as 6.069 Defined Daily Dose (DDD) per 1.000 population in 2015 (https://resistancemap.cddep.org/AntibioticUse.php; last accessed 19.09.2018) which can be compared to some European countries (e.g. Germany, Norway 6.647 DDD and 5.666 DDD, respectively). The highest value for Europe can be seen in Greece with 15.536 DDD followed by Spain (14.634 DDD), Romania (14.035 DDD) and France (13.040 DDD). Of note the Netherlands have the lowest rate with 4.127 DDD within Europe. The antibiotic consumption is therefore not higher than in other European countries and also the US (10.298 DDD) but still higher than in other world regions e.g. in West Africa (2.112 DDD) and Central America (1.695 DDD). This might indicate that *C. difficile* could represent a significant pathogen in Russia as it is in other European countries.

Due to the vast proportions of the Russian Federation it seems quite likely that *C. difficile* epidemiology might be influenced especially by European, Central and Far Eastern strains. This would include RT001 and RT027 which are the two most prevalent strains also in neighboring Finland as they are across Europe, potentially RT176 which is found in Poland and the Czech Republic. On the other hand strains like RT002 (also detected in Europe) and RT017 might strongly influence the Far Eastern regions of Russian since they are prevalent in East Asia. Of note the RT distribution may differ significantly between countries as seen in Europe and the Middle East.

To assess the epidemiological situation it is therefore necessary to conduct multi-center studies with study sites representable for the Russian Federation covering all major regions including clinical, molecular and phenotypical data. This can be considered the basis for further counter measures such as infection control programs (e.g. antibiotic stewardship). Since *C. difficile* epidemiology is permanently changing, ongoing surveillance procedures including molecular typing and susceptibility testing are necessary to detect emerging virulent strains and rising rates of antimicrobial resistance.

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Conflict of interest
None declared.

References

1. *Barbut F, Petit JC.* Epidemiology of *Clostridium difficile*-associated infections. Clin Microbiol Infect. 2001;7(8):405–10.
2. *Dubberke ER, Olsen MA.* Burden of *Clostridium difficile* on the healthcare system. Clin Infect Dis. 2012;55 Suppl 2: S88–92.
3. *Carter GP, Rood JI, Lyras D.* The role of toxin A and toxin B in *Clostridium difficile*-associated disease: Past and present perspectives. Gut Microbes. 2010;1(1):58–64.
4. *Gerding DN, Johnson S, Rupnik M, Aktories K. Clostridium difficile* binary toxin CDT: mechanism, epidemiology, and potential clinical importance. Gut Microbes. 2014;5(1):15–27.
5. *Natarajan M, Walk ST, Young VB, Aronoff DM.* A clinical and epidemiological review of non-toxigenic *Clostridium difficile*. Anaerobe. 2013;22:1–5.
6. *al Saif N, Brazier JS.* The distribution of *Clostridium difficile* in the environment of South Wales. J Med Microbiol. 1996;45(2):133–7.
7. *Moono P, Lim SC, Riley TV.* High prevalence of toxigenic *Clostridium difficile* in public space lawns in Western Australia. Sci Rep. 2017;7:41196.
8. *Kotila SM, Pitkanen T, Brazier J, Eerola E, Jalava J, Kuusi M, et al. Clostridium difficile* contamination of public tap water distribution system during a waterborne outbreak in Finland. Scand J Public Health. 2013;41(5):541–5.
9. *Hensgens MP, Keessen EC, Squire MM, Riley TV, Koene MG, de Boer E, et al. Clostridium difficile* infection in the community: a zoonotic disease? Clin Microbiol Infect. 2012;18(7):635–45.
10. *Vincent C, Manges AR.* Antimicrobial use, human gut microbiota and *Clostridium difficile* colonization and infection. Antibiotics (Basel). 2015;4(3):230–53.
11. *Noren T. Clostridium difficile* and the disease it causes. Methods Mol Biol. 2010;646:9–35.
12. *Furuya-Kanamori L, Marquess J, Yakob L, Riley TV, Paterson DL, Foster NF, et al.* Asymptomatic Clostridium difficile colonization: epidemiology and clinical implications. BMC infectious diseases. 2015;15:516.
13. *Matsuki S, Ozaki E, Shozu M, Inoue M, Shimizu S, Yamaguchi N, et al.* Colonization by *Clostridium difficile* of neonates in a hospital, and infants and children in three day-care facilities of Kanazawa, Japan. Int Microbiol. 2005;8(1):43–8.
14. *Gonzalez-Del Vecchio M, Alvarez-Uria A, Marin M, Alcala L, Martin A, Montilla P, et al.* Clinical Significance of *Clostridium difficile* in Children Less Than 2 Years Old: A Case-Control Study. Pediatr Infect Dis J. 2016;35(3):281–5.
15. *Lawson PA, Citron DM, Tyrrell KL, Finegold SM.* Reclassification of *Clostridium difficile* as *Clostridioides difficile* (Hall and O'Toole 1935) Prevot 1938. Anaerobe. 2016;40:95–9.
16. *Kazanowski M, Smolarek S, Kinnarney F, Grzebieniak Z. Clostridium difficile*: epidemiology, diagnostic and therapeutic possibilities-a systematic review. Tech Coloproctol. 2014;18(3):223–32.
17. *Lawson PA, Rainey FA.* Proposal to restrict the genus *Clostridium* Prazmowski to *Clostridium butyricum* and related species. Int J Syst Evol Microbiol. 2016;66(2):1009–16.
18. *Bartlett JG, Moon N, Chang TW, Taylor N, Onderdonk AB.* Role of *Clostridium difficil*e in antibiotic-associated pseudomembranous colitis. Gastroenterology. 1978;75(5):778–82.
19. *Yutin N, Galperin MY.* A genomic update on clostridial phylogeny: Gram-negative spore formers and other misplaced clostridia. Environ Microbiol. 2013;15(10):2631–41.
20. *Bauer MP, Notermans DW, van Benthem BH, Brazier JS, Wilcox MH, Rupnik M, et al. Clostridium difficile* infection in Europe: a hospital-based survey. Lancet. 2011;377(9759):63–73.
21. Centers for Disease C, Prevention. Vital signs: preventing *Clostridium difficile* infections. MMWR Morb Mortal Wkly Rep. 2012;61(9):157–62.
22. *Davies KA, Longshaw CM, Davis GL, Bouza E, Barbut F, Barna Z, et al.* Underdiagnosis of *Clostridium difficile* across Europe: the European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID). Lancet Infect Dis. 2014;14(12):1208–19.
23. *Borren NZ, Ghadermarzi S, Hutfless S, Ananthakrishnan AN.* The emergence of *Clostridium difficile* infection in Asia: A systematic review and meta-analysis of incidence and impact. PLoS One. 2017;12(5): e0176797.
24. *Forrester JD, Cai LZ, Mbanje C, Rinderknecht TN, Wren SM. Clostridium difficile* infection in low- and middle-human development index countries: a systematic review. Trop Med Int Health. 2017;22(10):1223–32.
25. *Indra A, Huhulescu S, Schneeweis M, Hasenberger P, Kernbichler S, Fiedler A, et al.* Characterization of *Clostridium difficile* isolates using capillary gel electrophoresis-based PCR ribotyping. J Med Microbiol. 2008;57(Pt 11):1377–82.
26. *van Dorp SM, Notermans DW, Alblas J, Gastmeier P, Mentula S, Nagy E, et al.* Survey of diagnostic and typing capacity for *Clostridium difficile* infection in Europe, 2011 and 2014. Euro Surveill. 2016;21(29).
27. *Kato H, Yokoyama T, Arakawa Y.* Typing by sequencing the slpA gene of Clostridium difficile strains causing multiple outbreaks in Japan. J Med Microbiol. 2005;54(Pt 2):167–71.
28. *Killgore G, Thompson A, Johnson S, Brazier J, Kuijper E, Pepin J, et al.* Comparison of seven techniques for typing international epidemic strains of *Clostridium difficile*: restriction endonuclease analysis, pulsed-field gel electrophoresis, PCR-ribotyping, multilocus sequence typing, multilocus variable-number tandem-repeat analysis,

amplified fragment length polymorphism, and surface layer protein A gene sequence typing. Journal of clinical microbiology. 2008;46(2):431–7.

29. *Bletz S, Janezic S, Harmsen D, Rupnik M, Mellmann A.* Defining and evaluating a core genome multilocus sequence typing scheme for genome-wide typing of *Clostridium difficile*. J Clin Microbiol. 2018;56(6).
30. *Knight DR, Elliott B, Chang BJ, Perkins TT, Riley TV.* Diversity and Evolution in the Genome of *Clostridium difficile*. Clin Microbiol Rev. 2015;28(3):721–41.
31. *Freeman J, Vernon J, Pilling S, Morris K, Nicholson S, Shearman S, et al.* The ClosER study: results from a three-year pan-European longitudinal surveillance of antibiotic resistance among prevalent Clostridium difficile ribotypes, 2011–2014. Clin Microbiol Infect. 2017.
32. *Färber J, Illiger S, Berger F, Gärtner B, von Müller L, Lohmann CH, et al.* Management of a cluster of *Clostridium difficile* infections among patients with osteoarticular infections. Antimicrobial Resistance & Infection Control. 2017;6(1):22.
33. *Freeman J, Vernon J, Morris K, Nicholson S, Todhunter S, Longshaw C, et al.* Pan-European longitudinal surveillance of antibiotic resistance among prevalent *Clostridium difficile* ribotypes. Clin Microbiol Infect. 2015;21(3):248 e9- e16.
34. *Lee JH, Lee Y, Lee K, Riley TV, Kim H.* The changes of PCR ribotype and antimicrobial resistance of *Clostridium difficile* in a tertiary care hospital over 10 years. J Med Microbiol. 2014;63(Pt 6):819–23.
35. *Pirs T, Ocepek M, Rupnik M.* Isolation of *Clostridium difficile* from food animals in Slovenia. J Med Microbiol. 2008;57(Pt 6):790–2.
36. *Alvarez-Perez S, Blanco JL, Pelaez T, Astorga RJ, Harmanus C, Kuijper E, et al.* High prevalence of the epidemic *Clostridium difficile* PCR ribotype 078 in Iberian free-range pigs. Res Vet Sci. 2013;95(2):358–61.
37. *Moono P, Putsathit P, Knight DR, Squire MM, Hampson DJ, Foster NF, et al.* Persistence of *Clostridium difficile* RT 237 infection in a Western Australian piggery. Anaerobe. 2016;37:62–6.
38. *Alvarez-Perez S, Blanco JL, Harmanus C, Kuijper EJ, Garcia ME.* Prevalence and characteristics of *Clostridium perfringens* and *Clostridium difficile* in dogs and cats attended in diverse veterinary clinics from the Madrid region. Anaerobe. 2017;48:47–55.
39. *Martin-Burriel I, Andres-Lasheras S, Harders F, Mainar-Jaime RC, Ranera B, Zaragoza P, et al.* Molecular analysis of three *Clostridium difficile* strain genomes isolated from pig farm-related samples. Anaerobe. 2017;48:224–31.
40. *Stein K, Egan S, Lynch H, Harmanus C, Kyne L, Herra C, et al.* PCR-ribotype distribution of *Clostridium difficile* in Irish pigs. Anaerobe. 2017;48:237–41.
41. *Visser M, Sephri S, Olson N, Du T, Mulvey MR, Alfa MJ.* Detection of *Clostridium difficile* in retail ground meat products in Manitoba. Can J Infect Dis Med Microbiol. 2012;23(1):28–30.
42. *Lim SC, Foster NF, Elliott B, Riley TV.* High prevalence of *Clostridium difficile* on retail root vegetables, Western Australia. J Appl Microbiol. 2018;124(2):585–90.
43. *Metcalf DS, Costa MC, Dew WM, Weese JS. Clostridium difficile* in vegetables, Canada. Lett Appl Microbiol. 2010;51(5):600–2.
44. *Ghavidel M, Salari Sedigh H, Razmyar J.* Isolation of *Clostridium difficile* and molecular detection of binary and A/B toxins in faeces of dogs. Iran J Vet Res. 2016;17(4):273–6.
45. *Razmyar J, Jamshidi A, Khanzadi S, Kalidari G.* Toxigenic *Clostridium difficile* in retail packed chicken meat and broiler flocks in northeastern Iran. Iran J Vet Res. 2017;18(4):271–4.
46. *Lawes T, Lopez-Lozano JM, Nebot CA, Macartney G, Subbarao-Sharma R, Wares KD, et al.* Effect of a national 4C antibiotic stewardship intervention on the clinical and molecular epidemiology of *Clostridium difficile* infections in a region of Scotland: a non-linear time-series analysis. Lancet Infect Dis. 2017;17(2):194–206.
47. *Peng Z, Jin D, Kim HB, Stratton CW, Wu B, Tang YW, et al.* Update on antimicrobial resistance in *Clostridium difficile*: Resistance mechanisms and antimicrobial susceptibility testing. J Clin Microbiol. 2017;55(7):1998–2008.
48. *Adler A, Miller-Roll T, Bradenstein R, Block C, Mendelson B, Parizade M, et al.* A national survey of the molecular epidemiology of *Clostridium difficile* in Israel: the dissemination of the ribotype 027 strain with reduced susceptibility to vancomycin and metronidazole. Diagn Microbiol Infect Dis. 2015;83(1):21–4.
49. *Baines SD, Wilcox MH.* Antimicrobial Resistance and Reduced Susceptibility in *Clostridium difficile*: Potential Consequences for Induction, Treatment, and Recurrence of *C. difficile* Infection. Antibiotics (Basel). 2015;4(3):267–98.
50. *Bauer MP, Notermans DW, van Benthem BH, Brazier JS, Wilcox MH, Rupnik M, et al.* Clostridium difficile infection in Europe: a hospital-based survey. Lancet (London, England). 2011;377(9759):63–73.
51. *Davies KA, Ashwin H, Longshaw CM, Burns DA, Davis GL, Wilcox MH, et al.* Diversity of *Clostridium difficile* PCR ribotypes in Europe: results from the European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID), 2012 and 2013. Euro Surveill. 2016;21(29).
52. *Nyc O, Pituch H, Matejkova J, Obuch-Woszczatynski P, Kuijper EJ. Clostridium difficile* PCR ribotype 176 in the Czech Republic and Poland. Lancet. 2011;377(9775):1407.
53. *van Dorp SM, Kinross P, Gastmeier P, Behnke M, Kola A, Delmee M, et al.* Standardised surveillance of Clostridium difficile infection in European acute care hospitals: a pilot study, 2013. Euro Surveill. 2016;21(29).
54. *Krutova M, Matejkova J, Kuijper EJ, Drevinek P, Nyc O.* Clostridium difficile PCR ribotypes 001 and 176 – the common denominator of C. difficile infection epidemiology in the Czech Republic, 2014. Euro Surveill. 2016;21(29).
55. *Baldan R, Trovato A, Bianchini V, Biancardi A, Cichero P, Mazzotti M, et al. Clostridium difficile* PCR ribotype 018, a successful epidemic genotype. J Clin Microbiol. 2015;53(8):2575–80.
56. *Mentula S, Kotila SM, Lyytikainen O, Ibrahem S, Ollgren J, Virolainen A. Clostridium difficile* infections in Finland, 2008–2015: trends, diagnostics and ribotypes. Eur J Clin Microbiol Infect Dis. 2017;36(10):1939–45.
57. *Isidro J, Santos A, Nunes A, Borges V, Silva C, Vieira L, et al.* Imipenem resistance in *Clostridium difficile* ribotype 017, Portugal. Emerg Infect Dis. 2018;24(4):741–5.
58. *Cheng JW, Xiao M, Kudinha T, Xu ZP, Hou X, Sun LY, et al.* The first two *Clostridium difficile* ribotype 027/ST1 isolates identified in Beijing, China – an emerging problem or a neglected threat? Sci Rep. 2016;6:18834.
59. *Cheng VC, Yam WC, Chan JF, To KK, Ho PL, Yuen KY. Clostridium difficile* ribotype 027 arrives in Hong Kong. Int J Antimicrob Agents. 2009;34(5):492–3.

60. *Lim PL, Ling ML, Lee HY, Koh TH, Tan AL, Kuijper EJ, et al.* Isolation of the first three cases of *Clostridium difficile* polymerase chain reaction ribotype 027 in Singapore. Singapore Med J. 2011;52(5):361–4.
61. *Collins DA, Hawkey PM, Riley TV.* Epidemiology of *Clostridium difficile* infection in Asia. Antimicrob Resist Infect Control. 2013;2(1):21.
62. *Huang H, Weintraub A, Fang H, Wu S, Zhang Y, Nord CE.* Antimicrobial susceptibility and heteroresistance in Chinese *Clostridium difficile* strains. Anaerobe. 2010;16(6):633–5.
63. *Liu XS, Li WG, Zhang WZ, Wu Y, Lu JX.* Molecular characterization of *Clostridium difficile* isolates in China from 2010 to 2015. Front Microbiol. 2018;9:845.
64. *Jin D, Luo Y, Huang C, Cai J, Ye J, Zheng Y, et al.* Molecular epidemiology of *Clostridium difficile* infection in hospitalized patients in Eastern China. J Clin Microbiol. 2017;55(3):801–10.
65. *Cheng VC, Yam WC, Lam OT, Tsang JL, Tse EY, Siu GK, et al. Clostridium difficile* isolates with increased sporulation: emergence of PCR ribotype 002 in Hong Kong. Eur J Clin Microbiol Infect Dis. 2011;30(11):1371–81.
66. *Cairns MD, Preston MD, Hall CL, Gerding DN, Hawkey PM, Kato H, et al.* Comparative genome analysis and global phylogeny of the toxin variant *Clostridium difficile* PCR ribotype 017 reveals the evolution of two independent sublineages. J Clin Microbiol. 2017;55(3):865–76.
67. *Kim H, Jeong SH, Roh KH, Hong SG, Kim JW, Shin MG, et al.* Investigation of toxin gene diversity, molecular epidemiology, and antimicrobial resistance of *Clostridium difficile* isolated from 12 hospitals in South Korea. Korean J Lab Med. 2010;30(5):491–7.
68. *Kim J, Kang JO, Kim H, Seo MR, Choi TY, Pai H, et al.* Epidemiology of *Clostridium difficile* infections in a tertiary-care hospital in Korea. Clin Microbiol Infect. 2013;19(6):521–7.
69. *Kim H, Lee Y, Moon HW, Lim CS, Lee K, Chong Y.* Emergence of *Clostridium difficile* ribotype 027 in Korea. Korean J Lab Med. 2011;31(3):191–6.
70. *Riley TV, Kimura T.* The Epidemiology of *Clostridium difficile* infection in Japan: A systematic review. Infect Dis Ther. 2018;7(1):39–70.
71. *Kato H, Ito Y, van den Berg RJ, Kuijper EJ, Arakawa Y.* First isolation of *Clostridium difficile* 027 in Japan. Euro Surveill. 2007;12(1): E070111 3.
72. *Putsathit P, Maneerattanaporn M, Piewngam P, Kiratisin P, Riley TV.* Prevalence and molecular epidemiology of *Clostridium difficile* infection in Thailand. New Microbes New Infect. 2017;15:27–32.
73. *Collins DA, Gasem MH, Habibie TH, Arinton IG, Hendriyanto P, Hartana AP, et al.* Prevalence and molecular epidemiology of *Clostridium difficile* infection in Indonesia. New Microbes New Infect. 2017;18:34–7.
74. *Riley TV, Collins DA, Karunakaran R, Kahar MA, Adnan A, Hassan SA, et al.* High prevalence of toxigenic and nontoxigenic *Clostridium difficile* strains in Malaysia. J Clin Microbiol. 2018;56(6).
75. *Hung YP, Huang IH, Lin HJ, Tsai BY, Liu HC, Liu HC, et al.* Predominance of *Clostridium difficile* ribotypes 017 and 078 among toxigenic clinical isolates in Southern Taiwan. PLoS One. 2016;11(11): e0166159.
76. *Vaishnavi C, Singh M, Mahmood S, Kochhar R.* Prevalence and molecular types of *Clostridium difficile* isolates from faecal specimens of patients in a tertiary care centre. J Med Microbiol. 2015;64(11):1297–304.
77. *Jamal WY, Rotimi VO.* Surveillance of antibiotic resistance among hospital- and community-acquired toxigenic *Clostridium difficile* isolates over 5-year period in Kuwait. PLoS One. 2016;11(8): e0161411.
78. *Jamal W, Pauline E, Rotimi V.* A prospective study of community-associated *Clostridium difficile* infection in Kuwait: Epidemiology and ribotypes. Anaerobe. 2015;35(Pt B):28–32.
79. *Berger FK, Rasheed SS, Araj GF, Mahfouz R, Rimmani HH, Karaoui WR, et al.* Molecular characterization, toxin detection and resistance testing of human clinical *Clostridium difficile* isolates from Lebanon. Int J Med Microbiol. 2018;308(3):358–63.
80. *Khoshdel A, Habibian R, Parvin N, Doosti A, Famouri F, Eshraghi A, et al.* Molecular characterization of nosocomial *Clostridium difficile* infection in pediatric ward in Iran. Springerplus. 2015;4:627.
81. *Jalali M, Khorvash F, Warriner K, Weese JS. Clostridium difficile* infection in an Iranian hospital. BMC Res Notes. 2012;5:159.
82. *Pepin J, Valiquette L, Cossette B.* Mortality attributable to nosocomial *Clostridium difficile*-associated disease during an epidemic caused by a hypervirulent strain in Quebec. CMAJ. 2005;173(9):1037–42.
83. *Tenover FC, Akerlund T, Gerding DN, Goering RV, Bostrom T, Jonsson AM, et al.* Comparison of strain typing results for *Clostridium difficile* isolates from North America. J Clin Microbiol. 2011;49(5):1831–7.
84. *Davila LP, Garza-Gonzalez E, Rodriguez-Zulueta P, Morfin-Otero R, Rodriguez-Noriega E, Vilar-Compte D, et al.* Increasing rates of *Clostridium difficile* infection in Mexican hospitals. Braz J Infect Dis. 2017;21(5):530–4.
85. *Aguayo C, Flores R, Levesque S, Araya P, Ulloa S, Lagos J, et al.* Rapid spread of *Clostridium difficile* NAP1/027/ST1 in Chile confirms the emergence of the epidemic strain in Latin America. Epidemiol Infect. 2015;143(14):3069–73.
86. *Jimenez A, Araya R, Paniagua D, Camacho-Mora Z, Du T, Golding GR, et al.* Molecular epidemiology and antimicrobial resistance of *Clostridium difficile* in a national geriatric hospital in Costa Rica. J Hosp Infect. 2018;99(4):475–80.
87. *Quesada-Gomez C, Lopez-Urena D, Acuna-Amador L, Villalobos-Zuniga M, Du T, Freire R, et al.* Emergence of an outbreak-associated *Clostridium difficile* variant with increased virulence. J Clin Microbiol. 2015;53(4):1216–26.
88. *Costa CL, Mano de Carvalho CB, Gonzalez RH, Gifoni MAC, Ribeiro RA, Quesada-Gomez C, et al.* Molecular epidemiology of *Clostridium difficile* infection in a Brazilian cancer hospital. Anaerobe. 2017;48:232–6.
89. *Balassiano IT, dos Santos-Filho J, Vital-Brazil JM, Nouer SA, Souza CR, Brazier JS, et al.* Detection of cross-infection associated to a Brazilian PCR-ribotype of *Clostridium difficile* in a university hospital in Rio de Janeiro, Brazil. Antonie Van Leeuwenhoek. 2011;99(2):249–55.
90. *Monteiro Ade A, Pires RN, Persson S, Rodrigues Filho EM, Pasqualotto AC.* A search for *Clostridium difficile* ribotypes 027 and 078 in Brazil. Braz J Infect Dis. 2014;18(6):672–4.
91. *Lopez-Urena D, Quesada-Gomez C, Miranda E, Fonseca M, Rodriguez-Cavallini E.* Spread of epidemic *Clostridium difficile* NAP1/027 in Latin America: case reports in Panama. J Med Microbiol. 2014;63(Pt 2):322–4.
92. *Lim SK, Stuart RL, Mackin KE, Carter GP, Kotsanas D, Francis MJ, et al.* Emergence of a ribotype 244 strain of *Clostridium difficile* associated with severe disease and related to the epidemic ribotype 027 strain. Clin Infect Dis. 2014;58(12):1723–30.

93. *Riley TV, Thean S, Hool G, Golledge CL*. First Australian isolation of epidemic *Clostridium difficile* PCR ribotype 027. Med J Aust. 2009;190(12):706–8.

94. *Richards M, Knox J, Elliott B, Mackin K, Lyras D, Waring LJ, et al.* Severe infection with *Clostridium difficile* PCR ribotype 027 acquired in Melbourne, Australia. Med J Aust. 2011;194(7):369–71.

95. *Knight DR, Giglio S, Huntington PG, Korman TM, Kotsanas D, Moore CV, et al*. Surveillance for antimicrobial resistance in Australian isolates of *Clostridium difficile*, 2013–14. J Antimicrob Chemother. 2015;70(11):2992–9.

96. *D.M. DA, Heffernan H, Dervan A, Bakker S, Freeman JT, Bhally H, et al.* Severe *Clostridium difficile* infection in New Zealand associated with an emerging strain, PCR-ribotype 244 NZMJ 2013;126(1280):10–4.

97. *Djebbar A, Sebaihia M, Kuijper E, Harmanus C, Sanders I, Benbraham N, et al.* First molecular characterisation and PCR ribotyping of *Clostridium difficile* strains isolated in two Algerian Hospitals. J Infect Dev Ctries. 2018;12(1):015–21.

98. *Rajabally N, Kullin B, Ebrahim K, Brock T, Weintraub A, Whitelaw A, et al.* A comparison of *Clostridium difficile* diagnostic methods for identification of local strains in a South African centre. J Med Microbiol. 2016.

99. *Janssen I, Cooper P, Gunka K, Rupnik M, Wetzel D, Zimmermann O, et al.* High prevalence of nontoxigenic *Clostridium difficile* isolated from hospitalized and non-hospitalized individuals in rural Ghana. Int J Med Microbiol. 2016;306(8):652–6.

100. *Seugendo M, Mshana SE, Hokororo A, Okamo B, Mirambo MM, von Müller L, et al. Clostridium difficile* infections among adults and children in Mwanza/Tanzania: is it an underappreciated pathogen among immunocompromised patients in sub-Saharan Africa? New Microbes New Infect. 2015;8:99–102.