COMMUNITY-ASSOCIATED *CLOSTRIDIUM DIFFICILE* INFECTION IN WESTERN AUSTRALIA:

EPIDEMIOLOGY AND IMPLICATIONS FOR PUBLIC HEALTH POLICY

by

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DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Lauren Edna Bloomfield

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SUMMARY

Clostridium difficile is a spore-forming, Gram-positive, anaerobic bacillus, which is capable of causing disease ranging from mild diarrhoea to fulminant colitis and toxic megacolon which can result in death. Rates of *C. difficile* infection (CDI) have increased in Australia, following significant rises in the rest of the world. Western Australian (WA) rates of hospital-identified CDI (HI-CDI) are reported by the WA Department of Health via Healthcare Infection Surveillance WA (HISWA).

Recent data show a trebling in CDI rates between January 2010 and December 2014. Enhanced surveillance, which involves applying a standard case definition to determine whether an infection is healthcare-associated (HA) or community-associated (CA), is encouraged at WA healthcare facilities (HCFs), although this has not been undertaken broadly to date.

The literature suggests a changing epidemiology of CDI, in which an increase in community cases is being observed, and disease is occurring in individuals who lack any of the classical risk factors: younger adults with no history of antibiotic use or recent hospitalisation. Locally, there has been little work done on establishing the burden of CA-CDI on the healthcare system.

In order to understand the epidemiology of CDI in WA, and further to determine potential risk factors behind this infection in the community, a retrospective review of HI-CDI cases reported to HISWA was conducted. This review of 2,962 cases from metropolitan public hospitals allowed determination of the proportions of HA and CA infection. Further analysis was conducted to establish ribotyping diversity, and to determine potential risk factors in CA cases based on demographic data collected.

There was a higher proportion of CA-CDI in 2014 compared to the baseline year (2010). CA-CDI cases comprised approximately 30% of all HI-CDI, with case numbers of both CA and HA increasing over the study period. CA cases were younger than HA cases, and more likely to be diagnosed at a non-tertiary hospital. These findings are in keeping with the international literature. A significantly higher proportion of CA-CDI in females aged 20 – 39 was a key finding, warranting further investigation. Analysis of prominent ribotypes showed the UK 014/020 group was the most common strain among both CA and HA cases, accounting for 28.3% of all

ribotyped cases. There was a significantly higher diversity of ribotypes among HA cases which suggests that imported cases from the community may be an important contributor to the overall burden of disease detected among inpatients.

Ribotyping data also identified the emergence of UK 012 –a seldom-isolated strain in WA prior to 2013; cases of this ribotype increased over the study period to become the second most prevalent strain in 2014. Although this strain did not cause severe disease, its appearance highlights the potential for one ribotype to rapidly emerge and dominate within a region.

In conclusion, CA-CDI represents a substantial proportion of CDI cases diagnosed in WA hospitals. Further work is required to determine the drivers behind disease acquired outside of hospitals, including investigation of food, animal and environmental sources. Improving our understanding of this infection in the community is essential in determining appropriate measures to control the spread of disease and protect the community from this increasing public health threat.

CHAPTER 1 – INTRODUCTION

1 *CLOSTRIDIUM DIFFICILE*

Clostridium difficile is a spore-forming, Gram-positive, anaerobic bacillus, which is a frequent cause of antibiotic-associated diarrhoea, especially amongst hospitalised patients (1). The spectrum of disease caused by *C. difficile* infection (CDI) can range from mild diarrhoea to severe conditions such as fulminant colitis and toxic megacolon which can result in death (1, 2). In 2010, the Society of Healthcare Epidemiology of America (SHEA) identified the epidemiology, pathogenesis, treatment and prevention of infection with *C. difficile* as one of the five most important clinical challenges facing the discipline of Healthcare Epidemiology (3).

C. difficile is certainly not a new organism. Indeed, modern molecular analysis suggests it is a very ancient bacterium (4). First described in neonates in 1935 (5), Spencer (1998) aptly coined this bacterium an 'orphan' for many years – that is to say it was "an organism looking for a disease" (6). This rapidly changed when its role in pseudomembranous colitis (PMC), a severe and potentially life-threatening disease of the bowel, was discovered in the late 1970s. From humble beginnings *C. difficile* was soon implicated in 90-100% of PMC cases, the majority of antibiotic-associated colitis cases and a significant proportion of antibiotic-associated diarrhoea cases (6).

2 PATHOGENESIS

Like many clostridial infections, disease due to *C. difficile* is toxin mediated. *C. difficile* produces three toxins either alone or in combination; toxin A (TcdA), toxin B (TcdB) and binary toxin (CDT), although the role of binary toxin is still being debated (7). TcdA is an enterotoxin and TcdB a potent cytotoxin (8); the two are thought to work synergistically, however the presence of both is not required for disease to occur (9). TcdA was initially considered to be critical for development of symptomatic disease (9), however TcdA-negative, TcdB-positive strains have since been found to cause clinical disease in humans and animals (7, 10). Different strains are capable of causing more severe disease, with more virulent strains shown to produce more TcdA (11). Non-toxigenic strains do not cause disease in humans or animals, and colonisation with these strains may in fact offer protection against symptomatic disease (12).

Infection with *C. difficile* requires disruption of the intestinal flora, most commonly through the use of antibiotics, coupled with exposure to the organism via the faecal-oral route. Under normal circumstances*, C. difficile* is unable to colonise and proliferate in the adult gastrointestinal (GI) tract, as the microflora present will not allow this to occur (in a phenomenon known as 'colonisation resistance'(13)). When such a disruption allows this to happen, the organism can establish itself in the colon, where toxin production commences and symptomatic infection may follow.

Life-threatening infection can occur in the absence of symptoms; however watery diarrhoea is the most common symptom of illness. Outcomes may vary; in many cases mild diarrhoea will be the only symptom of disease, resolving when the microflora returns to a normal state (14), with no long-term sequelae. In more severe cases, PMC or fulminant colitis can occur which can necessitate surgical intervention i.e. colectomy. In the case of severe or recurrent disease that is not responsive to treatment prognosis is poor, and development of high risk complications such as perforation and megacolon can ultimately result in death (14).

In most cases, withdrawal of the implicated antibiotic will allow re-establishment of the normal microbiota, and this alone will be sufficient for symptoms to cease and eventually for the organism to be eliminated from the body (15). In more severe cases, treatment with antibiotics (most commonly metronidazole or vancomycin) may be required to clear the infection (16). New treatment modalities, such as the antimicrobial fidaxomicin, monoclonal antibodies, and faecal microbiota transplants, are now being more widely used to treat patients with recurring symptoms (17). Approximately 20% of CDI cases experience recurrent disease, through either relapse or re-infection (17, 18). An overview of the pathogenesis of CDI is shown in Figure 1.

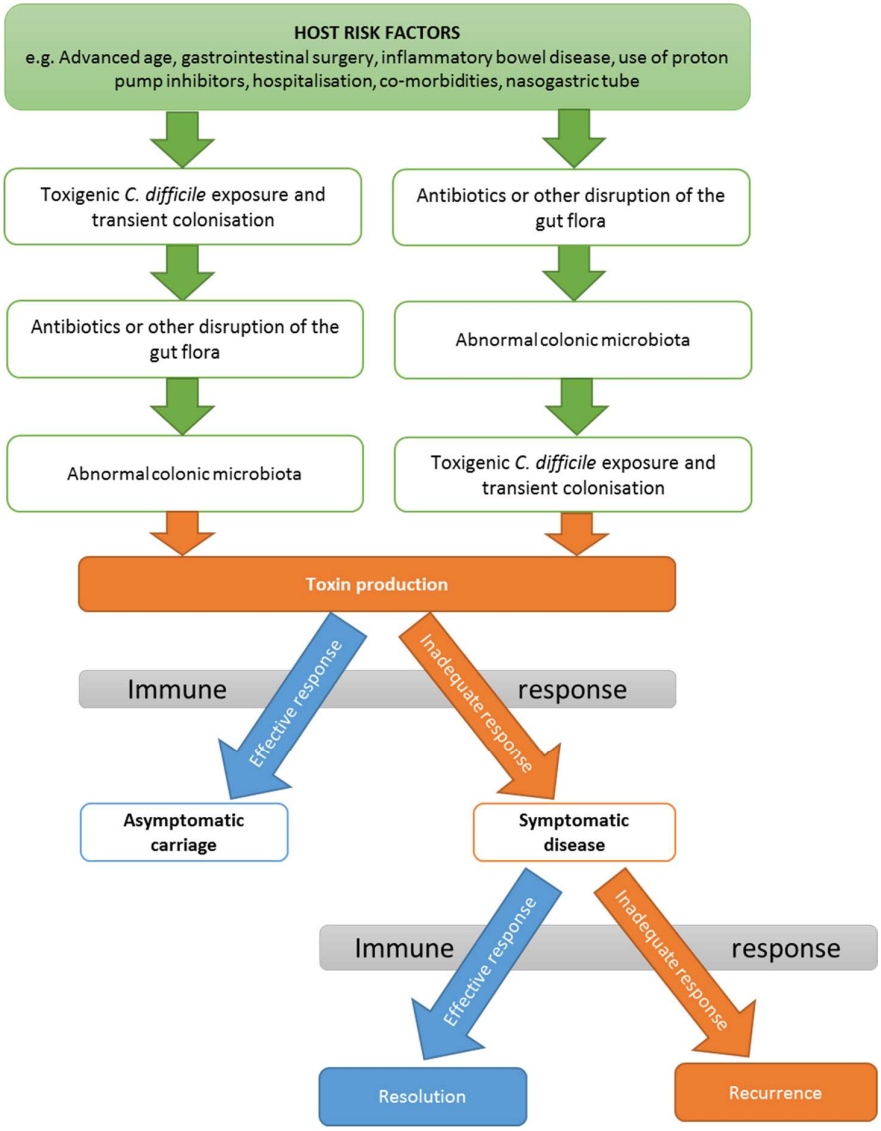


Figure 1. Pathogenesis of *Clostridium difficile* infection (adapted from Leffler and Lamont (19)).

Asymptomatic carriage occurs when an individual, either chronically or transiently, harbours the organism without development of disease. As the presence of normal flora does not normally allow *C. difficile* to establish or flourish, the rate of asymptomatic carriage is typically low, with prevalence studies showing asymptomatic carriage rates of between 1% - 3% in the healthy adult population (20). Asymptomatic carriage among neonates and infants is, however, markedly higher (reportedly 60% - 70% among healthy newborns (21)), with natural

resistance to disease believed to be the result of a lack of *C. difficile* toxin receptors in the colon (22). It is important, however, to note that disease has been documented in children from as young as 3 days old (23), indicating that perhaps not all neonates are protected from developing disease. This evidence needs to be balanced against the inability to conclusively exclude other causes of diarrhoea in children (23), which may make it more difficult to determine if this simply represents colonisation in a case with diarrhoea due to other causes. Investigations into receptor expression in hamster models has demonstrated that age-related susceptibility may be due to other factors (24), and this area needs further work.

C. difficile is different to most other enteric pathogens in that it is capable of producing spores at times when harsh environmental factors mean it can no longer survive and thrive in its vegetative state (25). These spores are incredibly hardy and can persist on contaminated surfaces for months or years. They are not able to be eradicated by the use of alcohol-based hand rub (ABHR) (26) – products which are in wide use in healthcare, and often take the place of traditional hand washing with soap and water if hands are not visibly soiled. This means that transmission can occur via fomites such as contaminated furniture, bed pans and medical equipment, or via the hands of healthcare workers who may be unaware they are still carrying the spores after performing hand hygiene with ABHR between patients (27).

Healthcare settings are an ideal environment in which *C. difficile* can spread. For some time, CDI was considered almost exclusively a nosocomial or 'healthcare-associated' infection (HAI) (28). While antibiotic use is also frequent in the community, exogenous acquisition of the organism from the hospital environment was thought to be the main source of colonisation and infection (29). Recent literature suggests a changing epidemiology, in which an increase in community cases is being observed, and disease is occurring in individuals who lack any of the classical risk factors: younger adults with no history of antibiotic use or recent hospitalisation (30). Coupled with the emergence of new so-called "hypervirulent" strains, the literature reflects a changing global landscape of CDI.

3 SURVEILLANCE

Rates of CDI have increased in Australia, following significant rises in the rest of the world (31-33). Western Australian (WA) rates of hospital-identified CDI (HI-CDI) are reported by the WA Department of Health via Healthcare Infection Surveillance WA (HISWA). Recent data show a trebling in aggregate rates between January 2010 and December 2014 (34). HI-CDI include all cases identified at a healthcare facility (HCF), including inpatients, outpatients, and those attending the emergency department. This definition does not differentiate between infections acquired in hospital and those acquired in the community but rather reflects the total burden of cases identified at WA HCFs.

Enhanced surveillance of CDI, which involves applying a standard case definition to determine whether an infection is healthcare- or community-associated, is encouraged at WA HCFs. As this enhanced surveillance is optional and the additional data are not reported to HISWA, establishing causes behind this increase in hospital-identified rates is very difficult at this stage. Furthermore, using only HI-CDI cases for surveillance, even with the addition of enhanced surveillance activities, will likely under-estimate the burden and over-estimate the severity of disease (32).

The importance of understanding the risk factors for and drivers of CDI cannot be overstated; at an estimated direct cost to the US healthcare system of up to USD4.8 billion per annum (35, 36), understanding epidemiology of CDI, particularly the source of infection, is central to prevention and control strategies. *C. difficile* is now reportedly the most common cause of healthcare-associated infections in the United States (37). Robust surveillance systems for diseases of public health concern are integral to disease control programs. The literature has highlighted marked decreases of incidence of CDI in the United Kingdom following the introduction of enhanced surveillance and rapid access to ribotyping (38).

3.1 COMMUNITY-ASSOCIATED DISEASE

While historically the lack of standardised definitions for community-associated (CA)-CDI has meant differential case classification in some studies, generally CA-CDI is understood to be disease in people who are not in hospital, and do not have a recent history of hospitalisation.

CA-CDI is an emerging area of concern for a number of reasons. First, the apparent increase in the prevalence of disease in this group over the last decade has led to a need to identify the drivers behind this trend. Second, the emergence of CDI in groups who may have previously been designated 'low risk', or who lack any of the traditional risk factors, means this infection may be under-reported and/or underdiagnosed in certain sectors of the community. Third, there is evidence that 'hypervirulent' strains capable of causing severe disease are circulating in the community. This leaves open the possibility of introduction to and subsequent transmission within the healthcare system, as patients move between hospitals and their homes. Finally, emerging evidence in the literature about potential reservoirs in animal, food and environmental sources have important public health implications, and should be further explored (39-42).

If individuals colonised in the community are developing disease after being admitted to hospital, rather than acquiring the organism from the hospital environment, this also presents a unique challenge for infection control professionals. Rather than traditional infection prevention and control strategies that may focus around environmental cleaning and hand hygiene, prevention of CDI in hospitals may call for a greater emphasis on the judicious use of antimicrobials, and/or the maintenance of healthy colonic flora by way of probiotics.

There is a clear mandate to understand the incidence of and risk factors for CA-CDI locally. While information collected by research institutions and clinicians in other jurisdictions can help to inform efforts, the nature of CDI suggests that a specific geographic region will have unique populations with varying levels of risk, divergent antimicrobial stewardship policies and different predominant strains. Without local surveillance, public health authorities have only the ability to infer what the magnitude of the problem is, and develop policy with incomplete information.

4 IMPLICATIONS FOR PUBLIC HEALTH POLICY

In order to inform public health policy around the prevention of CDI, establishing a solid foundation of knowledge surrounding the current situation is essential. With little known

regarding the current epidemiology of CA-CDI in WA, a research project was undertaken to determine the proportion of CA cases being reported using current enhanced surveillance definitions. By synthesising emerging knowledge from a review of the literature, and analysing data from enhanced surveillance activities, the project will contribute to the understanding of this emerging issue, and underpin the local public health response.

The inclusion of demographic data, routinely conducted ribotyping data (from 2011 onwards) and enhanced surveillance definitions presented a unique opportunity to analyse a large collection of CDI cases. These data were used to establish significant variations across groups, to highlight potential further areas for research, and to determine if any emerging strains appear to be of particular public health significance.

This project relied on well-established epidemiological techniques to inform public policy; the establishment of what is currently known locally, a review and analysis of existing data collections using consistent methodology, and comprehensive recommendations based on currently available data. The outputs of this work will have direct applicability to the development of disease control policy in WA, with a report outlining the research findings to be presented to the Western Australian Multi-Resistant Organism (WAMRO) Expert Advisory Group. Central to the undertaking of this work is a commitment to supporting evidence-based policy development, maintaining the focus on improving health in the population we serve.

CHAPTER 2 – LITERATURE REVIEW

1 INTRODUCTION

In Western Australia (WA), there has been a significant increase in the rate of hospital-identified *Clostridium difficile* infection (HI-CDI) since reporting commenced in 2010 (34). Rates of this organism have more than trebled over this period, increasing from 1.26 to 4.15 cases per 10,000 occupied bed days (34). This increase is unlikely to be wholly explained by changes in testing practices that have occurred recently in Australia. There is some evidence of an increase in test numbers because of greater awareness of CDI as an issue (unpublished data), but not enough to account for the increased rates seen. From 2010 – 2012, there was an approximate 25% increase in the number of tests for *C. difficile* in WA conducted by PathWest Laboratory Medicine (WA), the state public sector Pathology service, and during this same period the proportion of positive samples increased from approximately 2% to 5% (peaking at 7%). These unpublished data support a true increase in the number of CDI cases being detected in WA, and not just an artifact of increased testing.

In WA there was a small rise during 2010 around the time that testing moved to a more sensitive polymerase chain reaction (PCR)-based method (detection rate 90%) but this is unlikely to be associated with the rapid increase in rates of disease seen from mid-2011. Data from the WA Health Department's Healthcare Infection Surveillance program (HISWA) indicate that overall HI-CDI rates rose dramatically in mid-2011, from 2.5/10,000 occupied bed days (OBDs) in the second quarter (April-June) peaking at 5/10,000 OBDs in the first quarter of 2012 (Jan-Mar), before declining (43, 44).

The incidence of CDI cases defined as CA has also increased in other parts of Australia. A report on CDI in Tasmania concluded "the observed increase in CDI was most likely linked to transmission and infection pathways in the community, not inside hospitals" (45). If people are getting infected with *C. difficile* in the community, then a source or reservoir of *C. difficile* in the community is required. Very little is currently known about the epidemiology and risk factors of CA-CDI in WA.

To establish what is known about CDI in the community, and identify likely reservoirs of CDI in this setting, a literature review was conducted on CA-CDI both in Australia and around the

world. The results of this literature review were used to design an appropriate study to address the research question and build a knowledge base on which public health policy can be built.

2 HEALTHCARE- AND COMMUNITY-ASSOCIATED DISEASE – CASE DEFINITIONS

Defining what is considered a healthcare-associated (HA) vs a CA-CDI is essential in determining the validity of case classifications. Prior to an agreed definition of what 'community-associated' infection entailed, non-standardized definitions were applied by a number of authors (46-51). This includes misclassification due to a failure to determine hospitalisation history in cases presenting from the community (52).

Recommended standard case definitions were published by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for *Clostridium difficile* in 2006 (9), and in early 2007 the Centers for Disease Control and Prevention (CDC) ad hoc *C. difficile* working group recommended similar case definitions (53). These definitions have had wide acceptance and are commonly used to categorise cases into HA or CA. The definitions take into account the clinical and microbiological evidence to establish what a 'case' of CDI is, and further elucidate if a case should be classified as HA or CA.

The CDC working group notes that these definitions were "interim surveillance definitions and recommendations based on existing literature and expert opinion that can help to improve CDAD surveillance and prevention efforts" (53). Although these were published as 'interim' definitions, the literature does not show evidence of work to update these definitions since their release in 2007. The recently updated European Centre for Disease Prevention and Control (ECDC) *Clostridium difficile* surveillance protocol has not altered these original definitions (54). These have been adopted nationally in Australia and endorsed by the Australian Commission on Safety and Quality in Healthcare (293).

It is likely that these surveillance definitions can be improved. Further research locally could assist in this determination. As currently only HI-CDI is being reported locally, it would be particularly useful to determine if these definitions can be applied to HI-CDI cases to accurately

establish the burden of cases detected in hospitals that are community-associated. The details of the enhanced surveillance classification are shown in Table 1.

Locally, the terminology used in the international definitions (Table 1) has been adapted to reflect common use:

- HAI-HCFO (healthcare-associated infection, healthcare facility onset)
- HAI-CO (healthcare-associated infection, community onset)
- CAI (community-associated infection)
- Indeterminate
- Unknown

These terms align with the international definitions as shown below, and will be used throughout this dissertation.

Application of these definitions in non-hospitalised, community-dwelling patients may be a difficult undertaking for general practitioners; elucidating hospitalisation history would require additional resources and may result in misclassification of cases if not carried out correctly (55). Centralised epidemiological investigation of cases, with standardized applications of case definitions, is recommended to ensure consistent classification within a population (55).

One important distinction to make in the nomenclature is that of “community-acquired” vs. “community-associated” infection. These two terms have been used interchangeably in the literature; however, on the surface appear to have relatively different meanings. “Community-acquired” (and, moreover, hospital-acquired) gives the impression that the source of acquisition has been definitively determined. While in some cases (i.e. in the total absence of any healthcare contact) this may be possible to say with certainty, often the situation is more nuanced, with patients having varying levels of outpatient and other care. “Community-associated” (or healthcare-associated) implies the infection is associated with contact with a particular setting, without a more distinct inference to where the organism was physically acquired. This terminology is more appropriate, given the limitations of the enhanced surveillance definitions to definitively determine where the organism was acquired prior to the development of symptomatic disease.

Table 1. *C. difficile* enhanced surveillance definitions (Source: McDonald et al. (2007) (53)).

Classification	Definition
1. Healthcare facility onset, healthcare facility associated infection (HO-HCFA)	A case with symptom onset more than 48 h after hospital admission.
2. Community onset, healthcare facility associated infection (CO-HCFA)	A case with symptom onset in the community or 48 h or less after admission to an HCF, provided that symptom onset was less than 4 weeks after the last discharge from an HCF. Community-onset, HCF-associated cases should be attributed to the HCF from which the patient was last discharged, providing the patient was an inpatient of that HCF for more than 48 h.
3. Community-associated *Clostridium difficile* associated disease (CA-CDAD)	A case with symptom onset in the community or 48 h or less after admission to an HCF, provided that symptom onset was more than 12 weeks after the last discharge from an HCF.
4. Indeterminate	A case who does not fit any of the above criteria for an exposure setting, e.g. a patient who has symptom onset in the community but who was discharged from the same or another HCF 4-12 weeks before symptom onset.
5. Unknown	A case for whom the exposure setting cannot be determined because of lack of available data.

3 THE EMERGENCE OF COMMUNITY-ASSOCIATED CDI

Historically, CDI was considered largely nosocomial, with exogenous acquisition from the healthcare environment considered the main source of colonisation or infection (29). The capacity of *C. difficile* to cause disease in the community was reportedly recognised as early as 1982 (56), although this does not appear to have influenced further research in this area for

some time. Up until the late 1990s, the incidence of CA-CDI in United Kingdom (UK) studies was reported to be low, with "the rate of disease resulting in hospitalisation [reported to be] negligible" (57). Research from the United States (US) in the mid-1990s also noted that community-acquired CDI is "still uncommon" (46), demonstrating the relative obscurity of this disease in this population only 20 years ago. In Australia, CA disease was recognised in the 1990s (49, 50), although it arguably had a much lower profile than is apparent today.

It has been suggested that research into CDI can roughly be divided into 'early' (before 2000) and 'modern' (after 2000) eras (58). One of the major differences in the two eras is the shift from CDI as a predominantly nosocomial infection to an infection more increasingly diagnosed in the community. Moreover, nosocomial cases have also apparently become more frequent, and with more severe outcomes. (33, 59). The emergence of CA-CDI post 2000 both in Australia and internationally is explored further below.

4 COMMUNITY-ASSOCIATED CDI IN AUSTRALIA

In WA, attention has been given to CA-CDI since the 1980s, with a reported 4.7% prevalence of *C. difficile* among diarrhoeal samples from community patients as early as 1986 (48). Throughout the 1990s, the same research group documented the importance of this organism in general practice patients (prevalence between 5.5% and 10.7%), noting that *C. difficile* should be considered a potential cause of diarrhoeal illness in non-hospitalised patients (49, 50). Without systematic statewide surveillance, these estimates could not include a quantification of CA-CDI as a proportion of the overall burden of disease in WA.

There is little evidence available on the current prevalence of CA-CDI in Australia. In 2012, a study was conducted in Tasmania against a background of increased numbers of CDI detections at public hospitals. This study found an increasing incidence of CA-CDI from 10 cases per 100,000 population in 2010 to 17 cases per 100,000 population in 2011 (45). This was a relatively small study of 459 cases, however, it was beneficial in that it used population-based data rather than hospital-based data, which should have provided a more representative sample of the whole population.

Local data on the prevalence of CA-CDI in WA was not available at the commencement of the current study. HISWA reports overall HI-CDI rates, which have increased significantly since reporting commenced in 2010 (34). There is no centralised laboratory collection system of CDI cases in WA which is available to be analysed in order to determine population-based rates over this period. Further, the lack of hospitalisation histories for all cases makes determination of true CA-CDI impossible. Analysis of existing case data, coupled with review of case histories, is required to determine CA-CDI prevalence in WA.

5 COMMUNITY-ASSOCIATED CDI – INTERNATIONAL PERSPECTIVE

The emergence of, and risk factors for, CA-CDI have been outlined as major areas for research internationally (60-62). Most literature focusing on CDI epidemiology is traditionally based on hospital reports. Given the relative high proportions of disease and risk factors within this population, this is not unexpected. Similarly, epidemiological studies of CA disease often include cases that have been detected at a healthcare facility. This undoubtedly skews the data, as HI-CDI may be more severe (i.e. severe enough to warrant presentation at a hospital for treatment) and under-represented, as an acute care facility is likely not the primary source of healthcare for many people living in the community who develop gastroenteritis symptoms.

Reported incidence of CA-CDI is likely to vary based on the study population and local awareness and testing practices. Hospital-based studies looking at HI cases are suitable to compare prevalence across different regions, as this allows us to determine the proportion of CA disease in populations presenting to acute care facilities. This method is more reliable than trying to determine the relative incidence in a community, with methodological difficulties in determining catchment populations, and testing often at the discretion of the referring physician (63) which may lead to under-reporting. Community-based studies (i.e. conducted in general-practice) and laboratory-based studies were reviewed to determine risk factors, testing practices and ribotypes. However, for the purposes of comparing across regions, prevalence of CA-CDI as a proportion of all CDI in hospital-based studies was used.

5.1 EUROPE

Under-diagnosis of CDI, particularly among community cases, has been noted in Europe (64, 65). This may be owing to lack of clinical awareness, or non-sensitive laboratory diagnostic tests (64). Early reports showed that even when CA-CDI was considered relatively uncommon, cases were being identified in individuals with no recent hospitalisation history or links to outbreaks in hospitals (57), suggesting a potential source of infection in the community.

One might expect definitions of 'CAI' prior to the recommendations for standard case definitions (9) to be less uniform, however even since the introduction of these case definitions, some studies have continued to apply differing criteria to define HA and CA cases (66-68). A study in the Netherlands on 2005 data classified 36% as having 'community-onset' diarrhoea, however the after accounting for previous hospitalisation within the last month, the number who would be classified as 'CA-CDI' decreased to 20% (69). These data underscore the potential for different case definitions to significantly alter prevalence results.

Using a 90-day cut point for recent hospitalisation to define a case of 'probable community-acquired CDI', a study using administrative data in the England over a 12 year period demonstrated an increase in both the rate and proportion of CAI (67). The overall proportion of probable community-acquired CDI in this study increased from 7.1% to 13.5%. While this alternate definition for CA-CDI likely resulted in lower prevalence than would have been observed using ESCMID/CDC case definitions, consistent application of the same definition for surveillance purposes is sufficient to demonstrate a real increase over time.

A recent multi-centre study across 97 hospitals in 34 European countries applied ESCMID/CDC enhanced surveillance criteria to 506 CDI cases, and found 70/506 (14%) of cases were classified CA-CDI (70). The proportion of CA-CDI varied markedly across countries, ranging from 0% - 82%. As this was a hospital-based study, it is difficult to assess if the patient population and testing practices had a major influence on rates, from the limited information available. For example, hospitals with few CA-CDI may not have a large outpatient population or emergency department facilities, resulting in very low rates. Similarly, as mentioned by the authors, relying on physician requests for CDI testing may have meant some sites did not have many test requests in the outpatient population.

5.2 NORTH AMERICA

While CA-CDI is increasingly recognised in the United States, under-reporting is still suspected (71). The multi-centre study from Europe mentioned above demonstrates the potential for marked variation in the proportion of CA-CDI reported. The results of a prevalence study in one centre should, therefore, be interpreted with caution, and not generalized to a wider population.

With this limitation recognised, there have been several single-centre studies conducted in the US looking at CA-CDI prevalence, with varying reported prevalence (range 18% - 50%) (72, 73). These studies can be useful in determining risk factors, and indeed a significant finding was the differential characteristics of CA-CDI cases, who were found to be younger, more likely to be female, had fewer comorbidities, and were less likely to be exposed to antibiotics (72). CA-CDI cases were also less likely to have a severe infection. These data suggest a much different profile for CA-CDI cases as compared to HA cases.

As also applied in a United Kingdom study, a 90-day cut point for hospital admission was used in a US-based study investigating CA-CDI (74). This different application of case definitions resulted in 42% of cases being diagnosed as CAI, compared with 28% of cases if the CDC definitions had been applied (hospitalised within the last 4 weeks) (74). These data underscore the importance of standardized surveillance definitions to ensure that studies across various time points and geographical locations are uniformly reporting on the same patient groups, allowing for comparisons across time and regions.

Some large-scale CA-CDI studies have also been undertaken in the US. A six-centre study in North Carolina (USA) published in 2010 reported prevalence of CA-CDI of 20% (66). In another multi-centre US study involving eight geographic areas and 10,342 cases, the reported prevalence of CA-CDI was 32% (37). A further important finding of this study was that one in four patients with CA-CDI were hospitalised within 7 days of diagnosis, representing a significant cost and burden to the healthcare system. Others have reported even higher rates of hospitalisation (up to 40%) in CA-CDI cases (75).

Allard and colleagues surveyed 15 hospitals in Montreal 2005 – 2006, and of 2,297 cases of CDI, 599 (27%) were classified as CA-CDI, at a rate of 32 cases per 100,000 person-years (76).

Similar rates were observed across one reporting year in another Canadian province (Manitoba) by Lambert and colleagues, who also reported a prevalence of 27% and a rate of 23.4 cases per 100,000 person-years (77).

5.3 OTHER NATIONS

There are few studies on CA-CDI outside of North America and Europe, perhaps reflecting an overall lack of prevalence and typing studies conducted in these regions. A recent Singapore-based, single centre study found overall prevalence of 13.6% CA-CDI (78), lower than rates reported in other regions but nonetheless reflective of CA disease being of concern in this region.

One Kuwait-based study investigating diarrhoea in outpatients was found (79). Although this study was not set up to determine the proportions of CA-CDI and HA-CDI, a total of 16 cases were identified over a 2-year period, none of which had been hospitalised in the previous 6 months (79). These data suggest a low prevalence of CA-CDI presenting to this particular facility. No studies were found from South America or Africa describing CA-CDI. It is unclear if this represents a smaller burden of disease in these regions, a lack of public health awareness around the disease, a lower priority in terms of public health surveillance and activity, or a combination of these and other factors.

5.4 SUMMARY OF EVIDENCE

There are several limitations when it comes to the interpretation of apparent increases in CA-CDI in the international literature. An increased awareness and subsequent ascertainment bias has been acknowledged as a potential factor influencing the increase in incidence (38). It is logical that an increase in profile among physicians would result in increased testing and subsequent case ascertainment. In order for individual jurisdictions to monitor the impact of this bias on reporting, the proportion of positive cases should be reviewed alongside the raw numbers of requested tests. These data allow better interpretation of apparent increasing rates.

Another factor which needs to be taken into consideration is variable testing methodologies across different countries, and changes to more sensitive testing methodologies over time (38). The former may impact on prevalence and, at least partially, account for variable rates.

The latter may give the false appearance of increasing rates, when the reality is just more accurate detection methods. While evaluation of individual laboratory methods used in all studies was outside the scope of this review, differences were noted across various studies which may account for some of the heterogeneity in the results.

Taking these limitations into account, it is still apparent that CA-CDI is increasing, despite almost certainly being underdiagnosed in the community (80, 81). Disease in the community can be severe, with one study showing CA-CDI cases were more likely to develop severe infection than HA-CDI (82). Further, there is evidence of increasing severity of disease among community cases, using outcomes such as colectomy (83) as a measure. While hospital-based estimates vary between facility and region, overall about one third of CDI cases currently being detected in outpatients appear to be CA-CDI. It is clear that the relatively "uncommon" status of CA-CDI prior to 2000 no longer stands, and hospital-based case ascertainment likely represents 'the tip of the iceberg' in terms of overall CA-CDI prevalence in a population.

6 ESTABLISHED RISK FACTORS FOR COMMUNITY-ASSOCIATED CDI

There are several established risk factors for CA-CDI. Evidence from the literature suggests there may be some commonalities and some variations between risk factors in HA and CA cases, however it is generally accepted that there are overall differences between CA-CDI and HA-CDI patients (28). For HA-CDI, advanced age (>65), antibiotic treatment, and co-morbidities are all established risk factors (11, 84). CA-CDI, on the other hand, is frequently documented as occurring in younger populations who lack these traditional risk factors (30, 61, 80, 85-87).

Susceptibility to infection to some extent appears to vary on a case-by-case basis; while some CA-CDI cases may have many established risk factors, others seemingly lack the most important exposures, reflecting our lack of understanding of this entity. The degree to which host factors influence susceptibility and outcomes in disease is still not clear at this stage. Building a risk profile for CA-CDI may assist primary care providers in identifying these cases in the community setting.

6.1 ANTIBIOTIC EXPOSURE AND USE OF GASTRIC ACID SUPPRESSANTS

Antibiotic exposure is the most important risk factor for all CDI, including CA-CDI (51, 66, 88-91). While a meta-analysis conducted in the US, focusing on CA-CDI and antibiotics, supported recent antibiotic exposure being an important risk factor for developing CA-CDI (92), this was not uniform for all antimicrobials. Certain classes (clindamycin, fluoroquinolones and cephalosporins) presented the most significant risk, and others (e.g. tetracyclines) had no associated increased risk (92). A Canadian study conducted in the same year yielded similar results (93). The discrepant risk associated with different classes of antimicrobials has been found by other researchers (46). A case-control study conducted in the UK also found that exposure to antibiotics in the previous 4 weeks, particularly multiple agents, was significantly more frequent among CA-CDI cases than controls (94).

Although an important risk factor, US-based studies on CA-CDI cases have found 32% - 36% of those with a documented medication history had no previous antibiotic exposure in the preceding 3 months (95, 96). Further international studies have shown larger proportions (43% - 65%) of CA-CDI cases compared to HA-CDI cases had no previous antibiotic exposure (18, 97-99). These data support that antibiotics have an important, but perhaps not essential, role in CA-CDI, and that other yet to be determined factors may play a role. Healthcare providers in the community should be cognisant of the potential for CDI cases presenting with no prior history of antimicrobial use or hospitalisation.

Several studies have examined the use of gastric acid suppressants and their relationship with CDI (96, 100-102), with varying estimates of risk for CA-CDI (including nil significant findings) (103). The use of PPIs appears to be particularly significant for the subset of CA-CDI that is not associated with prior antimicrobial exposure (96), indicating that the disruption to the microbiota via PPI use may be sufficient to cause disease in the absence of antimicrobial therapy.

6.2 CO-MORBIDITIES AND OTHER PRE-EXISTING CONDITIONS

CDI is a significant disease in populations with inflammatory bowel disease (IBD) (including Crohn's Disease), with a high incidence, severe disease, and evidence of increasing rates over time (104-106). As patients with IBD often have diarrhoea, and as non-diarrhoeal specimens

are not routinely tested for CDI, this leaves open the possibility that this is being detected more in this group due to surveillance bias (30). This is especially true among Crohn's patients, half of whom have no have colonic involvement in disease (107). This is, however, unlikely to account for all of the increase in disease observed in IBD cases, with IBD patients appearing to have a different acquisition pattern to the general population, including increased susceptibility to a wider range of sources in the community (107).

Other comorbid conditions are associated with increased risk of CA-CDI, including chronic kidney disease, immunodeficiency (through infection or drug therapy), malignant lesions and solid organ transplants (61, 108, 109). Severe comorbid conditions such as these increase the risk of CDI due to prolonged use of antimicrobials, and frequent contact with healthcare facilities (61). As current definitions require an inpatient stay of >48 h prior to diagnosis in order to classify an infection as HAI, frequent, short stay hospital visits (such as those for dialysis or chemotherapy) are not captured in this determination.

6.3 CONTACT WITH CHILDREN <2 YEARS OF AGE

Identification of *C. difficile* from neonates has been long-established; the first isolation of this pathogen in 1935 was from the stool of healthy infants (5). Although relatively rare in healthy adults, asymptomatic colonization with toxigenic *C. difficile* occurs commonly among infants and children <2 years old (21, 110, 111). Acquisition can either occur during the neonatal period, or later on (between 4 – 6 months of age), which corresponds to the weaning period (110). Risk factors for development of disease in children <2 years appear to differ from the rest of the population (112), and, as previously noted, true disease as opposed to concurrent carriage in diarrhoeal patients may be difficult to discern. More information is required to determine the scope of magnitude of CDI in this population (113).

The literature has also shown that CA-CDI occurs more frequently in females than males (114, 115). A 2006 study conducted in Connecticut found females had nearly twice the incidence of CA-CDI than males (95), although no hypothesis was offered for this discrepancy. CA-CDI has also been described in increasing numbers of peripartum women, many of whom do not have any other predisposing factors (116, 117). Studies have identified contact with infants ≤2 years old as having a significant association with CA-CDI (79, 94, 96), and children have been

previously identified as potential reservoirs in the community (118). As common primary care givers for neonates and young infants, the possibility that neonates are responsible for causing disease in women in the community warrants further investigation.

6.4 SUMMARY OF EVIDENCE

Literature from the last decade has suggested that testing for CA-CDI may be appropriate in patients with no established risk factors. While there is certainly some commonality in risk factors, HA- and CA-CDI cases may not present with the same history and exposures. Building a risk profile for CA-CDI is important to allow primary care providers to recognise and appropriately diagnose CDI outside of the hospital.

Some patients with co-morbid conditions are more likely to present with CDI in the community. These include chronic conditions for which long term use of antimicrobial or immunosuppressive agents is required, and/or the patient requires frequent outpatient contact with healthcare facilities for treatment (e.g. chemotherapy or dialysis). There should be heightened awareness among primary care providers for diagnosis of CDI in this patient group.

The late acquisition of *C. difficile* in infants, which coincides with the introduction of solid foods, raises some important questions about likely sources of this introduction. There is also evidence to suggest that children may be a reservoir in the community, particularly for their primary carers. In addition, the body of evidence suggests patients with CA-CDI are younger, more likely to be female and are less likely to have received antibiotics prior to infection, compared with HA cases (30, 71, 97, 100, 119, 120). This includes cases in the community in otherwise 'healthy' children, who do not have comorbid conditions or antibiotic exposure, and who frequently develop recurrent and complicated disease at a higher rate than HA-CDI cases in the same population (121).

There is clearly more work to be done in determining risk factors for CA-CDI cases, and some work in attempting to quantify the objective risk to individuals based on the presence (or absence) of a number of host and environmental factors. Further research in this area is essential to be able to communicate with primary care providers, and provide further advice concerning testing in the community.

7 SEASONALITY AND ANTIMICROBIAL USE

Seasonality in CDI has been demonstrated previously, primarily in patients who are admitted to hospital with a severe respiratory tract infection that is treated with antibiotics and who then develop CDI in hospital (122, 123). As disruption of the gut microflora due to antimicrobial use is the leading risk factor for development of CDI, it might be expected that rates would increase during times of peak antimicrobial consumption. Peaks in CDI rates during the winter months in the Northern Hemisphere have previously been observed (77).

In WA, peaks observed towards the end of reporting years may be suggestive of an annual, seasonal pattern of outbreaks. Antibiotic trend data published by the Pharmaceutical Benefits Scheme (PBS) shows a slight decline over the available data in recent years (2008 onwards) in defined daily doses (DDD) per 1,000 people per day (Figure 2), which suggests that overall increases in antimicrobial prescribing are likely not a leading driver in increased CDI rates in Australia during this time. Local data should be reviewed to test for correlation between prescribing figures and the rate of CA-CDI in WA.

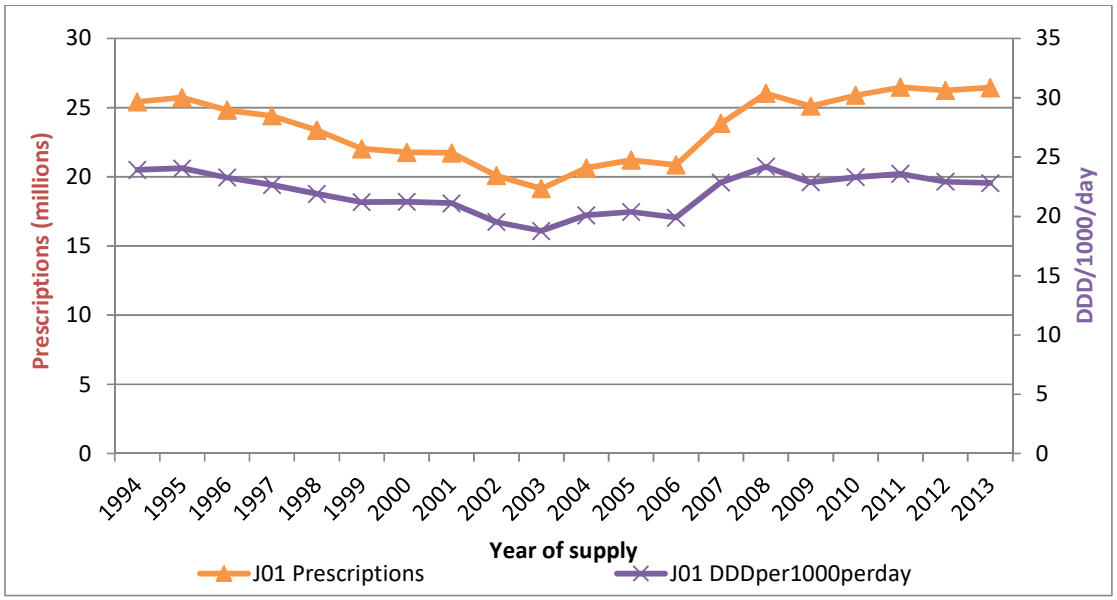


Figure 2. Quantity of systemic antibiotics dispensed under the PBS/RPBS, Australia, 1994 - 2013 (Source: Pharmaceutical Benefits Scheme (124)).

8 SIGNIFICANT STRAINS OF *CLOSTRIDIUM DIFFICILE*

Comparative phylogenomics (consisting of whole-genome sequence comparisons and Bayesian phylogenomics) has been used to group *C. difficile* into distinct genetic groups or clades (125). Based on genomic data there are 5 clades (or groups) of *C. difficile* circulating in the world (126). Within these clades, there are over 400 identified strains, with only toxin-producing strains within a clade capable of causing disease (14). The lack of standard nomenclature and typing systems used to catalogue *C. difficile* strains has been highlighted as a complicating factor in understanding prominent strains of *C. difficile* in human and animal populations (39). Polymerase chain reaction (PCR) ribotyping, restriction endonuclease analysis (REA), pulsed-field gel electrophoresis (PGFE) and toxinotyping are all methods used globally, with some regional methodological preferences (39), however it is not always clear, especially with 'new' strains and in the absence of a reference sample, if strains documented in the published literature are unique. PCR ribotyping is arguably the most common method used internationally, and is the method used by the reference laboratory in WA.

8.1 SIGNIFICANT STRAINS IN HUMAN DISEASE– GLOBAL PERSPECTIVE

Literature from Europe and North America describes increases in both rates and severity of CDI in the last 10–15 years (71, 127-130). These increases have attracted attention from the scientific community, with several prominent researchers seeking to explain these increases through epidemiological studies. An important outcome from this research has been the documentation of a reportedly 'hypervirulent' strain, the BI/NAP1/027 strain, particularly in the Quebec region of Canada (26, 131, 132). The strain is known by these various names because of the methodology used for specific strain identification; BI by restriction endonuclease analysis, North American pulse field gel electrophoresis type 1 (NAP1) or polymerase chain reaction (PCR) ribotype UK 027 (130).

This epidemic strain is often associated with more severe disease and increased morbidity and mortality, and is often refractory to treatment with demonstrated resistance to fluoroquinolones (133). There are serious public health implications should an outbreak of this strain, or one with similar characteristics, occur in a healthcare facility (134, 135). UK 027 is characterised by the production of greater amounts of TcdA and TcdB, and an additional binary

toxin. Although there have been three separate known introductions of UK 027 into Australia (once in 2008 (136) and twice in 2010 (137)), the strain has not flourished in this region, likely due to Australia's more conservative policies regarding prescribing of fluoroquinolones.

This particular ribotype is possibly the most significant due to the well-documented impact on patient outcomes and health systems. Sixteen European countries reported cases of UK 027 following increased surveillance, with incidence varying from sporadic and important individual cases to widespread outbreaks (138). Based on analysis from the *Clostridium difficile* ribotyping network (CDRN) data (England), UK 027 has been significantly associated with mortality (OR = 1.9) (38). A 19% decrease in the prevalence of UK 027 in England between 2007 and 2008 resulted in a 29% decrease in CDI-related deaths over the same period (38).

The emergence of UK 027 has been widely documented in North America, with all Canadian provinces and at least 40 states in the USA reporting cases (38). This again was punctuated by an increase in the incidence and severity of cases (139). A large US-based study also found UK 027 to be the most commonly isolated type among CA cases, demonstrating the potential for this strain to cause disease both inside and outside of the hospital setting (37).

It is important to note that severe disease is not attributable to UK 027 in all cases; some European counties have reported lower mortality than observed in North America, and not all North American studies have demonstrated increased severity and mortality as compared with non-UK 027 strains (38). There is also evidence that UK 027 has a two-fold higher MIC_{90} to metronidazole (a common first treatment option), although the clinical significance of this finding is not clear (140). Piecing together the factors that result in poorer outcomes in patients with different strains of CDI is necessary to develop a suitable tool for determining prognosis and course of treatment.

Differential prevalent ribotypes have been reported among CA and HA infections (141, 142), which could be due to different antibiotic use between groups, or different exposure sources. Overall, ribotypes UK 014/020 and UK 002 are among the most prevalent in Europe (70). Data from the UK also show the rapidly evolving nature of circulating *C. difficile* strains, with 45% of cases within this geographical region demonstrating genetic distinction from other cases over a three-year period (143).

8.2 SIGNIFICANT STRAINS IN HUMAN DISEASE - AUSTRALIA

Molecular epidemiology undertaken at the PathWest/UWA laboratory in WA has identified a new strain of *C. difficile* (UK 244) that is related to the epidemic UK 027 strain seen in North America and Europe. To date UK 244 CDI cases have been found in several Australian states (144, 145) and, following whole genome sequencing at Oxford University in the UK, the strains from various states were found to be clonal (identical) suggesting a common source (146). Furthermore, the majority of CDI cases caused by UK 244 appear to be CA-CDI and mainly detected in patients who presented to hospital emergency departments rather than from hospital inpatients (146). An Australian study of UK 244 infections found a 13-fold increase in mortality compared to non-UK 244 cases (145). A recent study found that some US strains of *C. difficile* previously identified as UK 027 were new ribotypes that had evolved from UK 027, including UK 244 (147). Both of these ribotypes reside in clade 2, suggesting that UK 244 may have been introduced to Australia from the USA.

8.3 SIGNIFICANT STRAINS IN ANIMALS

C. difficile has also been isolated from a number of non-human animals. Ribotyping has previously shown fewer ribotypes isolated from animal samples than human samples (40, 148), however recently more substantial diversity among animal ribotypes has been reported (149). Several ribotypes of *C. difficile* have been epidemiologically linked to production animals outside of Australia, particularly UK 078 (150, 151). This binary-toxin producing strain is the predominant strain found in porcine and bovine isolates, responsible for >80% of infections in some surveys (150). UK 078 is significant as it is associated with a growing proportion of human disease (151-153), particularly CA-CDI (154).

Investigations into the relatedness of human and animal isolates if UK 078 have revealed that human and porcine strains are genetically related, which may reflect a common source (152, 155). Further genetic study into human and animal isolates suggests several other common strains (148). An important distinction between human and animal strains is the high prevalence of binary-toxin producing strains among animal populations; with Rupnik and colleagues showing approximately 40% of horse isolates, 80% of pig isolates and 100% of calf isolates are binary toxin positive (42).

8.4 SUMMARY OF EVIDENCE

There are significant strains of *C. difficile* circulating in both hospital and community settings. Furthermore, animal populations carry ribotypes causing significant disease in production animals, including a majority of strains that produce binary toxin. These animal strains comprise an increasing proportion of disease-causing strains among human populations. There is also evidence that emerging strains can appear rapidly within an established population. The literature also describes the potential benefits of prospective strain typing to further explore genetic diversity and likely sources of transmission in the community (61).

From the data available, it is clear that different geographical areas have different strains of interest and public health relevance. While strains that are capable of causing severe disease in the international context are noteworthy, local epidemiological evidence is necessary to determine which strains pose particular public health concerns in the local context. It is essential in the case of imported food products that prevalent strains in animal populations from exporting nations are monitored, however local strains that appear *de novo* and are not linked to international travellers or imports require ongoing surveillance.

9 LABORATORY TESTING FOR *CLOSTRIDIUM DIFFICILE* INFECTION

Different diagnostic techniques by laboratories have been highlighted as an area of concern when estimates of CDI prevalence are being conducted across multiple sites or countries (156). Several diagnostic tests are available for CDI. There are advantages and disadvantages of various testing methods, often with trade-offs between sensitivity, turn-around time and costs. The available tests can loosely be grouped into those that detect the organism, those that detect the toxin, and those that determine if the organism is capable of producing toxin by detecting toxin genes. A summary of the various testing methods is shown in Table 2.

The ability of different tests to detect different targets will clearly limit the ability of some studies to correctly determine which patients have active disease, which are asymptomatically colonised with toxigenic strains, and which are harboring non-toxigenic strains. To account for variable positive predictive values (PPVs) in populations with low prevalence, both ESCMID and the CDC recommend a two-step testing process, with a sensitive screening test as the first test

(157, 158). No single test is suitable under all circumstances, and the outcome (e.g. diagnosis of infection, public health surveillance) must be taken into account when performing diagnostic testing.

Table 2. Diagnostic methods for the detection of *C. difficile* (adapted from Rupnik et al. (2009) (60)).

Diagnostic method	Advantages	Disadvantages
Culture	• Sensitive	• Does not differentiate toxigenic and non-toxigenic strains • Slow
Antigen detection (glutamate dehydrogenase [GDH])	• High negative predictive value • Fast	• Non-specific (requires supplementary testing)
Cytotoxin assay	• Sensitive • High specificity for infection	• Slow
Enzyme immunoassay	• Fast	• Low positive predictive value, particularly in population with low prevalence
Membrane assays	• Fast	• Low positive predictive value, particularly in population with low prevalence
Real-time PCR	• Rapid	• Uncertain specificity for infection
Cytotoxigenic culture	• High sensitivity	• Uncertain specificity for infection • Slow
Toxin B gene detection	• High sensitivity • Fast	• Uncertain specificity for infection • High cost

10 TRANSMISSION OF *CLOSTRIDIUM DIFFICILE* IN THE COMMUNITY

As *C. difficile* has traditionally been treated as a nosocomial infection, much of the literature around transmission focuses on the hospital environment, extending out into long term and similar care facilities. The recognition of this organism as a cause of diarrhoeal illness in the

community has driven more recent research efforts towards understanding the acquisition and transmission of this disease outside of the hospital setting.

In 2010, Otten and colleagues developed a transmission model of CA-CDI as an initial step towards risk assessment of this pathogen in the community (159). The model developed contains eight infection states; susceptible, gastrointestinal exposure, colonized, diseased, deceased, clinically resolved colonized, relapse diseased, and cleared, with directional transfers between the states (Figure 3). The model represents a complex relationship between epidemiological states in which a susceptible individual, lacking protective factors, becomes exposed to the organism, which in some cases leads to a diseased state. Sources of exposure and risk factors for developing disease once exposed are key areas for public health intervention in order to prevent disease and halt transmission.

Potential sources of exposure in the community are discussed in greater details below, but can be categorised broadly into consumption (ingestion of spores from a contaminated food product), person-to-person contact (transmission from another infected or colonised person), animal-to-person contact (transmission from an infected or colonised domestic or wild animal), and environment-to-person (ingestion of spores after exposure to a contaminated environmental source).

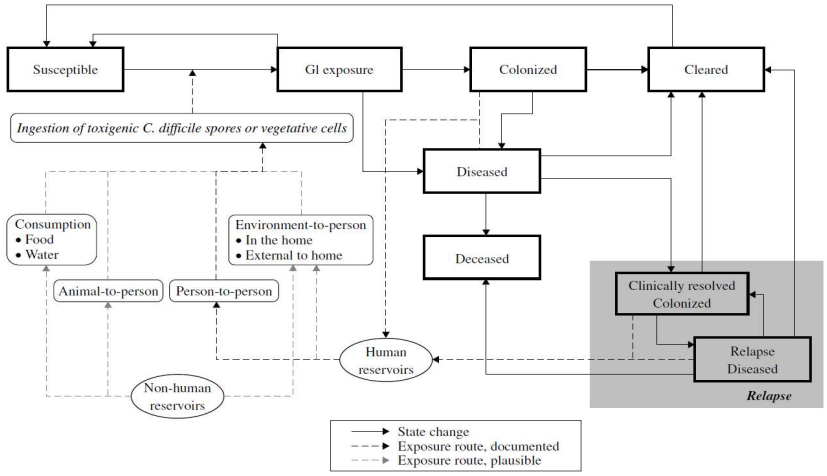


Figure 3. Transmission model of community-associated *C. difficile* (Source: Otten, 2010 (159)).

11 *CLOSTRIDIUM DIFFICILE* IN THE ENVIRONMENT

If humans and animals are both developing CDI, a common environmental source (e.g. contaminated soil or water) is a potential reservoir. *C. difficile* from the environment may cause disease after entering the household environment via contaminated fomites, or via ingestion at the source. Household prevalence sampling has been undertaken by numerous authors to determine common the magnitude of contamination, and common sites. Environmental sampling, including drinking and natural water sources, has also been undertaken by a number of researchers. Although there has not been a large amount of work dedicated to environmental sampling, the evidence available is summarised below.

11.1 *C. DIFFICILE* IN HOUSEHOLDS

Weese and colleagues undertook a sampling study which included looking at both pets (dogs and cats) and the household environment in 84 homes in Canada (160). A total prevalence of 5.3% was reported from household samples, with the most common areas for isolation including toilet and kitchen areas (160). While pet bowls and eating areas were also common sites of isolation, only 15% of households with *C. difficile* detected also had a pet which tested positive, indicating that actively shedding pets were not the source of environmental contamination in the majority of cases. Importantly, UK 027 was the most common environmental ribotype isolated in this study.

Alam and colleagues found a prevalence of 32.3% in 127 environmental samples from 30 households in Houston, Texas (161). The samples included soles of shoes, swabs from bathrooms and other surfaces, and household dust samples, and all contained toxigenic strains, suggesting a potential reservoir for toxigenic *C. difficile* infection in the community. This study did not collect demographic information such as recent hospitalisation history or occupational history from the residents, which may have provided further insight into likely sources of introduction into the household environment.

11.2 *C. DIFFICILE* IN WATER

In 2013, a Finnish study documented *C. difficile* contamination of public tap water. Treated sewage effluent was introduced to drinking water in local town via the wastewater treatment

plant, which resulted in a community-based gastroenteritis outbreak. A sample of symptomatic patients was tested for CDI, along with environmental sampling of contaminated water and sewage effluent. The study found indistinguishable ribotypes cultured from symptomatic patients and contaminated tap water. Without more discriminatory methods, it was not possible to conclusively link the contamination event to cases in the community, however contaminated drinking water may be a potential reservoir, based on these findings (162).

One other study on drinking water reported on a markedly different environment. Simango tested household water samples in rural Zimbabwe, including household stored water and borehole/well water (163), and found toxigenic *C. difficile* in 14/234 (6.0%) of water samples, in the absence of any documented contamination event. Presence of animals living close to homes and water sources in this study could have been the source of water contamination (163).

Wastewater treatment plants (WWTPs) as potential reservoirs of CA-CDI have also been investigated. In a recent Iranian study, *C. difficile* was found in digested sludge samples and waste stablisation ponds (164). A limitation in this study, however, was the lack of ribotyping data available, without which an epidemiological link to CDI cases in the community could not be drawn. Romano and colleagues also recovered *C. difficile* from 18/18 (100%) of both treated and untreated WWTP samples in Switzerland (165). In Canada, a WWTP study also found a high prevalence of *C. difficile* among raw sludge (108/117. 92%) and digested sludge (106/110. 96%) samples, in addition to river sediments (25/64, 39%), demonstrating environmental dissemination of the organism via WWTPs (166). A significant finding of both the Swiss and Canadian studies was the overlap in common strains isolated from water samples and cases in the local human population.

The use of stablised domestic sewage in agriculture is widespread, with approximately 60% of these biosolids used for this application in the United States (167). In Australia, piggery effluent is treated to remove pathogens, and subsequently used to water farm land (63). Residual spores that survive this process may contaminate produce or infect animal stock using this land (63), and *C. difficile* has been isolated from biosolids previously in the United States (167).

Al Saif and Brazier published one of the most comprehensive environmental studies (168), albeit confined to a single geographic area (South Wales) and nearly 20 years ago. Water sampled in this study included rivers, lakes, drainage channels, the ocean, pools and tap water. *C. difficile* was isolated from all water samples with varying prevalence between 5.5% (tap water) and 87.5% (rivers), demonstrating wide dissemination of this organism in aquatic environments. A high prevalence (68%) of *C. difficile* in rivers has also been reported Slovenia (169), with overlap between environmental and human ribotypes.

11.3 *C. DIFFICILE* IN SOIL

As noted by Levett some 30 years ago, the literature surrounding environmental isolation of *C. difficile* is primarily focused on soil or peat (170). As far back as 1955, *C. difficile* was isolated from environmental soil samples in Korea (171). Al Saif and Brazier also tested soil samples as part of their large environmental study in Wales, and 22/104 (21%) of soil samples, taken from random sites near Cardiff were positive for *C. difficile* (168).

Sampling of soil from animal housing areas yielding positive results has previously been conducted (172, 173), although these findings are likely representative of shedding by colonised or symptomatic animals, as outlined in section 12. Simango conducted a study in rural Zimbabwe, investigating *C. difficile* in the environment, which found a high prevalence (54/246, 37.0%) of toxigenic strains around households tested (163). This was attributed to shedding by domestic chickens, again highlighting the potential for animals to contaminate their surrounding environments.

11.4 SUMMARY OF EVIDENCE

C. difficile in households, even excluding those of known index cases, appears to be relatively common. Positive findings on boots/shoes suggests an introduction from contaminated soil from outside the home, whereas presence on kitchen surfaces and refrigerators may indicate transfer from food products. If the data collected to date are representative of a typical household, people may expect to come into contact with *C. difficile* in their home environments on a regular basis.

The significance of finding low absolute counts of *C. difficile* in environmental samples is unclear – while the 'infectious dose' remains unknown, detection in any number of sources

may or may not be of public health significance (40). It is possible that people are coming into regular contact with low levels of *C. difficile* either inside or outside of their home, which are rarely capable of causing disease. Discrepant individual practices around hand hygiene, particularly through hand washing before eating, may also impact on the significance of environmental contamination and the likelihood of disease development.

The reports on *C. difficile* outside of the hospital environment demonstrate that this organism is ubiquitous in natural settings, including soils and waterways, and inevitably present in environments where human faecal matter is treated, i.e. WWTPs. In addition, treated animal effluent used to water agricultural products, or manure used for fertilizer, are other potential environmental sources, with treatment practices not sufficient to eliminate the spores from the end product. Thus, there are large numbers of potential environmental sources for CA-CDI. Local sampling with highly discriminatory typing methods may help to narrow down potential local environmental sources, including assessment of the ability of water treatment processes to remove spores, and prevent further spread in the environment.

12 *CLOSTRIDIUM DIFFICILE* IN ANIMALS

One potential source of *C. difficile* transmission outside of the hospital environment is via animals. Although many clostridial organisms cause disease in both humans and animals, these have not traditionally been considered zoonotic agents (174). Molecular studies have, however, demonstrated common *C. difficile* isolates in production animals, companion animals and humans (150-152, 175-177), particularly UK 078. As in humans, diarrhoea and *C. difficile* colitis has long been associated with antimicrobial therapy in animals (178). Outbreaks of CDI have been reported at veterinary hospitals, affecting hospitalised dogs (179).

This opens the possibility of *C. difficile* sources for human infection in production (food) animals, companion animals and native/wild animals. As in neonatal humans, young animals are colonised by *C. difficile*, which is displaced as the microflora matures (180). Animals are also susceptible to CDI, which can result in severe diarrhoeal disease and mortality among herds with widespread infection. Unlike humans, neonatal animals (especially piglets) develop

severe disease including diarrhoea, respiratory distress, and demonstrated high levels of morbidity and mortality (180-182). Table 3 summarises the available evidence of *C. difficile* in animals. Animals were assumed to be healthy (i.e. lacking symptoms of gastrointestinal disease) unless the study explicitly stated otherwise. Common strains (if available) are included only when they corresponded to an international collection (or reference) number, in order to be able to compare accurately across studies.

Table 3. Summary of studies reporting prevalence of *C. difficile* in animals.

Year of publication	Country	Animal	Disease status	Most prevalent ribotype(s)/ toxinotype(s)	Prevalence n/N (%)	Ref.
Production (food) animals						
North America						
2002	USA	Piglets	Diarrhoeic	NA	29/100 (29.0%)	(182)
2006	Canada	Calves	Healthy	UK017, UK078, UK027	20/134 (14.9%)	(183)
2006	Canada	Calves	Diarrhoeic	UK017, UK078, UK027	11/144 (7.6%)	(183)
2008	USA	Calves	Healthy	UK078	19/53 (35.9%)	(184)
2008	USA	Calves	Diarrhoeic	UK078	94/253 (37.2%)	(184)
2009	USA	Swine	Healthy	Toxinotype V	131/1008 (13.0%)	(185)
2010	USA	Neonatal piglets	Diarrhoeic	Toxinotype V	241/513 (50.0%)	(186)
2010	Canada	Piglets	Healthy	UK078	116/121 (95.8%)	(187)
2011	Canada	Calves	Healthy	UK078	122/200 (61.0%)	(188)
2011	Canada	Slaughter-aged pigs	Healthy	UK078	30/436 (6.9%)	(189)
2011	USA	Swine	Healthy	Toxinotype V	252/2,963 (8.6%)	(190)
2011	USA	Swine	Healthy	NA	55/345 (15.9%)	(191)
2011	USA	Dairy cattle	Healthy	NA	32/1,325 (2.4%)	(191)
2011	USA	Beef cattle	Healthy	NA	188/2,965 (6.3%)	(191)
2011	USA	Steers	Healthy	NA	24/186 (12.9%)	(192)
2012	Canada	Cattle	Healthy	UK078	36/874 (4.1%)	(193)
2013	Canada	Slaughter pigs (manure)	Healthy	UK078	16/20 (80.0%)	(194)
2013	Canada	Pigs	Healthy	UK078	68/225 (30.2%)	(194)
2014	USA	Poultry	Healthy	NA	1/340 (0.3%)	(195)
2014	USA	Swine	Healthy	NA	1/150 (0.67%)	(195)
2014	USA	Cattle	Healthy	UK078	2/330 (0.61%)	(195)
Europe						
2008	Slovenia	Chickens	Healthy	UK023	30/61 (62.3%)	(196)
2008	Slovenia	Piglets	Diarrhoeic	Toxinotypes V, 0	133/257 (51.8%)	(176)

2008	Slovenia	Calves	Diarrhoeic	UK033	1/56 (1.8%)	(176)
2009	Austria	Pigs	Healthy	UK126	2/61 (3.3%)	(41)
2009	Austria	Chickens	Healthy	UK420, UK014/0	3/59 (5.0%)	(41)
2009	Austria	Cows	Healthy	UK001, UK446	3/67 (4.5%)	(41)
2009	Spain	Piglets	Healthy	NA	82/287 (28.6%)	(197)
2009	Spain	Piglets	Diarrhoeic	NA	58/254 (22.8%)	(197)
2009	Slovenia	Pigs	Healthy and diarrhoeic	UK006, UK029	247/485 (50.9%)	(177)
2009	Slovenia	Calves	Healthy and diarrhoeic	UK022, UK077	4/42 (9.5%)	(177)
2009	Slovenia	Horses	Healthy and diarrhoeic	UK033	1/20 (5.0%)	(177)
2011	The Netherlands	Piglets	Healthy	UK078	71/71 (100.0%)	(198)
2011	The Netherlands	Slaughter pigs	Healthy	UK078	58/677 (8.6%)	(199)
2012	Switzerland	Calves	Healthy	UK033, UK003, UK066. UK070	6/47 (12.7%)	(200)
2012	Switzerland	Cows	Healthy	UK137	1/63 (1.5%)	(200)
2012	Switzerland	Goats	Healthy	UK001	3/40 (7.5%)	(200)
2012	Belgium	Slaughter cattle	Healthy	UK002, UK014, UK081, UK087	14/202 (6.9%)	(201)
2012	Belgium	Calves	Healthy	UK078	4/18 (22.2%)	(201)
2012	Belgium	Slaughter pigs	Healthy	-	0/194 (0.0%)	(201)
2012	Belgium	Piglets	Healthy	UK078, UK002	18/23 (78.3%)	(201)
2012	The Netherlands	Pigs	Healthy	-	0/100 (0.0%)	(202)
2012	The Netherlands	Pigs	Diarrhoeic	UK078	9/36 (25.0%)	(202)
2012	The Netherlands	Cattle	Healthy	UK012	7/200 (3.5%)	(202)
2012	The Netherlands	Cattle	Diarrhoeic	-	0/5 (0.0%)	(202)
2012	The Netherlands	Sheep	Diarrhoeic	UK015, UK097	2/11 (18.2%)	(202)
2012	The Netherlands	Poultry	Healthy	UK014, UK010	5/100 (5.0%)	(202)
2012	The Netherlands	Poultry	Diarrhoeic	UK014, UK010	2/21 (9.5%)	(202)
2012	Czech Republic	Piglets	Healthy and diarrhoeic	Toxinotype 0	19/30 (63.3%)	(203)
2013	Belgium	Cattle (intestinal contents)	Healthy	UK078, UK014, UK029	10/101 (9.9%)	(204)
2013	Belgium	Pigs (intestinal contents)	Healthy	UK 078	1/100 (1.0%)	(204)
2013	Belgium	Cattle (carcasses)	Healthy	UK023, UK015	8/101 (7.9%)	(204)
2013	Belgium	Pigs (carcasses)	Healthy	UK014, UK081	7/100 (7.0%)	(204)

2013	Spain	Piglets (free range)	Healthy	UK078	41/160 (25.6%)	(205)
2013	Germany	Piglets	Healthy	UK078, UK126	39/51 (76.5%)	(206)
2013	Germany	Piglets	Diarrhoeic	UK078, UK126	108/150 (72.0%)	(206)
2013	Germany	Calves	Diarrhoeic	UK033, UK078, UK045	176/999 (17.6%)	(207)
2013	Sweden	Piglets	Healthy	UK046	45/67 (67.2%)	(208)
2014	Slovenia	Goats	Healthy	UK045, UK014/020, UK010	10/109 (9.2%)	(209)
2014	Slovenia	Sheep	Healthy and diarrhoeic	UK056	6/105 (5.7%)	(209)
2015	Italy	Veal calves	Healthy and diarrhoeic	UK078, UK012, UK126	87/420 (20.7%)	(210)
Other						
2006	Zimbabwe	Chicken	Healthy	NA	31/115 (27.0%)	(163)
2006	Zimbabwe	Cattle	Healthy	NA	3/59 (5.1%)	(163)
2006	Zimbabwe	Goats	Healthy	NA	5/56 (8.9%)	(163)
2006	Zimbabwe	Ducks	Healthy	NA	0/4 (0.0%)	(163)
2006	Zimbabwe	Turkeys	Healthy	NA	0/3 (0.0%)	(163)
2006	Zimbabwe	Rabbits	Healthy	NA	2/25 (8.0%)	(163)
2006	Zimbabwe	Pigeons	Healthy	NA	0/8 (0.0%)	(163)
2006	Zimbabwe	Guinea Pigs	Healthy	NA	0/5 (0.0%	(163)
2006	Zimbabwe	Pigs	Healthy	NA	1/1 (100.0%)	(163)
2008	Zimbabwe	Chickens	Healthy	NA	29/100 (29.0%)	(172)
2013	Australia	Sheep	Healthy	-	1/156 (0.6%)	(211)
2013	Australia	Lamb	Healthy	UK101, UK056, UK137	14/215 (6.5%)	(211)
2013	Australia	Cattle	Healthy	UK127, UK033, UK056, UK126	209/975 (22.7%)	(212)
2014	Iran	Calves	Healthy	NA	90/150 (60.0%)	(213)
2015	Korea	Slaughter pigs	Healthy	UK078	2/659 (0.3%)	(214)
2015	Australia	Piglets	Healthy	UK014, UK033, UK237	90/150 (60.0%)	(215)
Companion animals						
1987	USA	Foals	Healthy	-	0/18 (0.0%)	(216)
1987	USA	Foals	Diarrhoeic	NA	27/43 (62.7%)	(216)
1987	USA	Horses	Healthy	-	0/62 (0.0%)	(216)
2003	Canada	Dogs (hospitalised)	Diarrhoeic	NA	48/93 (51.6%)	(179)

2006	Canada	Dogs	Healthy	NA	58/102 (56.9%)	(217)
2008	Canada	Dogs (hospitalised)	Healthy and diarrhoeic	NA	70/360 (19.4%)	(218)
2008	Canada	Cats (hospitalised)	Healthy and diarrhoeic	NA	3/42 (7.1%)	(218)
2010	Canada	Cats	Healthy	Toxinotype 0	3/14 (21.4%)	(160)
2010	Canada	Dogs	Healthy	Toxinotype 0, IX	14/139 (10.1%)	(160)
2011	Canada	Horses	Healthy	UK001, UK027	52/742 (7.0%)	(219)
2012	Canada	Horses	Healthy	UK078. UK001	10/25 (40.0%)	(220)
2012	Canada	Horses	Healthy	UK078, UK001	14/135 (10.3%)	(221)
2012	Germany	Dogs	Healthy	UK010	9/165 (5.5%)	(222)
2012	Germany	Cats	Healthy	UK014/020	5/135 (3.7%)	(222)
2012	The Netherlands	Dogs	Diarrhoeic	UK010, UK014	29/116 (25.0%)	(202)
2012	The Netherlands	Cats	Diarrhoeic	UK010, UK014	18/115 (15.7%)	(202)
2012	The Netherlands	Horses	Diarrhoeic	UK010, UK014	24/135 (17.8%)	(202)
2013	Sweden	Dogs	Healthy	UK009. UK010	2/50 (4.0%)	(223)
2013	Sweden	Dogs	Diarrhoeic	UK014	2/20 (10.0%)	(223)
2014	USA	Horses	Healthy	UK078, UK027	11/55 (20.0%)	(195)
2014	Belgium	Horses (hospitalised)	GI disorder	NA	5/41 (12.2%)	(224)
2014	Belgium	Horses (hospitalised)	No GI disorder	UK014	5/32 (15.6%)	(224)
2015	Spain	Puppies	Healthy	UK056, UK010	14/18 (77.7%)	(225)
Native/wild animals						
2011	USA	Feral pigs	Healthy	Toxinotypes V, 0	7/161 (4.4%)	(226)
2011	Slovenia	Wild passerine birds	Healthy	-	0/98 (0.0%)	(227)
2012	Canada	Wild mammals	Healthy	Toxinotypes 0, II, IV, XIII	5/109 (4.6%)	(228)
2012	Canada	Wild raccoons	Healthy	-	0/216 (0.0%)	(228)
2014	Spain	Zoo animals	Healthy	UK078	7/199 (3.5%)	(229)
2014	Brazil	Wild carnivore species	Healthy and diarrhoeic	UK053, UK046	2/34 (5.9%)	(230)
2014	Brazil	Coati	Healthy	UK053, UK014/020, UK106	3/46 (6.5%)	(231)
2014	Canada	Wild urban rats	Healthy	UK001, UK078	95/724 (13.1%)	(232)
2014	Slovenia	Barn swallows	Healthy	UK078, UK002, UK014	7/175 (4.0%)	(233)

NA – Not available

12.1 PRODUCTION ANIMALS

C. difficile infection is of particular significance in production animals, with the majority of animal research conducted in this group. As shown in Table 3, much of this research has centered on cattle, swine and poultry. Studies also have been conducted on both healthy and diarrhoeic animals, although most were prevalence surveys of healthy animals.

In North America, CDI is considered the most significant cause of neonatal diarrhoea in swine (234). Production animals have historically been given broad spectrum antimicrobials mixed in with feed as a prophylactic measure for infectious disease, and as growth promoters (235, 236). The use of antimicrobials alters the microflora in livestock as it does in humans, leaving them susceptible to CDI. Once the organism is introduced to a herd, a large number of susceptible animals living in close quarters can rapidly become infected. CDI in production animals is of concern to industry due to potential growth delays in infected herds (237), loss of stocks (238), and potential contamination of meat and dairy products, which may damage consumer confidence. Piglets can also become colonised within an hour of birth, in the absence on antimicrobial treatment (198).

Kiss and Bilkei demonstrated postpartum mortality from CDI in sows receiving antimicrobial therapy for mastitis metritis agalactia, and noted the negative impacts on economic factors (such as replacement and production costs) and employee morale, along with increased concerns about animal welfare (181). The increase in postpartum sow mortality was observed in the absence of increase neonatal piglet deaths from CDI at the same farms (181). This, combined with a subsequent reduction of mortality following withdrawal of antimicrobial administration, indicates prior colonisation in sows with *C. difficile,* with infection precipitated by antimicrobial treatment, rather than an increase in a common environmental reservoir leading to increased disease (181).

Norman and colleagues found significant variation ($p<0.001$) in the prevalence of *C. difficile* in at integrated swine operation, with the prevalence among suckling piglets (61/122, 50.0%) higher than lactating sows (34/143, 23.8%) and breeding boars and sows (7/180, 3.9%) (185). Others have also found a significant decrease in prevalence among piglets over time (187, 194). Different carriage rates have also been reported among cattle (183, 188, 210, 212) and

chickens (196) of different ages. These data suggest that prevalence studies in animals need to take age at testing into account, as rates will vary markedly among animals of different ages.

Aside from detection in production animals and the environment in which they are housed, research has been conducted into the contamination of the surrounding environment. Keessen and colleagues demonstrated widespread aerial dissemination of *C. difficile* on a pig farm, with personnel activity contributing to an increase in numbers (239). While the consequences of these findings for human health are not clear, detection of *C. difficile* occurred 20 metres from the facility which raises the potential for wider contamination of the surrounding environment.

Common strains are reported in both animal populations and human isolates within the same geographical region (201, 204, 219). Common strains being found in both humans and production animals leaves open the possibility that transmission is occurring from human to animal rather than the alternative. A small study conducted by Keessen and colleagues among pig farm workers found daily to weekly contact with pigs versus monthly to less than yearly contact was significantly associated with an intestinal presence of *C. difficile* ($p = 0.003$) (240). These data support the alternative hypothesis, with workers more likely to be colonised via frequent contact with the animals and their environment.

There is a suggestion that there may be seasonal influences on the carriage of *C. difficile* in animals, with a United States study finding relatively low prevalence during a summer sampling period (195). In addition, Thitaram and colleagues also demonstrated different faecal isolation methodologies yielded discrepant prevalence among different species (191). These data, combined with evidence from other authors about the influence of animal age on prevalence, strongly suggest that numerous factors may influence outcomes in prevalence studies, making valid comparison across different studies particularly difficult.

12.2 COMPANION ANIMALS

In a study investigating *C. difficile* in household pets, Weese and colleagues detected similar ribotypes among both humans and companion animals, suggestive of interspecies transmission (160). The results of this study, however, leave open the possibility that dogs and their owners may be being colonised by a common environmental source and, further, the association between detection of *C. difficile* in dogs and immunocompromised owners leaves

open the potential that transmission is occurring from human to animal, rather than the alternative.

A high prevalence of *C. difficile* carriage in healthy dogs was found by Lefebvre and colleagues (56.9%), these findings are significant as these dogs were selected because they had visited inpatients in hospital as part of a community programme (217). Whether this high rate of colonisation was due to contact with the hospital environment or with hospitalised patients in unclear, however it underscores the potential for companion animals to become colonised after interaction with humans and the environment. These findings were further supported by Weese and colleagues, who reported dogs living with an immunocompromised person to be 7.9 times more likely to be colonised with *C. difficile* (160).

C. difficile has long been known to affect horses, with diarrhoeal illness reportedly the most common clinical abnormality in young foals (216). Fatal colitis associated with CDI has been reported in horses (237, 241), and *C. difficile* can cause disease in foals without prior antimicrobial exposure (216).

12.3 NATIVE/WILD ANIMALS

Several studies have explored the prevalence of *C. difficile* in wild animal populations (Table 3). The presence of *C. difficile* in these populations confirms the presence of *C. difficile* in the environment outside of animal healthcare and housing facilities. Brazilian studies have found ribotypes in wild animals that have also been reported in human cases (UK 014/020 and UK 106), the latter ribotype considered uncommon in other countries (231).

Jardine and colleagues isolated *C. difficile* from wild raccoons found on farms in Canada (4/52, 7.7%), but in contrast found no *C. difficile* in raccoons living on the grounds of a zoo (0/219, 0.0%), although these studies were conducted 3 years apart (228). These findings are, however, still of interest, as both wild raccoon populations presumably had contact with the environment surrounding animal enclosures, however only those with close proximity to production animal farms (beef, swine and dairy), suggesting a possible link between domesticated and wild animal species.

While not strictly 'wild' animals, an outbreak was reported among five Asian elephants at a zoo in Denmark (242). This resulted in the deaths of two animals from enterocolitis, with a third animal suffering serious disease (242). *C. difficile* was identified as the most likely cause of this outbreak and while no prior antimicrobials use was reported, in this event the consumption of a large amount of broccoli was purported to be a potential risk factor due to the antimicrobial effect of sulforaphane (242). This unique case again demonstrates the potential for CDI to occur in animal populations in the absence of antimicrobial exposure; the source of spores in this case was presumably environmental or foodborne contamination.

12.4 SUMMARY OF EVIDENCE

Varying prevalence of *C. difficile* has been documented in both healthy and diseased animals. It has been noted that recovery methods may account for variation in prevalence across studies (243). The diseased status, age, and species of animal also influence the reported prevalence. Interestingly, some studies found a higher prevalence among healthy animals as compared to their diseased counterparts (197, 206). Depending on the level of mixing within populations, this may suggest a high overall carriage rate among healthy animals, or high susceptibility of exposure and carriage when symptomatic animals are in a herd.

The frequent detection of *C. difficile* in animal populations, and common isolates found in both humans and animals is suggestive that transmission between humans and animals is likely occurring, either directly or indirectly. Evidence that ribotypes that were prevalent among animals are now causing disease in increasing numbers in humans implies that transmission is occurring from animals to humans.

While animal-human or human-animal transmission is biologically plausible, a common source in the environment which allows transmission to both groups is an equally valid suggestion. The ubiquity of *C. difficile* in the environment supports this hypothesis. In the case of wild animal populations, it seems more plausible that presence in these animals is indicative of *C. difficile* present in the environment (e.g. via contamination from treated waste) rather than a route of human-animal transmission (233).

In order to establish *C. difficile* as a zoonotic organism, research must establish an epidemiological link between animals and humans who do not share a common environment

which may be a common source of infection. The most likely scenario in which this could occur is via the food chain, or via water systems contaminated with the excrement of colonised or infected animals. Monitoring the prevalence of *C. difficile* in animal populations is useful in veterinary medicine, and may further inform decisions on the use of antimicrobials in this population. Moreover, longitudinal analysis of predominant strains in animals is required to establish links with changes to strains causing disease in human populations.

13 *CLOSTRIDIUM DIFFICILE* IN FOOD

Leading on from the discovery of *C. difficile* in animals, and in particular production (food) animals, the possibility of transmission via the food chain has been examined by several authors. This includes transmission as a result of contamination of retail meat, vegetables that may have been indirectly contaminated via fertilization of the soil in which they are grown with the faeces of infected or colonised animals, and other ready-to-eat products. Table 4 summarises the available evidence of *C. difficile* contamination in food.

The first study to evaluate the presence of *C. difficile* food products for human consumption was documented in 1983, with a prevalence survey focused mainly on food served in hospitals which found no evidence of *C. difficile* contamination in any of the foods sampled (244). Early investigations of *C. difficile* as a foodborne pathogen in the published literature were patchy, with little reported until this century. There has been a sustained increase in studies published since 2009, which follows the increase of reports of *C. difficile* in animals (Figure 4).

Table 4. Summary of studies reporting prevalence of *C. difficile* in food samples.

Year of publication	Country	Food	Most prevalent ribotype(s)/ toxinotype(s)	Prevalence n/N (%)	Reference
Meat Products					
North America					
1983	USA	Fish, poultry, beef, lamb, pork	NA	0/15 (0.0%)	(244)
2007	Canada	Ground meat	UK077, UK014	12/60 (20.0%)	(245)
2009	USA	Cooked/uncooked beef	UK078, UK027	14/33 (42.4%)	(246)
2009	USA	Ground cooked/uncooked pork	UK078, UK027	19/46 (41.3%)	(246)
2009	USA	Turkey	UK078, UK027	4/9 (44.4%)	(246)
2009	Canada	Ground beef	UK078, UK027	14/115 (12.2%)	(247)
2009	Canada	Ground pork	UK078, UK027	14/115 (12.2%)	(247)
2009	Canada	Ground beef	UK027, UK077, UK014	10/149 (6.7%)	(248)
2009	Canada	Veal	UK027, UK077, UK014	3/65 (4.6%)	(248)
2010	Canada	Ground pork and pork chops	UK027	7/393 (1.8%)	(249)
2010	Canada	Chicken	UK078	26/203 (12.8%)	(250)
2011	USA	Chicken	Toxinotype V	4/32 (12.5%)	(251)
2011	USA	Retail meat	Toxinotype V	23/243 (9.5%)	(252)
2012	USA	Ground veal	Toxinotype V	4/50 (8.0%)	(253)
2012	USA	Ground beef	NA	0/617 (0.0%)	(254)
2012	USA	Pork chops	NA	0/265 (0.0%)	(254)
2012	USA	Ground turkey	NA	0/614 (0.0%)	(254)
2012	USA	Chicken breast	NA	0/259 (0.0%)	(254)
2012	USA	Pork sausage	UK078	13/103 (12.7%)	(255)
2012	Canada	Ground beef	NA	2/24 (8.3%)	(256)
2012	Canada	Ground pork	NA	1/24 (4.2%)	(256)
Europe					
2009	Sweden	Ground beef	NA	2/82 (2.4%)	(257)
2009	Austria	Pork	NA	0/27 (0.0%)	(41)

2009	Austria	Chicken	NA	0/6 (0.0%)	(41)
2010	France	Ground beef	UK012	2/105 (1.9%)	(258)
2010	France	Pork sausage	NA	0/59 (0/0%)	(258)
2010	Austria	Ground beef	NA	0/30 (0.0%)	(259)
2010	Austria	Ground beef/ground pork	UK053	3/70 (4.3%)	(259)
2010	Switzerland	Ground beef/ground pork	NA	0/46 (0.0%)	(260)
2011	The Netherlands	Pork	NA	0/63 (0.0%)	(261)
2011	The Netherlands	Beef/calf	NA	0/164 (0.0%)	(261)
2011	The Netherlands	Lamb	UK045	1/16 (6.3%)	(261)
2011	The Netherlands	Chicken	UK003	7/247 (2.7%)	(261)
2015	Belgium	Various meals (incl. pork sausage)	UK078	1/188 (0.5%)	(262)
Other					
1996	New Zealand	Spoiled meat (venison)	NA	1/4 (25.0%)	(263)
2012	Costa Rica	Beef	UK029	1/67 (1.5%)	(264)
2012	Costa Rica	Pork	UK029	2/66 (3.0%)	(264)
2012	Costa Rica	Poultry	UK029	1/67 (1.5%)	(264)
2013	Iran	Chicken	NA	19/120 (15.8%)	(265)
2014	Cote d'Ivoire	Cooked beef (kidney and flesh)	NA	49/395 (12.4%)	(266)
2014	Iran	Beef	UK078	2/121 (1.7%)	(267)
2014	Iran	Cow	-	1/106 (0.9%)	(267)
2014	Iran	Sheep	-	1/150 (0.7%)	(267)
2014	Iran	Buffalo	UK078	6/67 (9.0%)	(267)
2014	Iran	Goat	UK078	3/92 (3.3%0	(267)
2014	Iran	Camel	NA	0/124 (0.0%)	(267)
2014	Iran	Beef	UK078	3/54 (5.6%)	(268)
2014	Iran	Hamburgers (fresh and defrosted)	NA	4/56 (7.1%)	(268)
2014	Iran	Chopped beef	NA	1/35 (2.8%)	(269)
2014	Iran	Ground beef	NA	1/46 (2.1%)	(269)
2014	Iran	Chopped mutton	NA	2/55 (3.6%)	(269)
2014	Iran	Ground mutton	NA	4/64 (6.2%	(269)

Vegetables					
North America					
2010	Canada	Vegetables	UK078	5/111 (4.5%)	(249)
Europe					
1996	United Kingdom	Raw vegetables	NA	7/300 (2.3%)	(168)
2009	United Kingdom	Ready-to-eat salads	UK001, UK017	3/40 (7.5%)	(270)
2013	France	Ready-to-eat raw vegetables	UK001, UK014/020/077, UK015	3/104 (2.9%)	(271)
Other					
2014	Iran	Onions	NA	0/14 (0.0%)	(268)
Other products					
North America					
1983	USA	Beverages	NA	0/15 (0.0%)	(244)
1983	USA	Eggs and egg products	NA	0/10 (0.0%)	(244)
1983	USA	Fruits (raw and canned)	NA	0/15 (0.0%)	(244)
1983	USA	Dairy products	NA	0/10 (0.0%)	(244)
1983	USA	Starches (breads and cereals)	NA	0/20 (0.0%)	(244)
1983	USA	Vegetables (raw and cooked)	NA	0/30 (0.0%)	(244)
1983	USA	Desserts, jellies, soups	NA	0/15 (0.0%)	(244)
1983	USA	Raw fruits and vegetables	NA	0/20 (0.0%)	(244)
1983	USA	Raw milk products	NA	0/10 (0.0%)	(244)
1983	USA	Spices	NA	0/20 (0.0%)	(244)
2005	Canada	Dog and cat food	NA	1/25 (4.0%)	(272)
2011	Canada	Seafood and fish	UK078	5/119 (4.2%)	(273)
Europe					
1996	United Kingdom	Fish gut contents	NA	0/107 (0.0%)	(168)
2010	Austria	Raw milk	NA	0/50 (0.0%)	(259)
2012	Italy	Edible bivalve molluscs	UK014/020, UK078, UK010	26/53 (49.0%)	(274)
2015	Italy	Edible bivalve molluscs	UK078/126, UK010, UK001	36/925 (3.9%)	(275)

Other					
2013	Egypt	Infant formula	NA	16/100 (16.0%)	(276)
2014	Iran	Textured soy protein	NA	0/14 (0.0%)	(268)
2014	Iran	Seasoning	NA	0/17 (0.0%)	(268)

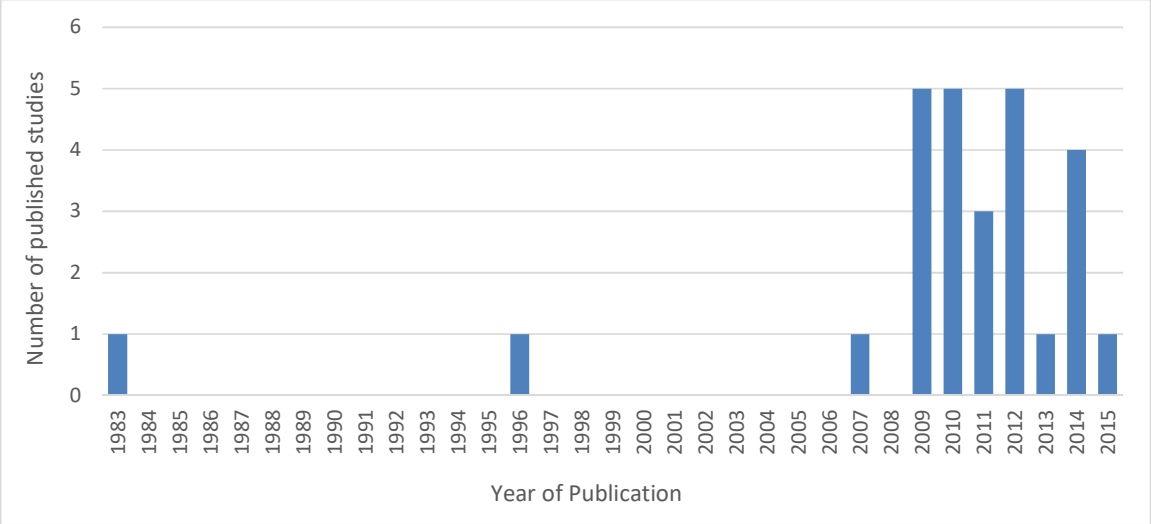


Figure 4 Studies in the published literature reporting the prevalence of *C. difficile* in food.

Internationally, the high prevalence of UK 078 in production animals, and increases of this ribotype in human infections, has sparked interest in a potential foodborne source. Goorhuis and colleagues found an increase in human CDI caused by UK 078 from 3% to 13% across a 3-year period, with a higher proportion of these cases being younger and having CA-CDI (154).

Ribotypes UK 078 and UK 027 dominated meat prevalence surveys in North America. This aligns with the common ribotypes from animal studies, and suggests that meat is being contaminated at some point in processing, rather than another external source. Ribotyping in meat products from Europe and other regions did not demonstrate such a high prevalence of UK 078, which may be reflective of the greater variability in prevalent animal strains in these regions. Although not all ribotypes were able to be matched to reference laboratory samples, matches between ribotypes found in food samples and local human cases have been reported (256, 259, 264). A lack of standard typing information across all studies highlights current inadequacies in nomenclature in the international literature, and the impact this can have on identifying significant strains across regions.

13.1 REGIONAL PREVALENCE TRENDS

The majority of studies on *C. difficile* in food have occurred in North America (USA and Canada) and Europe. Breaking down the published prevalence data into regions provides some distinct trends. The prevalence of CDI in meat samples from North America was, on average, higher

than that observed in Europe (mean 5.0% vs 1.5%). Aside from one landmark study in 1983 in hospital foods which included a range of products, few studies from the USA have looked at *C. difficile* in foods other than meat.

Explanations for this variance in retail meat may be due to differing sensitivities in testing methods, divergent sampling techniques, or may truly represent lower prevalence in Europe. If meat is becoming contaminated during slaughter and processing, a higher prevalence in production animals leading on from increased use of antimicrobials may be driving this increase down the chain.

Although the majority of studies focused on meat for human consumption, some authors expanded the scope out to other products. Weese and colleagues have been the only group to explore *C. difficile* in pet food (272) – a potential pathway of contamination for those who handle this food or who come into contact with the waste of animals who may be transiently colonised. In addition, a single study in Egypt looking at infant formula found a high prevalence (16%), although the majority of these strains were non-toxigenic, and unlikely to have a major role in disease transmission (276).

13.2 TRENDS OVER TIME

There is no evidence of any research groups conducting systematic testing of products over time to establish a baseline and a variation in prevalence over time. Without this information, an isolated sampling of food to establish the presence of *C. difficile* does not allow inferences about the impact of *C. difficile* in food on the apparent increasing incidence of disease in the community. While overall the surveys demonstrated marked variation in the prevalence of *C. difficile* (the proportion of contaminated samples ranged from 0.0% - 44.4%), it is unclear from these studies, with relatively narrow sampling frameworks, if these proportions are representative of varying trends over time, or represent a deviation. One study suggested potential a potential association between seasonality and prevalence (277), which could have implications for interpreting variable prevalence results.

A very crude analysis can be performed by grouping studies by year of publication and product to establish if there appears to be any increasing prevalence. As shown in Figure 5, there was no increase in prevalence over time in studies examining *C. difficile* in meat products. The

relatively low number of studies in vegetables, and the very diverse groups of 'other' foods investigated, means they are not suitable for longitudinal investigation.

There are clear limitations in combining the outcomes of studies performed by different groups in different regions and these data should be interpreted with caution. Limitations include differences in the sensitivity of tests, range of products, sampling methods and number of samples tested. Systematic sampling and testing of a range of food products over time is essential for determining changes in prevalence. An annual survey of suspect food products conducted by the same research group on foods sourced from similar outlets would be useful in establishing variance over time, and this is a recommended area for future research.

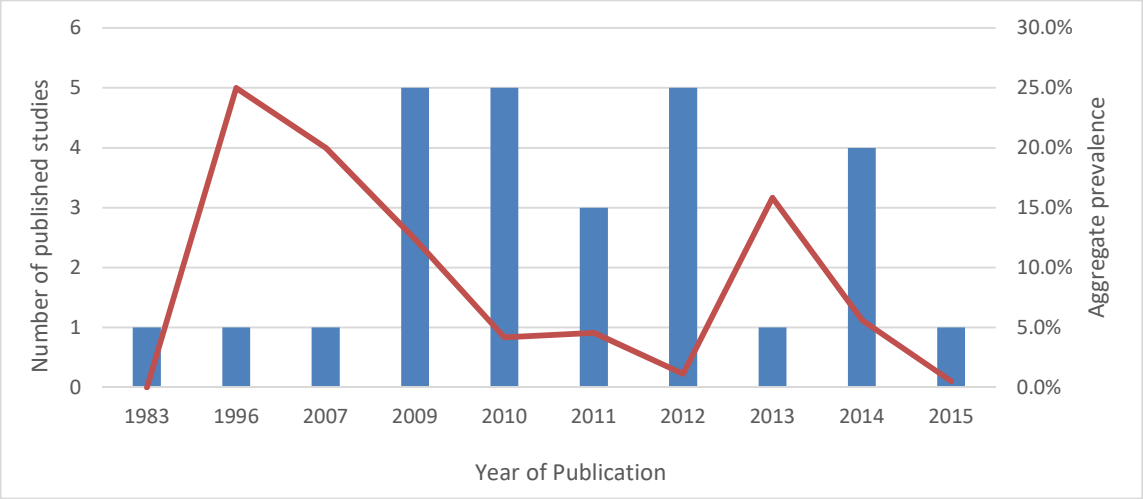


Figure 5. *C. difficile* in meat products - published studies and reported prevalence.

13.3 PRODUCTS OF INTEREST

The majority of studies found in this review were of retail meats, especially beef, pork and poultry. Presumably the detection of genetically similar strains in animal herds and humans has led to an increased awareness of the potential for *C. difficile* as a foodborne pathogen. The potential for secondary contamination of vegetables e.g. onions and salads has been explored to a lesser extent, despite early evidence of these foods as a potential source (168).

Looking at global prevalence surveys in food to date, beef and veal products, pork products and mixed/other were all similar at approximately 5% aggregate prevalence (Figure 6). These findings are supported by a population level case-control study conducted by Søes and

colleagues, to establish certain foods and other risk factors that may be correlated with development of CDI in the community. This study suggested consumption of beef was a risk factor for CDI in adults (OR 5.5, CI_{95} 2.0 – 15), along with antibiotic treatment and hospitalisation (112).

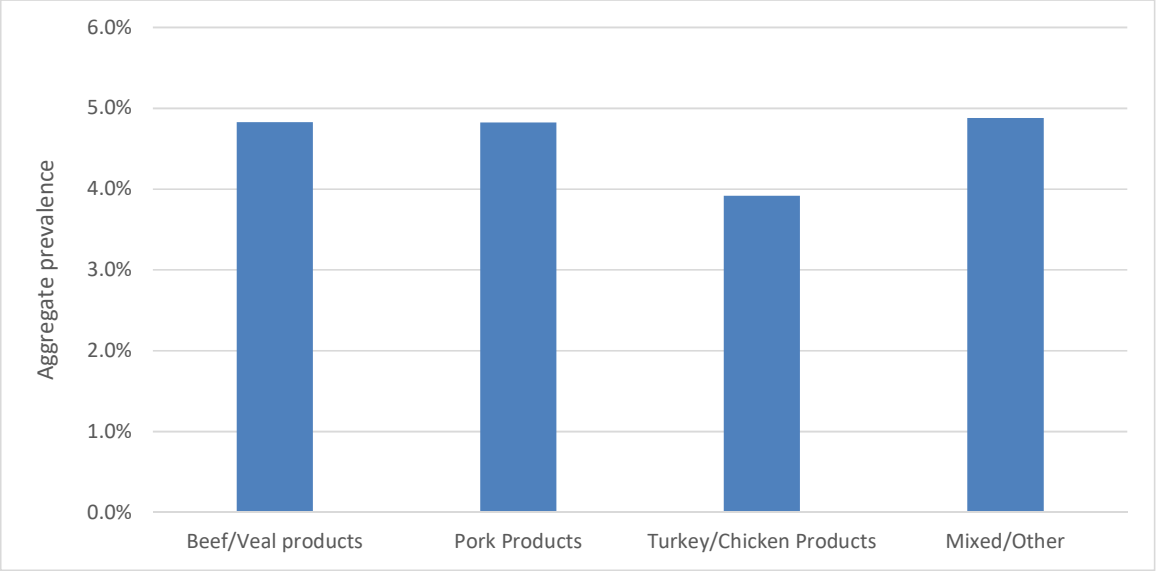


Figure 6. Prevalence of *C. difficile* in meat products – aggregated studies 1983 – 2015.

13.4 RESILIENCE DURING STORAGE AND PREPARATION

C. difficile spores can survive in low-temperature conditions (e.g. freezing at -80°C and -20°C, including numerous freeze-thaw cycles) for up to 4 months (278). It is therefore a reasonable proposition that *C. difficile* can remain present in food products for a significant time following processing. In addition, *C. difficile* spores also reportedly survived heating to 71°C (the recommended cooking temperature for ground meat in the United States), and 10% of spores survived reheating at 85°C (279). As the authors noted, this raises concerns for potential post-heating germination of survivor spores, as has been found for other clostridial spores. These studies demonstrate the potential for *C. difficile* spores on contaminated food products to survive storage and cooking processes.

13.5 SUMMARY OF EVIDENCE

Although the mechanism for infection via contaminated food is established, there is insufficient evidence linking the consumption of contaminated food to the increases of CA-CDI. There are a number of factors that may mediate the development of CDI, which makes this different from other potential foodborne outbreaks. Unlike salmonellosis or another equivalent gastrointestinal pathogen, exposure to the organism, even in a large group of people, may not result in many or even one case of infection. It is likely that the only scenario in which a foodborne outbreak could be established is a large group of susceptible individuals consuming a contaminated product at a discreet event. Outside of the hospital or residential aged care environment, it is difficult to imagine a scenario where such an exposure would occur.

With foodborne pathogens, the infectious dose (the level at which the organism needs to be detected in food to present a risk for infection) is of interest. This has not been established for *C. difficile*, and indeed it is not clear if there is a minimum infectious dose required for a susceptible host. If this is the case, then even very low levels of contamination may be sufficient of cause disease. Weese and colleagues noted of all positive samples of retail chicken were only positive on enrichment culture (250); other studies that used detection methods with lower negative predictive value may have under-reported the prevalence. Further, the possibility of laboratory contamination has been suggested as a potential explanation for evidence of *C. difficile* in food products (280), which can be dealt with by future researchers with the inclusion of additional highly discriminatory techniques, such as whole genome sequencing.

From the somewhat limited number of published studies available, the potential for *C. difficile* as a foodborne pathogen undoubtedly exists. The presence of toxigenic spores on ready-to-eat foods, as well as the demonstrated potential for spores to survive freezing and cooking processes suggests that ingestion of spores from contaminated food products and subsequent infection of a susceptible host is a plausible scenario. In addition, ribotyping data showing identical strains in human and animal isolates and some evidence of an increased risk of CDI in the community linked to the consumption of beef are all indicative of a potential link to the food chain.

Australia does not import beef or chicken meat, or dairy products, from overseas, but does import a substantial amount of fruit and vegetables, and some pork that cannot be sold unprocessed (i.e. cooked or cured). With the pork caveat, the evidence of *C. difficile* found in meat products outside Australia is not of particular significance in the Australian context, however systematic, longitudinal testing (as previously mentioned) could provide local evidence of any local retail meat contamination. There is a possibility that imported root vegetable(s) grown in soil contaminated or fertilised with animal manure containing *C. difficile* could result in foodborne transmission in the community in Australia. Further evidence is required to substantiate this hypothesis.

Certainly, more evidence is required to provide an epidemiological link between the consumption of contaminated food and the development of CDI, and to establish if foodborne transmission occurs in the community at a frequency to cause public health concern. Further, longitudinal studies demonstrating changes in prevalence over time could be used to strengthen the case for a correlation between increased prevalence in food and increased case numbers in the community. Evidence of possible seasonal influences on prevalence should be considered when conducting these studies.

Following on from this, if a link is established in future between contaminated food and disease in humans, the public health implications and actions are unclear; if any level of exposure in the community might be enough to precipitate disease development, then prevention needs to be directed 'up the chain', with implications for the use of antimicrobials in production animals, and handling of carcasses and meat processing.

Failing the ability to mitigate risk associated with contaminated food products, public health messages may best be targeted at the susceptible population in the community, who may not always be easily identified and who may not be able to fully eliminate their risk through avoidance of potentially contaminated food, or consumption of probiotics. While the literature clearly demonstrates the potential for food products as one reservoir for CA-CDI, a great deal more work is needed in this area.

14 *CLOSTRIDIUM DIFFICILE* IN HUMAN CONTACTS

While *C. difficile* spores are ubiquitous in the environment, the potential for transmission via either direct human-human contact or a contaminated shared environment (i.e. surfaces in the home of an infected or colonised contact) has also been explored. There are particular groups who are at increased risk of shedding the organism in the community, including recovering index cases, asymptomatic carriers, and young children.

14.1 CHILDREN <2 YEARS OF AGE

Children under the age of 2 years are reportedly common asymptomatic carriers of *C. difficile* (281, 282). An English study from 1984 demonstrated that the stools of 71% of infants in a special care nursery contained *C. difficile*, with 94% of these producing toxin in vitro (283). The study concluded that acquisition from the environment (rather than maternal transmission) was the likely source of colonisation, as evidenced from progressive acquisition during the course of hospitalisation (283). Common asymptomatic carriage in this group leaves open the possibility that young children may transmit this organism to susceptible household members in the community. This is a plausible route of transmission, particularly between neonates and their primary carers, who would come in to frequent contact with stool. Wilcox and colleagues showed in a study of 57 patients with CA-CDI who were diagnosed by their GPs an association between CDI and contact with infants under 2 years of age in univariate analysis (62).

14.2 PATIENTS RECENTLY DISCHARGED FROM HOSPITAL

An apparent rise in CA-CDI occurring alongside an increase in HA-CDI leaves open the hypothesis that recently discharged CDI cases are shedding spores in the home environment, leading to an increase in community-acquired disease. If this were true, then we would expect the literature to demonstrate higher rates of CA-CDI among household contacts of recently discharged CDI cases. To date, there is no evidence supporting this hypothesis (29), however studies have examined the potential spread of CDI in the community from recently discharged cases (284, 285).

In a study among household contacts of recently discharged index cases, (1,562 spouses or children), Pepin and colleagues found a moderate increase in the risk of household contacts

developing CDI for a few months post-discharge, however not enough to justify specific interventions (other than avoidance of unnecessary antimicrobials) (284). In a smaller study, Jury and colleagues found 14/44 (31.8%) of recent CDI cases visiting an outpatient clinic had contamination of one or more skin sites, and 12/44 (27.2%) shed spores to one or more 'high-touch' surfaces in examination rooms (286). These data present a potential source of CDI in susceptible patients, that under current case definitions would not be attributable to a HCF.

Robust genetic data is required to epidemiologically link cases occurring either within hospitals, or among contacts of cases in the community. Eyre and colleagues undertook such a study of 1,200 CDI cases in the UK over a 3-year period, and demonstrated that 45% of cases had sufficient genetic diversity to represent transmission other than a symptomatic case from the same geographical region (143). A further 13% of cases who were genetically linked had no plausible previous contact, either in hospital or the community. These data suggest contact with asymptomatic carriers, or an environmental source, as the likely mode of transmission (143).

14.3 OTHER ASYMPTOMATIC CARRIERS

Asymptomatic colonisation refers to the carriage of *C. difficile* in the GI tract in the absence any signs or symptoms of infection. Asymptomatic long-term gastrointestinal (GI) tract colonisation with *C. difficile* is rare, although transient colonisation occurs frequently, reflecting transmission via environmental contamination. Carriage rates in healthy adults who have not been recently hospitalised or taken a course of antibiotics have been shown to be 1 – 3% (287, 288), although this may be an over-estimate due to inappropriate choice of controls on some studies.

People who are colonised with *C. difficile* may spread spores into the environment and act as reservoirs of transmission, although the role of asymptomatic carriers in the spread of CDI is not fully understood (289). Infection prevention and control measures for *C. difficile* in a healthcare facility are usually focused around suspected or documented CDI cases, i.e. those showing symptoms of disease (289). Although current evidence appears to support this, asymptomatic carriage may have important implications for transmission in both endemic and epidemic settings

14.4 SUMMARY OF EVIDENCE

The potential for asymptomatic carriers of *C. difficile* to shed the organism into the home environment and cause disease in other contacts has certainly been demonstrated. This potential may be amplified in the case of children under 2 years of age and their primary carers; close contact with faecal matter as part of caring for these children potentially exposes carers to a higher level of spores than might normally be found on contaminated surfaces in an average home. While the role of asymptomatic carriers is not well established this is a plausible mechanism for transmission in the community, and contact with potential asymptomatic carriers as a driver of CA-CDI should be explored further.

15 CONCLUSIONS

This literature review documents a growing incidence of CA-CDI, with cases in the community resulting in severe disease. While 'traditional' risk factors for CDI are well established, cases in the community lacking in traditional risk factors are being documented, suggesting that CA cases may have different risk factors for disease. Importantly, the literature has demonstrated paucity in knowledge around the epidemiology and risk factors for CA-CDI, not just in a local context but on a global scale.

The available evidence suggests close contacts, the environment, animals (companion, production and native) and food as potential sources of this infection in the community. While there has been demonstrable plausibility for each of these routes of transmission for CA-CDI, no study has definitively identified one or a combination of these as the primary source of infection in the community. At this stage, the presence of *C. difficile* in these potential reservoirs does not conclusively suggest a causative link; animals and food remain potential but unproven sources of CDI in the community. Locally, evidence is required to quantify the burden of CA infection, and establish if any of these suggested risk factors appear to have a causative role in CA-CDI.

To date, no studies have conclusively documented a transmission route between animals and humans. Common strains have been found in humans and animals, however while *C. difficile* is potentially a zoonotic organism, further research needs to be undertaken to establish the

path of transmission. At best the evidence is patchy and further research into the epidemiology and risk factors of CA-CDI is clearly required.

Current Australian clinical practice guidelines preclude routine laboratory testing for acute episodes of diarrhoeal illness in the community, as most are self-limiting, however, microbiological testing is indicated for severe disease, recent antimicrobial use or hospital admission (290). Data from the Netherlands suggest GPs test for *C. difficile* in only 7% of stool samples, and about 40% of these are positive (291). If this is similar in Australia, there will need to be significant changes in current clinical practice if CA-CDI is to be detected in this setting, given the variable risk profile and clinical presentation of these cases.

Based on the available evidence, CDI, particularly in the community, sits firmly under the 'One Health' umbrella, in which human health, animal health and the environment are inextricably linked. CDI affects human and animal populations, although the links between the two require better definition. While the issue of CA-CDI is undoubtedly of high public health significance, the messages around reducing risk in the community are not clear. From a local perspective, understanding the burden of disease in the community is paramount. Current data on HI-CDI is the most reliable, accurate data source available which contains validated cases detected at healthcare facilities.

The documentation of 'hypervirulent' strains (i.e. UK 027) undoubtedly demonstrates outbreaks associated with severe disease and poor outcomes across multiple geographic regions. The lack of severe disease in all cases, and studies that document lower mortality in different regions, both suggest that ribotype alone is likely not enough to predict a poor outcome in a patient. Multiple host factors such as advanced age and comorbidities can influence both susceptibility to infection and the outcome. In a hospital environment, where one might expect a generally older and sicker population, the potential impact of these strains is more apparent.

However, in a community setting the impact of infections with different strains is less clear; further investigations may shed light on whether public health concerns lie more with the health outcomes in community-based patients, or the potential of introduction from the community into a healthcare setting, with a more vulnerable patient population.

This review has highlighted several gaps in the current knowledge surrounding CA-CDI. In the first instance, existing local data should be used to establish the burden of community infection, including changes over time since data collection commenced in WA in 2010. Following the application of enhanced surveillance definitions, a risk profile can be developed based on demographic data to establish if HA-CDI and CA-CDI appear to be increasing overall, and if the proportions of each are varying over time. WA is in a unique position to be able to build up local knowledge, and further contribute to the international understanding of this disease.

16 GAPS IN THE LITERATURE

The literature review highlighted several gaps in the current literature related to the local epidemiology of CA-CDI. Most notably:

- There is limited data on the prevalence of CA-CDI in Australia, and no available data on the number of CA-CDI cases occurring in WA, as a proportion of total HI-CDI cases reported to HISWA.
- Ribotyping data, while available since 2011, has not been integrated into the HISWA dataset.
- There is no available information on prevalent strains among HA and CA cases in WA.
- There is no available information on which groups, if any, are at higher risk of developing CA-CDI in WA.

17 RESEARCH QUESTIONS, AIMS AND OBJECTIVES

Based on the literature review and current gaps, the following research questions, aims and objectives were proposed:

Question 1 What is the incidence of CA-CDI in WA, and is it increasing?

- **Aim 1** To determine the incidence of CA-CDI cases, as a proportion of HI-CDI cases, reported to HISWA between 1 January 2010 and 31 December 2014.

- **Objective 1.1** Apply internationally accepted enhanced surveillance definitions to HI-CDI cases, to determine if they are CA-CDI or HA-CDI cases.
- **Objective 1.2** Describe the characteristics of HI-CDI cases for the study period.
- **Objective 1.3** Determine evidence for increasing proportions of CA-CDI among HI-CDI cases, using trend analysis.
- **Objective 1.4** Describe patient characteristics of CA-CDI cases.

Question 2 Which ribotypes are prevalent among CA-CDI and HA-CDI cases?

- **Aim 2** To determine common ribotypes among HISWA cases between 11 October 2011 and 31 December 2014
  - **Objective 2.1** Integrate available ribotyping data into the HISWA dataset.
  - **Objective 2.2** Describe common ribotypes among HI-CDI cases, and within population sub-groups, including analysis of strain diversity.
  - **Objective 2.3** Document emerging strains and perform analysis for severe disease on ribotypes of interest.

CHAPTER 3 –METHODS

1 ETHICS APPROVAL AND CONSIDERATIONS

Initial ethics approval for research into the research project, titled *Risk Factors for Emerging Community-Associated* Clostridium difficile *infection in Western Australia* was granted on 24 January 2014 (Flinders Social and Behavioural Research Ethics Committee Project 6359). An amendment to this project to allow expanded an enhanced surveillance application was approved on 28 October 2014.

This project involved the analysis of an existing patient dataset, with review of individual medical records required in order to make a determination of case classification. To this end, maintaining patient confidentiality was the primary ethical consideration in undertaking this research. No patient names, addresses or other readily identifiable information were recorded during the analysis of these data.

The unique medical record number (UMRN) was the only variable which would potentially allow identification of cases, and any datasets containing this variable were stored on secure, password protected Department of Health computers. No confidential or identifiable information is presented in the publication or dissemination of any study results, with patient confidentiality maintained throughout the duration of the study.

2 STUDY DESIGN

This was a descriptive epidemiological study using the HISWA database to identify *C. diffiicle* cases reported between 01 January 2010 and 31 December 2014. A nested case-control design was used to compare the demographic characteristics of CAI and HAI cases.

2.1 SETTING

HISWA is an online infection control surveillance database, and was established in 2005 as a voluntary reporting program for healthcare associated infections (HAI) for both public and private hospitals in WA. Mandatory reporting of select indicators for public facilities commenced in 2007. In January 2010, reporting of hospital identified (HI) CDI was added to the mandatory HISWA data collection set.

Case definitions are outlined in the *HISWA Surveillance Manual* (292). A HI-CDI case includes any patient attending any area of the hospital i.e. inpatients, those presenting to and emergency department (ED) and outpatients. A case is 'identified' by a hospital by merit of a positive specimen result, with the pathology request generated from the facility.

2.2 PARTICIPANTS

Study participants included cases within the HISWA data collection. This comprises an online database, with HAIs reported by infection control personnel at each hospital. In addition to some standard HISWA fields, the *Clostridium difficile* module collects the following variables on each participant:

- Unique patient identifier
- Patient DOB
- Patient postcode
- Laboratory specimen number
- Specimen date of collection
- Ribotype (if known)

Cases include those who meet the standard case definitions (section 3). In addition, denominator data is collected in order to be able to generate a rate of infection. The HISWA denominator for HI-CDI is bed-days, and includes all occupied bed-days for the surveillance period, including all inpatient wards, hospital in the home (HITH) admissions, and same-day ward admissions (i.e. day procedure units). Boarders, children under 2 years old, and ED or outpatient attendance data are excluded from denominator data.

3 *CLOSTRIDIUM DIFFICILE* INFECTION CASE DEFINITIONS

A case of CDI needs to meet standard case definitions in order to be accepted by HISWA. All cases are validated centrally by the Department of Health against the criteria in order to ensure only valid cases are being recorded. HISWA case definitions are based on nationally accepted case definitions. As described in the Australian Commission on Safety and Quality in Health

Care *Implementation Guide for Surveillance of Clostridium difficile infection* (293), a CDI case meets the following criteria:

- The sample must be a diarrhoeal specimen i.e. must be unformed and take the shape of the container, and
- The stool sample yields a positive result in a laboratory assay for *C. difficile* toxin A and/or B, or
- A toxin-producing *C. difficile* organism is detected in the stool sample by culture or other means

The following criteria are exclusions:

- Formed stools
- Cases where a known previous positive test has been obtained within in the last 8 weeks
- The patient is less than 2 years old at the date of admission

A HI-CDI case is any case diagnosed in a patient attending a hospital (including admitted patients, outpatients and those presenting to emergency departments).

3.1 SEVERE DISEASE

According to the Australian Commission on Safety and Quality in Health Care *Implementation Guide for Surveillance of Clostridium difficile infection* (293) the following criteria are associated with severe CDI:

- age over 60 years
- temperature greater than 38.3oC
- serum albumin less than 25 g/L
- peripheral white blood cell count greater than 15,000 cells/microL
- deteriorating renal function
- elevated serum lactate

- endoscopic evidence of pseudomembranous colitis or treatment in the intensive care unit
- subtotal colectomy performed
- toxic megacolon diagnosed

4 APPLICATION OF STANDARD ENHANCED SURVEILLANCE DEFINITIONS

All cases of HI-CDI reported by metropolitan public hospitals between 01 January 2010 and 31 December 2014 were reviewed. Case review was limited to public hospitals in the metropolitan area due to the availability of centralised, electronic patient notes, which allowed review of visit histories to other public metropolitan healthcare facilities, pathology results and discharge summaries. Ethics approval was not sought for access to patient records from private facilities, and the lack of electronic patient records available centrally from non-metropolitan hospitals precluded review of these cases.

Cases were identified using the HISWA database. For all cases, the patient records were reviewed, and data were collected on age, sex and hospitalisation history (including relevant hospitalization in the 12 weeks prior to diagnosis, principle diagnosis on admission, and length of stay. Using the CDC enhanced surveillance definitions, cases were differentiated into community-associated infection (CAI), healthcare-associated infection healthcare facility onset (HAI HCFO), healthcare-associated infection community onset (HAI CO), indeterminate and unknown. Following the application of enhanced surveillance definitions, patient characteristics were analysed comparing CA and HA cases, including review of differences between sex, age, ribotype, and hospital of detection.

4.1 DEFINITIONS

The CDC/ECDC enhanced surveillance definitions were applied to the HISWA dataset in order to categorise cases into CA and HA infections (9, 53). These are internationally accepted definitions which are commonly used to categorise cases HA or CA, and are shown in Figure 7. In the event that ribotyping data revealed cases of a ribotype of interest, e.g. a potentially

'hypervirulent' or emergent strain, further analysis of the medical record was done to determine if the cases met the criteria for severe disease, as outlined above.

5 SOCIO-ECONOMIC INDEXES FOR AREAS (SEIFA) SCORES

Postcode data is routinely collected as part of the HISWA dataset, with the postcode of patient residence entered into the database by infection control personnel. The Index of Relative Socio-economic Advantage and Disadvantage (IRSAD) from the Australian Bureau of Statistics (ABS) was applied to the postcode data (294). The IRSAD summarises information about the economic and social conditions of people and households within an area, including both relative advantage and disadvantage measures (295). Lower scores are indicative are lower levels of advantage/higher levels of disadvantage.

6 RIBOTYPING

Routine ribotyping of isolates recovered by PathWest Laboratory Medicine commenced in October 2011. Both toxin profiling and PCR ribotyping was undertaken. Template DNA was prepared by suspension of cells in 5% (wt/vol) Chelex-100 (Sigma-Aldrich, Castle Hill, NSW, Australia). All isolates of *C. difficile* were characterised by PCR for the presence genes encoding large clostridial toxins (*tcdA* and *tcdB*) (296) and binary toxin (*cdtA/B*) (297), and for variations in 16S-23S rRNA intergenic spacer region (PCR ribotype) (298). PCR ribotypes were identified by comparison with banding patterns in the PathWest reference library.

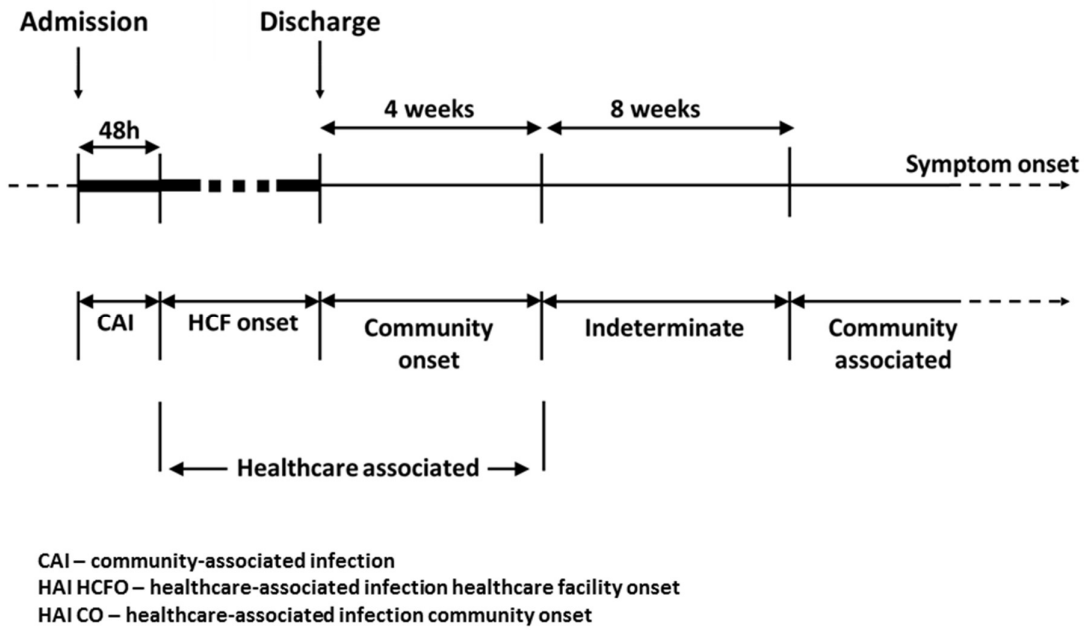


Figure 7 Current enhanced surveillance definitions (adapted from McDonald et al. (53)).

7 DATA ANALYSIS

Data were analysed using STATA v. 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

7.1 ENHANCED SURVEILLANCE

Trend analysis was conducted on annual count data of cases. Proportions were compared year-on-year by classification, sex, age group and hospital type using the ptrend function (http://www.stata.com/support/faqs/statistics/test-for-trend/). A Kruskal-Wallis test was used to compare median age.

For the purposes of comparing CA and HA cases in, only HA cases with healthcare facility onset were included (i.e. those classified HAI HCFO) in order to remove the potential ambiguity in the classification of community-onset cases (i.e. all HA cases were diagnosed while an inpatient of the facility). Univariate logistic regression was used to determine particular sub-groups with significantly higher probabilities of reporting CA infection within demographic categories including year of infection, sex, age group, hospital type, diagnosing hospital and IRSAD score.

Differences across age groups, by sex, were further explored using chi-squared tests to determine if either gender was over-represented within a particular age group. A chi-squared test was also used to determine if there were significant increases in rates over the 5-year period, comparing the first and last quarter of reported data.

7.2 RIBOTYPING DATA

All available ribotyping data was integrated into the existing HISWA dataset. Once integrated, overall proportions of ribotypes, along with prevalence by classification, age and hospital, were determined. Any emerging strains, or strains of particular significance, were explored further for markers of severity and prevalence among CA and HA cases. Within-group diversity of ribotypes was calculated using Simpsons Index (299), with 95% confidence intervals calculated using methods outlined by Grundmann et al. (300).

7.3 ANTIMICROBIAL PRESCRIBING TRENDS

The seasonal pattern seen with CA-CDI may be related to variations in antibiotic use in the human population. Seasonal respiratory illnesses are associated with an increase in health-seeking behaviour and subsequent seasonal increases in antimicrobial prescribing during winter/spring months. Antimicrobial prescribing data for Western Australia was sought from Medicare Australia via the Pharmaceutical Benefits Scheme/Repatriation Pharmaceutical Benefits Scheme (PBS/RPBS) for the study period (http://medicarestatistics.humanservices.gov.au/statistics/pbs_item.jsp). The number of prescriptions of pharmaceuticals classified as 'J01 antibacterials for general use' (see Appendix 2 for full list of item numbers) was extracted from the PBS dataset.

Antimicrobial prescribing trends by class of antimicrobial, and totals, were tested for trend and correlated with numbers of CA-CDI using the Pearson product-moment correlation coefficient.

CHAPTER 4 – RESULTS

1 SURVEILLANCE DATA

For the period 01 January 2010 – 31 December 2014, a total of 3,863 cases of *Clostridium difficile* infection were reported to HISWA. Of these, 2,962 were reported by facilities in metropolitan Perth. The rates of HI-CDI collected by HISWA are shown in Figure 8. All hospital groups saw a significant increase in overall HI-CDI rates between the first and last reporting period, using a chi-squared test for trend ($p<0.01$); with some notable peaks at the end of the 2011, 2012 and 2014 calendar years (Figure 8).

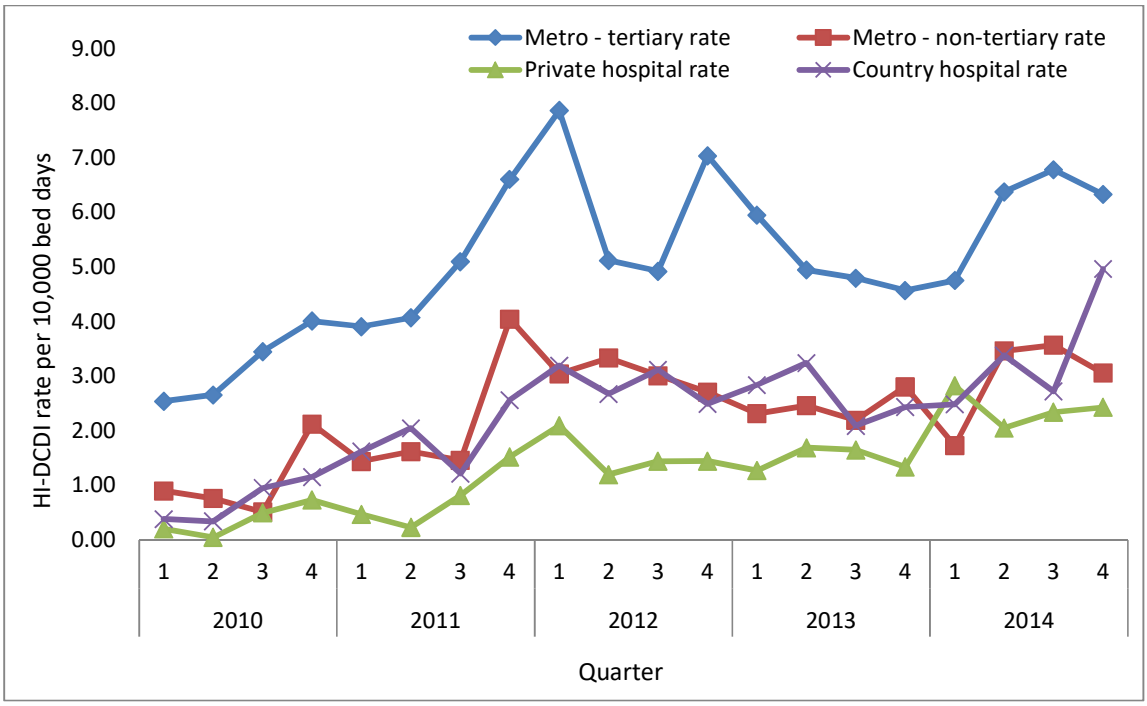


Figure 8 . Rates of HI-CDI, by hospital group, Western Australia, 2010 – 2014.

2 ENHANCED SURVEILLANCE

A total of 2,962 cases (2,491 from tertiary hospitals (TER) and 471 from metropolitan non-tertiary (MNT) hospitals) were reviewed for the period 2010 – 2014 for the purposes of enhanced surveillance. There was no significant variation across the 5-year period of the proportions of cases by classification, with the exception on unknown classifications which increased, due largely to a higher proportion of transfers with an unknown previous length of

stay (Table 5). The data did, however, show a significant increase in proportions of CAI between 2010 and 2011 (24.7% to 31.1%, p=0.037); however, the proportion then remained relatively stable (no significant variations) across the rest of the reporting period (hence no significant trend). There were more than double the case numbers of CA-CDI reported in 2014 (n = 207), as compared to 2010 (n = 84). This represents a 146% increase between 2010 and 2014, whereas HA-CDI (healthcare facility onset) case numbers increased by 70% over the same period. When comparing 2014 to 2010 (assuming 2010 as a baseline year), there was a borderline non-significant increase in the proportion of cases between the first and last year of the study period (p=0.061).

Trend analysis also showed a significant increase in the proportion of cases diagnosed at a non-tertiary hospital. There was a significant increase in cases aged 2 – 19 years, but no other significant differences by age group. Overall, approximately half (52.2%) of cases were classified as HAI HCFO, with approximately one third of cases (29.3%) classified as CA. There were more females (55.4%) than males, and 15.9% of cases were diagnosed at non-tertiary hospitals. A summary of enhanced surveillance data is shown in Table 5.

Table 5. Summary of enhanced surveillance of HI-CDI cases, Western Australia, 2010 – 2014.

		2010 n (%) (n=340)	2011 n (%) (n=578)	2012 n (%) (n=764)	2013 n (%) (n=604)	2014 n (%) (n=676)	TOTAL n (%) (n=2,962)	p value (test for trend)
CLASSIFICATION	CAI	84 (24.7)	180 (31.1)	220 (28.8)	177 (29.3)	205 (30.3)	**866 (29.2)**	0.2814
	HAI CO	36 (10.6)	75 (13.0)	78 (10.2)	51 (8.4)	77 (11.4)	**317 (10.7)**	0.4485
	HAI HCFO	195 (57.4)	289 (50.0)	400 (52.4)	332 (60.0)	332 (49.1)	**1,548 (52.2)**	0.1795
	INDETERMINATE	24 (7.1)	32 (5.5)	56 (7.3)	37 (6.1)	50 (7.4)	**199 (6.7)**	0.5712
	UNKNOWN	1 (0.3)	2 (0.3)	10 (1.3)	7 (1.2)	12 (1.8)	**32 (1.1)**	**0.0083**
SEX	FEMALE	188 (55.3)	324 (56.1)	422 (55.2)	334 (55.3)	374 (55.3)	**1,642 (55.4)**	0.8891
	MALE	152 (44.7)	254 (43.9)	342 (44.8)	270 (44.7)	302 (44.7)	**1,320 (44.6)**	
AGE	MEDIAN (RANGE)	67.25 (2.55 - 98.97)	67.00 (2.05 - 100.27)	69.65 (2.29 - 106.49)	66.40 (2.05 - 100.32)	65.40 (2.29 - 100.07)	**67.15 (2.05 – 106.49)**	**0.019^**
	>65 years	181 (53.2)	316 (54.7)	442 (57.8)	314 (52.0)	342 (50.6)	**1,595 (53.8)**	0.1159
AGE GROUP	2y - 19y	26 (7.6)	33 (5.7)	41 (5.4)	47 (7.8)	65 (9.6)	**212 (7.2)**	**0.0221**
	20y - 39y	32 (9.4)	79 (13.7)	82 (10.7)	85 (14.1)	88 (13.0)	**366 (12.4)**	0.1759
	40y - 59y	69 (20.3)	115 (19.9)	144 (18.8)	115 (19.0)	135 (19.9)	**578 (20.0)**	0.8816
	60y - 79y	126 (37.1)	188 (32.5)	282 (36.9)	199 (32.9)	232 (34.3)	**1,027 (34.7)**	0.5752
	80+ years	87 (25.6)	163 (28.2)	215 (28.1)	158 (26.2)	156 (23.1)	**779 (26.2)**	0.1071
HOSPITAL TYPE	NON-TERTIARY	40 (11.8)	86 (14.9)	122 (16.0)	100 (16.6)	123 (18.2)	**471 (15.9)**	**0.0081**
	TERTIARY	300 (88.2)	492 (85.1)	642 (84.0)	504 (83.4)	553 (81.8)	**2,491 (84.1)**	

^Kruskal-Wallis test

CAI – Community-associated infection; HAI CO – Healthcare associated infection, community onset; HAI HCFO - Healthcare associated infection, healthcare facility onset

3 RATES OF COMMUNITY- AND HEALTHCARE-ASSOCIATED INFECTIONS

The HISWA dataset uses bed days as a denominator for CDI to allow surveillance of variations in rate across time. HAI HCFO infection rates are reported per 10,000 bed-days. As identified CA cases were only a subset of all CA cases (cases presenting to primary care facilities and smaller hospitals were not included), simple counts of CA cases per quarter are presented for comparison. In order to be satisfied that HA cases were not the result of community acquisition post-discharge, rates of HAI CO cases were excluded from further analysis. These results are shown in Figure 9. Over the 5-year study period; there was a significant increase in the rate of HAI HCFO infections (p=0.001) and an increase in case numbers of CAI. The peak for both occurred in late 2011/early 2012, with the increase in CAI case numbers preceding the increase in HAI HCFO rates.

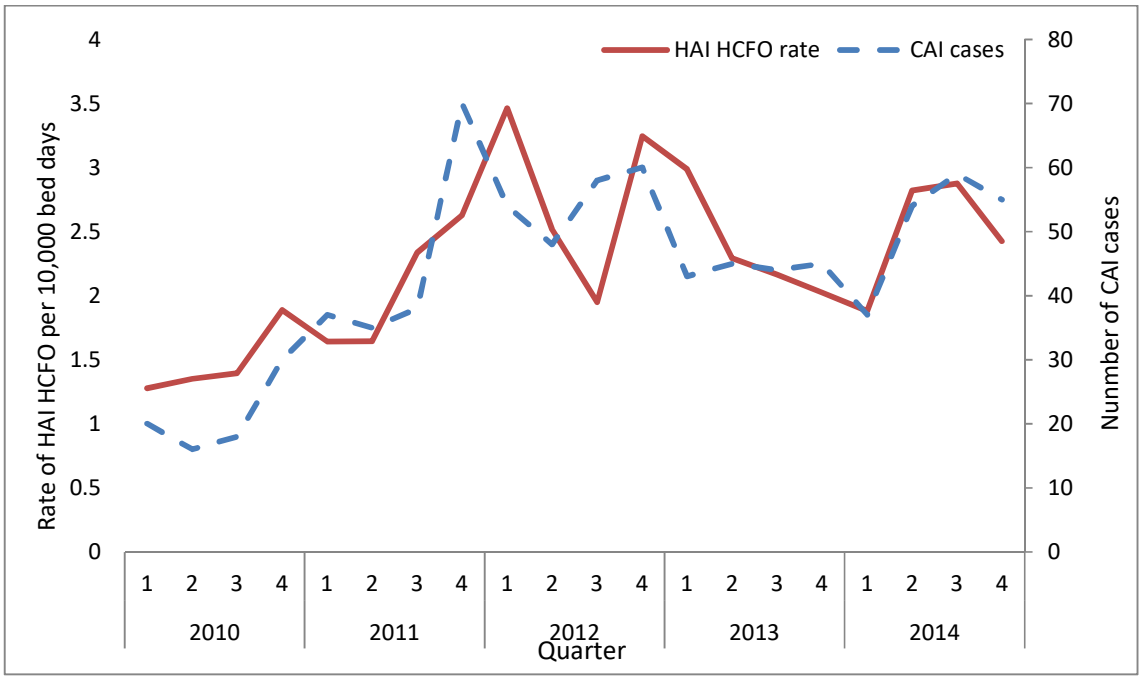
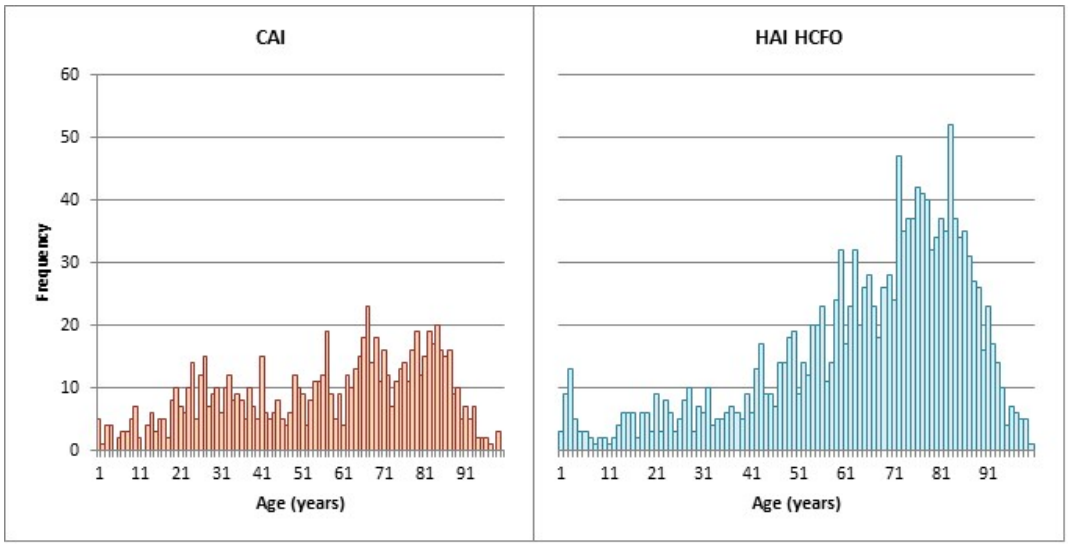


Figure 9. Rates of HAI HCFO infections and counts of CAI cases, Western Australia, 2010 – 2014.

4 COMPARISON OF PATIENT DEMOGRAPHICS

A total of 866 CAI and 1,548 HAI HCFO cases were reviewed. Factors associated with CA-CDI diagnosis at metropolitan hospitals are shown in Table 6. Cases were significantly less likely to be diagnosed with CAI in the first reporting year (OR = 0.69, p=0.023), with overall case numbers were lower for this period. CA-CDI cases were significantly more likely to be diagnosed at a MNT hospital (OR = 1.57, p<0.001). The reported odds ratios varied by site, however these were higher for all MNT hospitals that reported cases. Significantly higher proportions of CA-CDI diagnosed were in the younger age groups, particularly the 20 – 39 years group (OR = 3.71, p<0.001). The odds of CA-CDI diagnosis were lower in those with an IRSAD score of 5 (OR = 0.65, p=0.005), although no other significant variations by IRSAD score were observed.

The age profiles for CA-CDI and HA-CDI cases are shown in Figure 10; while HA-CDI cases exhibit the classic positively skewed distribution (i.e. a higher proportion of cases diagnosed in an older age group), CA cases were more frequently observed in younger patients. While overall, CA-CDI cases were not significantly more likely to be female (OR 1.10, p=0.27), females in the 20-39 year age group were significantly more likely to have CA-CDI (OR 1.91, p=0.006) (Table 7).



CAI – Community-associated infection; HAI HCFO - Healthcare associated infection, healthcare facility onset.

Figure 10. Age distribution of CA-CDI and HA-CDI, Western Australia, 2010 – 2014

Table 6. Factors associated with CA-CDI diagnosis at a metropolitan public hospital. Western Australia, 2010 – 2014.

		Community-associated infections	
		n (%)	OR (CI_{95})
TOTAL		866 (29.2)	-
YEAR OF INFECTION	**2010**	84 (9.7)	**0.69 (0.51 – 0.95)**
	2011	180 (20.8)	1.01 (0.78 – 1.30)
	2012	220 (25.4)	0.89 (0.70 – 1.13)
	2013	177 (20.4)	0.86 (0.67 – 1.11)
	2014	205 (23.7)	Ref
SEX	**FEMALE**	494 (57.0)	1.10 (0.93 - 1.30)
	MALE	372 (43.0)	Ref
AGE GROUP	**2 – 19 years**	63 (7.3)	**1.96 (1.35 – 2.84)**
	20 – 39 years	181 (20.9)	**3.71 (2.79 – 4.94)**
	40 – 59 years	171 (19.7)	**1.52 (1.18 – 1.97)**
	60 – 79 years	258 (29.8)	1.06 (0.85 – 1.33)
	80+ years	193 (22.3)	Ref
HOSPITAL TYPE	**MNT**	166 (19.2)	**1.56 (1.25 - 1.96)**
	TER	700 (80.7)	Ref
DIAGNOSING HOSPITAL	**A (TER)**	229 (26.4)	**0.66 (0.53 - 0.83)**
	B (TER)	173 (20.0)	0.84 (0.66 - 1.08)
	C (TER)	49 (5.7)	1.36 (0.90 - 2.05)
	D (TER)	3 (0.3)	2.41 (0.40 - 14.55)
	E (TER)	246 (28.5)	Ref
	F (TER)	0 (0.0)	-
	G (MNT)	56 (6.5)	**2.09 (1.36 - 3.22)**
	H (MNT)	0 (0.0)	-
	I (MNT)	52 (6.0)	**1.82 (1.19 - 2.79)**
	J (MNT)	0 (0.0)	-
	K (MNT)	57 (6.6)	**2.29 (1.49 - 3.54)**
	L (MNT)	0 (0.0)	-
	M (MNT)	1 (0.1)	-
IRSAD SCORE	**1**	9 (1.0)	0.65 (0.29 - 1.44)
	2	32 (3.7)	1.34 (0.80 – 2.22)
	3	52 (6.0)	0.70 (0.48 - 1.02)
	4	40 (4.6)	0.78 (0.51 – 1.20)
	5	103 (11.9)	**0.65 (0.48 – 0.88)**
	6	154 (17.8)	1.01 (0.76 - 1.33)
	7	70 (8.1)	0.92 (0.65 - 1.30)
	8	93 (10.7)	0.96 (0.70 - 1.33)
	9	141 (16.3)	0.90 (0.68 - 1.20)
	10	172 (19.9)	Ref

Table 7. CA-CDI and HA-CDI in females, by age group, Western Australia, 2010 – 2014.

Age group	CAI n (%)	HAI HCFO n (%)	OR (CI_{95})
2 - 19 years	29 (44.6)	36 (55.4)	1.02 (0.52 – 1.98)
20 - 39 years	109 (67.3)	53 (32.7)	**1.91 (1.20 – 3.05)**
40 - 59 years	99 (39.3)	153 (60.7)	1.11 (0.75 – 1.63)
60 - 79 years	141 (31.8)	302 (68.2)	1.18 (0.88 – 1.58)
80+ years	116 (27.7)	303 (72.3)	0.86 (0.61 – 1.21)
Total	**494 (36.8)**	**847 (63.2)**	**1.09 (0.93 – 1.30)**

CAI – Community-associated infection; HAI HCFO - Healthcare associated infection, healthcare facility onset

5 RIBOTYPING DATA

Between 01 October 2011 and 31 December 2014, 2,252 isolates from metropolitan hospitals were eligible to be ribotyped. Of these, 1,358 (60.3%) eligible isolates had ribotyping data available. In total, 168 unique ribotypes were identified from the HISWA dataset. The ribotype most commonly isolated during the study period was the UK 014/020 group; accounting for almost one third of all ribotyped isolates (Table 8). These ribotypes are often combined due to the difficulty in distinguishing their ribotyping patterns (301).

No isolates of UK 027 (the 'hypervirulent' ribotype that caused major outbreaks across North America and Europe) were detected during the study period. There were no reported cases of UK 078 – the 'animal-associated' ribotype that has been detected in other Australian jurisdictions and countries.

As shown in Figure 16, the relative frequency of the ribotypes changed over time. In particular, ribotype UK 012 emerged from relative obscurity, to become a highly prevalent strain. The emergence of ribotype UK 244, a binary toxin producing ribotype, towards the beginning of the reporting period was another clinically significant finding. This ribotype appears to be closely related to UK 027 (both ribotypes are from clade 2) and is reportedly of similar high virulence (301). Of the more commonly reported ribotypes; the UK 014/020 group, UK 244, UK 056 and UK 012 were considered ribotypes of particular significance and were analysed further.

Table 8. Most commonly isolated ribotypes, specimens collected 2011 – 2014.

Ribotype	Total	%
UK 014/020 [G]	384	28.3%
UK 002	111	8.2%
UK 056	86	6.3%
UK 054	45	3.3%
UK 012	43	3.2%
UK 046	41	3.0%
UK 018	39	2.9%
UK 005	30	2.2%
UK 015/UK 193	30	2.2%
UK 103	29	2.1%
UK 010	28	2.1%
UK 017	28	2.1%
UK 053	28	2.1%
UK 070	25	1.8%
QX 076	23	1.7%
QX 001	19	1.4%
UK 087	18	1.3%
QX 014	14	1.0%
UK 244	14	1.0%
QX 026	11	0.8%
UK 064	11	0.8%
Other	301	22.2%

***For a complete list, see Appendix 1**

5.1 RIBOTYPE UK 014/020 GROUP

The UK 014/020 group was the most commonly isolated ribotype in both CA and HA cases, and overall comprised 28.2% of all ribotypes isolated between 1 October. 2011 and 31 December 2014. The proportion of cases of HI-CDI caused by this ribotype group peaked in 2014, accounting of 39% of cases in this year. The UK 014/020 group was significantly more prevalent among CAI cases (OR = 1.45, p=0.004) than other ribotypes, accounting to 34% of ribotyped CAI cases.

Mapping of monthly case counts showed that the UK 014/020 group sharply declined between September 2012 and October 2013, with only 7 cases of this ribotype reported in total over this 12-month period, compared with an average of 14 cases per month before and after this time (Figure 11). No other strain replaced this group as the dominant ribotype during this period; the most prevalent ribotype during this period (UK 002) accounted for only 9% of ribotypes.

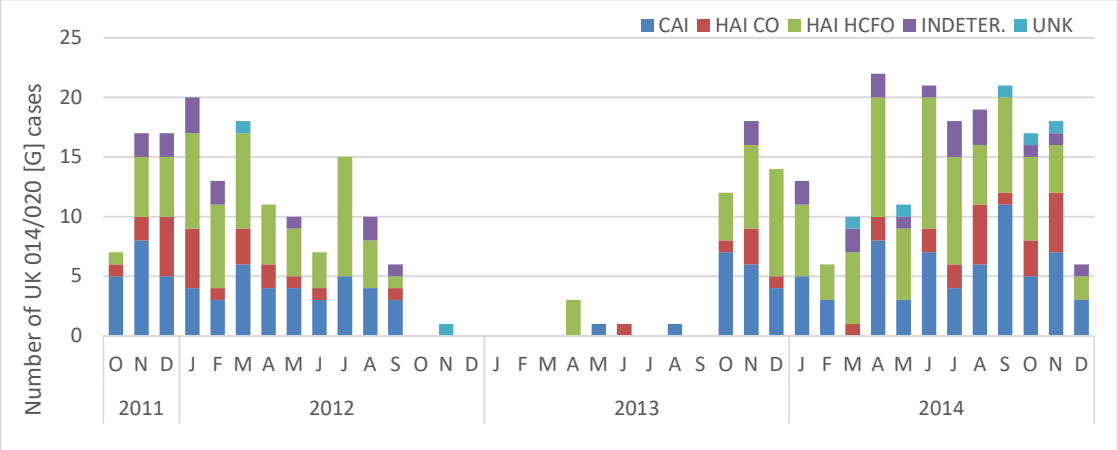


Figure 11. UK 014/020 [G] cases of CDI reported to HISWA, Western Australia, 2011 – 2014.

5.2 RIBOTYPE UK 244

During the study period, UK 244 was the 19th most prominent ribotype, with 14 detections accounting for 1% of cases. The majority of detections in the HISWA dataset occurred in 2011 – 2012, with no detections in 2014. UK 244 was significantly more likely to be diagnosed among CAI cases (p=0.0224), with CAI accounting for 57% of cases. The initial 3 cases of UK 244 in WA, diagnosed in October 2011, were all classified as CAI (Figure 12). As shown in Table 9, 2/14 (14%) of cases were determined to be severe, 1 CAI case and 1 HAI HCFO case.

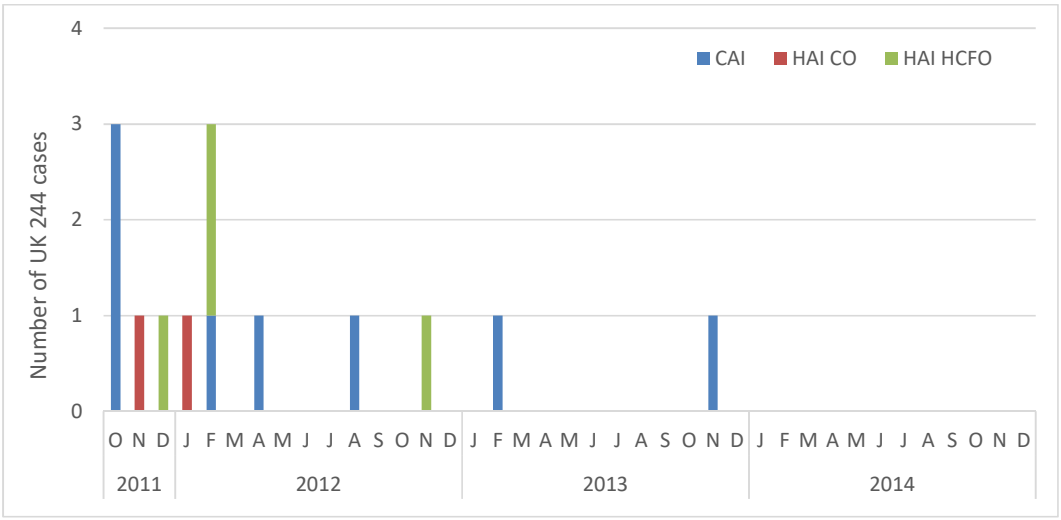


Figure 12. UK 244 cases of CDI reported to HISWA, Western Australia, 2011 – 2014.

Table 9. UK 244 CDI cases, Western Australia, 2011 – 2014, - assessment for severe disease.

CASE	CLASSIFICATION	AGE	SEX	INFECTION MONTH	INFECTION YEAR	>60 YEARS	TEMP > 38.3°C	ALBUMIN <25g/L	WBC> 15,000 c/mL	↓RENAL FUNCTION	↑SERUM LACTATE	PMC	ICU ADMISSION	COLECTOMY	TOXIC MEGACOLON	SEVERE DISEASE
1	CAI	26.1	M	10	2011	X	x	X	X	X	-	**√**	X	X	X	N
2	CAI	87.8	M	10	2011	**√**	X	X	X	**√**	I	**√**	X	X	X	N
3	CAI	21.2	M	10	2011	X	-	X	X	X	-	X	X	X	X	N
4	HAI CO	9.1	M	11	2011	X	-	X	X	X	-	X	X	X	X	N
5	HAI HCFO	83.2	M	12	2011	√	-	X	X	X	X	X	X	X	X	N
6	HAI CO	67.7	M	1	2012	√	-	X	X	**√**	-	X	X	X	X	N
7	HAI HCFO	32.4	F	2	2012	X	-	X	X	X	-	X	X	X	X	N
8	**CAI**	**58.5**	**F**	**2**	**2012**	**X**	**X**	**√**	**√**	**√**	**-**	**√**	**X**	**X**	**X**	**Y**
9	HAI HCFO	89.0	F	2	2012	√	-	X	X	**√**	-	X	X	X	X	N
10	CAI	25.2	F	4	2012	x	X	X	X	**√**	I	X	X	X	X	N
11	CAI	89.3	F	8	2012	√	-	√	√	x	√	X	X	X	X	N
12	**HAI HCFO**	**89.2**	**M**	**11**	**2012**	**√**	**√**	**√**	**√**	**√**	**-**	**√**	**√**	**X**	**X**	**Y**
13	CAI	20.3	F	2	2013	X	-	X	X	X	-	X	X	X	X	N
14	CAI	27.7	F	11	2013	X	-	-	**√**	X	-	X	X	X	X	N

5.3 RIBOTYPE UK 056

Overall, ribotype UK 056 was the third most prevalent ribotype in WA, comprising 6.3% of ribotyped isolates. This strain has been previously detected in Western Australian sheep (211), and was detected in 7.7% of <7-day-old calves in another recent Western Australian study (212). As shown in Figure 13, just over half (51.2%) of UK 056 cases were determined to be HAI HCFO, with 31.4% classified as CAI. There were no significant differences in the proportion of CA-CDI reported among UK 056 compared to other HI-CDI cases (OR = 1.10. p = 0.683).

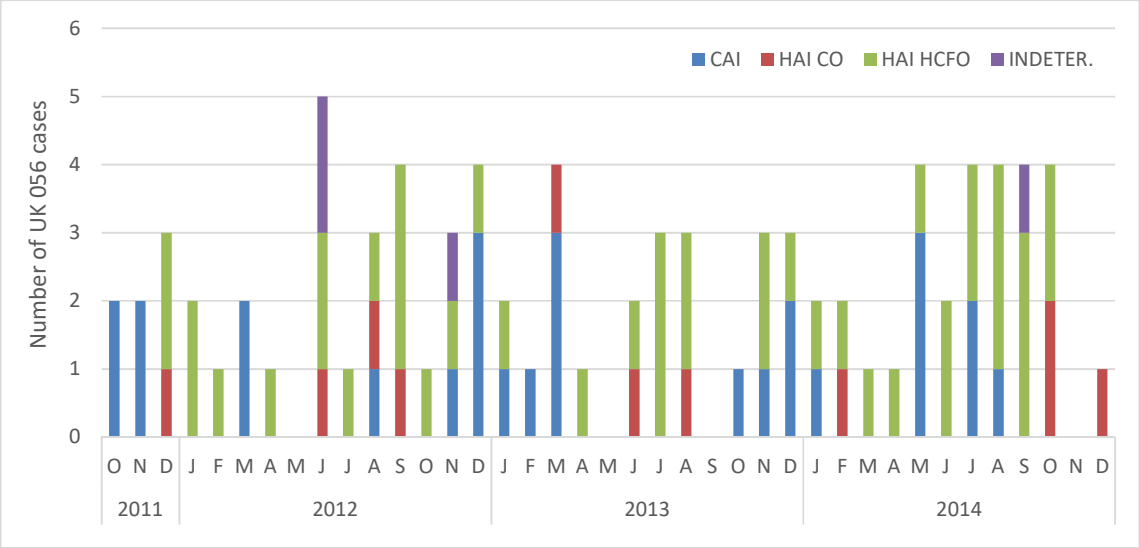


Figure 13. UK 056 cases of CDI reported to HISWA, Western Australia, 2011 - 2014

5.4 RIBOTYPE UK 012

Ribotype UK 012 is of interest due to its emergence from a previously uncommon ribotype in WA to become one of the most prevalent ribotypes. Prior to 2013, there was only one documented cases of UK 012 in the HISWA dataset (Figure 14). Between 01 October 2011 and 31 December 2014, 43 cases of UK 012 were detected in metropolitan hospitals via HISWA. Following an initial case detected in September 2012, and another single case in February 2013, there was a cluster of cases commencing September 2013. From a previously unknown strain, UK 012 was the equal seventh most prevalent strain in 2013 and equal second most prevalent in 2014 (Table 10). For the overall study period 2011 - 2014, UK 012 was the 5th most commonly isolated ribotype (Table 8).

Table 10. Prevalent ribotypes in metropolitan hospitals, Western Australia, 2011 – 2014.

	2011			2012			2013			2014		
	Strain	N	%	Strain	N	%	Strain	N	%	Strain	N	%
1	UK 014/020 [G]	41	31%	UK 014/020 [G]	111	25%	UK 014/020 [G]	50	16%	UK 014/020 [G]	182	39%
2	UK 054	12	9%	UK 002	56	13%	UK 056	23	7%	UK 002	31	7%
3	UK 002	8	6%	UK 056	27	6%	UK 002	16	5%	**UK 012**	31	7%
4	UK 018	8	6%	UK 053	15	3%	QX 076	15	5%	UK 056	29	6%
5	UK 015/UK 193	7	5%	UK 010	14	3%	UK 054	14	5%	UK 046	14	3%
6	UK 056	7	5%	UK 015/UK 193	14	3%	UK 046	12	4%	UK 103	12	3%
7	UK 064	5	4%	UK 017	13	3%	UK 005	11	4%	UK 010	9	2%
8	UK 244	5	4%	UK 018	12	3%	**UK 012**	11	4%	UK 018	8	2%
9	UK 046	4	3%	UK 005	11	2%	UK 018	11	4%	UK 054	8	2%
10	QX 001	3	2%	UK 046	11	2%	UK 017	10	3%	UK 070	8	2%

Enhanced surveillance data demonstrated 18/43 (42%) of UK 012 cases CA-CDI and 24/43 (56%) were HA-CDI (21 with hospital onset and 3 with community onset) (Figure 14). UK 012 cases were more likely to be CA-CDI (OR 1.75, CI_{95} 0.94 – 3.24) and female (OR 1.81, CI_{95} 0.95 – 3.46) compared to other CDI cases, although these differences were not statistically significant (p = 0.077 and 0.071, respectively), likely due to low case numbers in each category. Each case's admission notes were assessed for indicators of severe disease. As shown in Table 11, none of the 43 cases was deemed to be severe, based on assessment against these criteria.

Table 11. UK 012 CDI cases, Western Australia, 2011 -2014 - assessment for severe disease.

CASE	CLASSIFICATION	AGE	SEX	INFECTION MONTH	INFECTION YEAR	>60 YEARS	TEMP > 38.3°C	ALBUMIN <25g/L	WBC> 15,000 c/mL	↓RENAL FUNCTION	↑SERUM LACTATE	PMC	ICU ADMISSION	COLECTOMY	TOXIC MEGACOLON	SEVERE DISEASE
1	CAI	53	F	9	2012	X	-	X	√	X	√	X	X	X	X	N
2	HAI HCFO	75	M	2	2013	√	-	X	X	√	-	X	X	X	X	N
3	HAI HCFO	50	F	9	2013	X	-	X	X	√	-	X	X	X	X	N
4	CAI	2	M	9	2013	X	-	-	-	-	-	-	-	-	-	N
5	HAI HCFO	73	M	9	2013	√	-	√	√	X	√	X	X	X	X	N
6	HAI HCFO	62	M	9	2013	√	-	X	X	X	X	X	X	X	X	N
7	CAI	66	M	10	2013	√	X	√	X	X	-	X	X	X	X	N
8	HAI HCFO	65	F	10	2013	√	-	√	√	√	X	X	X	X	X	N
9	HAI HCFO	76	M	10	2013	√	-	X	X	√	-	X	X	X	X	N
10	HAI HCFO	93	M	10	2013	√	-	X	X	√	-	X	X	X	X	N
11	HAI HCFO	46	F	10	2013	X	-	X	X	√	-	X	X	X	X	N
12	CAI	86	M	12	2013	√	X	X	√	X	√	X	X	X	X	N
13	HAI CO	76	F	1	2014	√	-	X	X	√	-	X	X	X	X	N
14	HAI HCFO	53	F	1	2014	X	-	√	X	X	-	X	X	X	X	N
15	CAI	42	F	2	2014	X	X	X	X	X	-	X	X	X	X	N
16	CAI	30	M	2	2014	X	X	X	X	X	-	X	X	X	X	N
17	CAI	54	F	2	2014	X	-	X	√	√	√	X	X	X	X	N
18	CAI	15	F	2	2014	X	-	-	-	-	-	-	-	-	-	N
19	HAI HCFO	75	F	3	2014	√	-	X	X	X	X	X	X	X	X	N
20	HAI CO	61	F	4	2014	√	√	√	X	√	-	X	X	X	X	N
21	HAI HCFO	61	F	4	2014	√	-	X	√	√	-	X	X	X	X	N
22	HAI HCFO	78	F	4	2014	√	-	-	√	√	-	X	X	X	X	N
23	HAI HCFO	88	M	4	2014	√	-	√	X	√	√	X	X	X	X	N
24	CAI	73	F	5	2014	√	X	X	X	X	-	X	X	X	X	N
25	HAI HCFO	85	F	5	2014	√	-	X	√	X	√	X	X	X	X	N

26	HAI HCFO	91	F	5	2014	√	-	X	X	X	I	X	X	X	X	N
27	CAI	30	F	6	2014	X	X	X	X	X	-	X	X	X	X	N
28	HAI CO	61	F	6	2014	√	X	√	X	X	√	X	X	X	X	N
29	CAI	32	F	7	2014	X	-	X	X	X	-	X	X	X	X	N
30	HAI HCFO	60	M	7	2014	X	-	X	X	X	-	X	X	X	X	N
31	CAI	80	F	7	2014	√	X	X	X	X	-	X	X	X	X	N
32	CAI	80	M	8	2014	√	X	X	X	X	-	X	X	X	X	N
33	HAI HCFO	95	F	8	2014	√	-	X	X	X	-	X	X	X	X	N
34	CAI	57	F	9	2014	X	-	X	X	X	-	X	X	X	X	N
35	HAI HCFO	89	F	9	2014	√	-	X	X	X	-	X	X	X	X	N
36	HAI HCFO	89	F	9	2014	√	-	X	X	X	-	X	X	X	X	N
37	CAI	72	M	9	2014	√	X	X	X	X	X	X	X	X	X	N
38	HAI HCFO	84	M	9	2014	√	-	X	X	X	-	X	X	X	X	N
39	CAI	22	F	10	2014	X	-	√	X	X	X	X	X	X	X	N
40	HAI HCFO	70	F	10	2014	√	-	X	X	X	-	X	X	X	X	N
41	CAI	4	F	11	2014	X	-	X	X	X	-	X	X	X	X	N
42	INDETER.	89	F	11	2014	√	-	X	X	X	-	N	N	N	X	N
43	CAI	73	F	11	2014	√	-	√	X	√	-	X	X	X	X	N

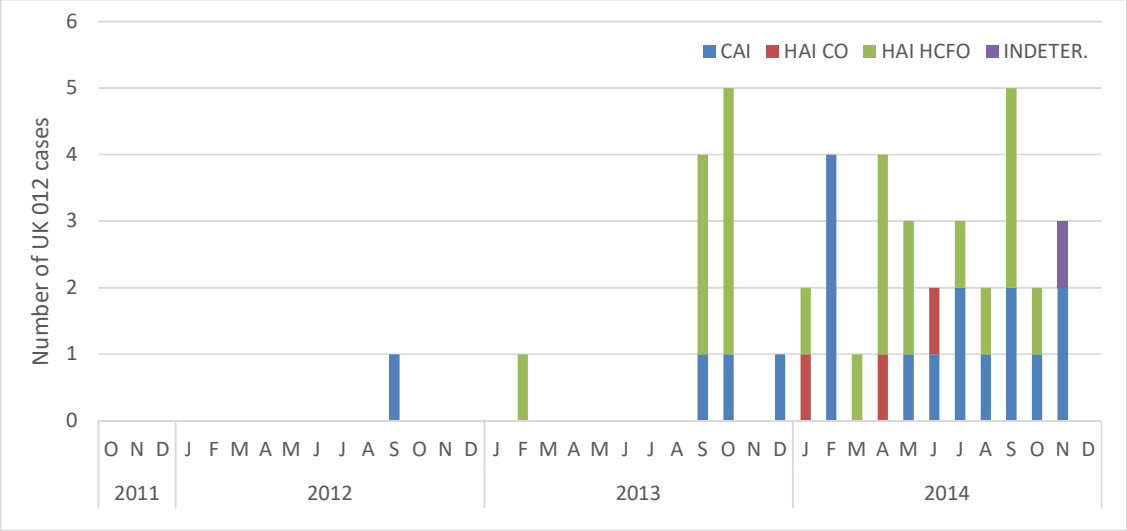


Figure 14. UK 012 cases of CDI reported to HISWA, Western Australia, 2011 – 2014.

5.5 COMPARISON OF COMMUNITY- AND HEALTHCARE-ASSOCIATED RIBOTYPES

For CAI and HAI HCFO, 400 and 697 specimens, respectively, had ribotyping data available. CAI was caused by 83 unique ribotypes and HAI HCFO by 119 unique ribotypes. The UK 014/020 group was the most commonly isolated ribotype in CA cases, accounting for 34% of the total isolates for which ribotyping data were available. As previously mentioned, ribotype UK 012 was the equal 4th most prevalent ribotype among CA cases, accounting for 6% of the total. The UK 014/020 group was also the most common ribotype isolated from HAI HCFO cases, accounting for 23% of the total.

There were similarities in the most commonly isolated strains in both groups, with 51 ribotypes common among both CAI and HAI HCFO cases. Thirty-three ribotypes occurred in CAI only and 69 in HAI HCFO only. The unique ribotypes were more often unique occurrences (median 1 case count, range 1 - 5), with 18/20 of the most prevalent CAI strains also present in HAI HCFO cases, and vice versa. The relative frequencies of common ribotypes from isolates of CAI and HAI HCFO infections are shown in Figure 15.

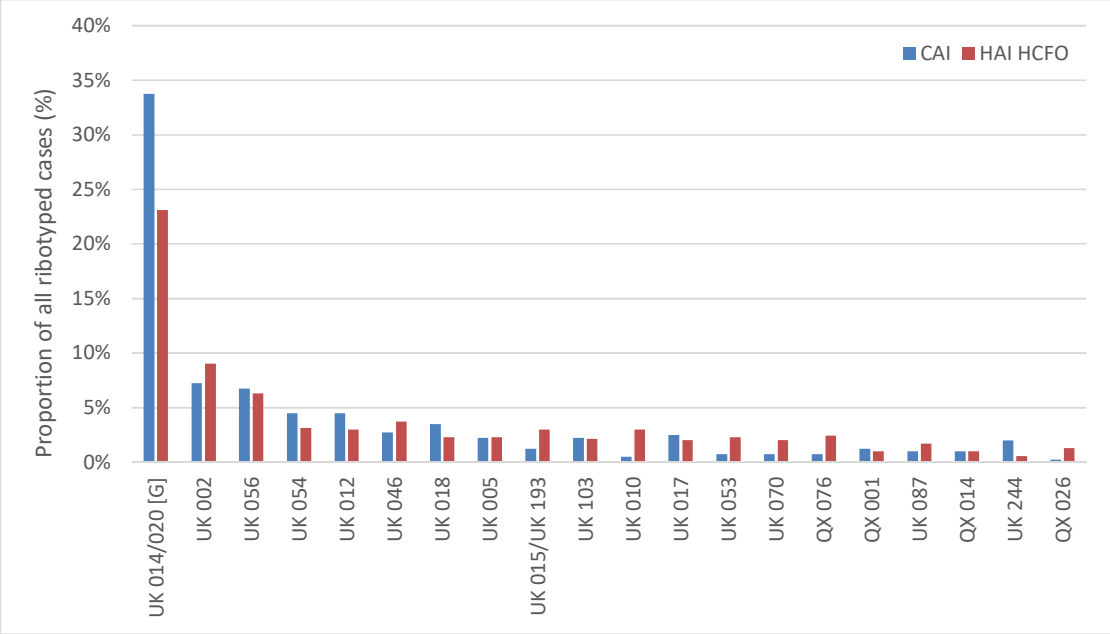
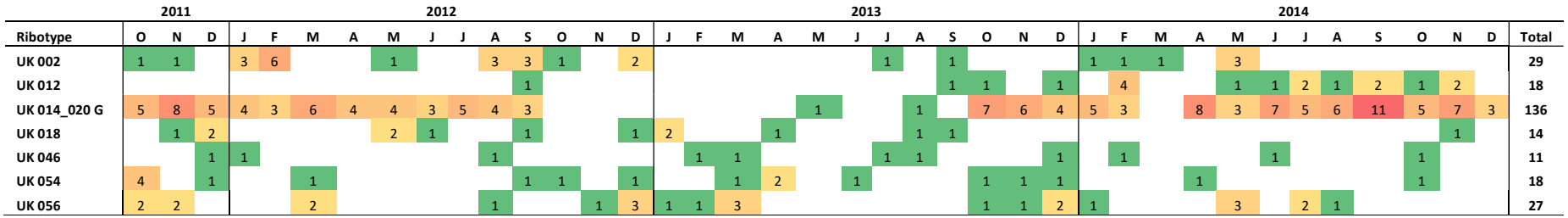


Figure 15. Proportions of common ribotypes causing CDI from CAI and HAI HCFO cases, Western Australia, 2011 – 2014.

Monthly case counts of common ribotypes (>10 over the study period) isolated from HI-CDI cases are shown in Figure 16. The emergence of UK 012 is evident, along with the decline in UK 244 cases in 2013, and eventual disappearance in 2014. No other ribotypes stand out in terms of emergence or decline over the study period; some appear more consistently than others in the majority of reporting months. A similar pattern emerges when comparing ribotype patterns of CA (Figure 17) and HA (Figure 18) ribotype patterns, with emergent UK 012 shown in both groups.

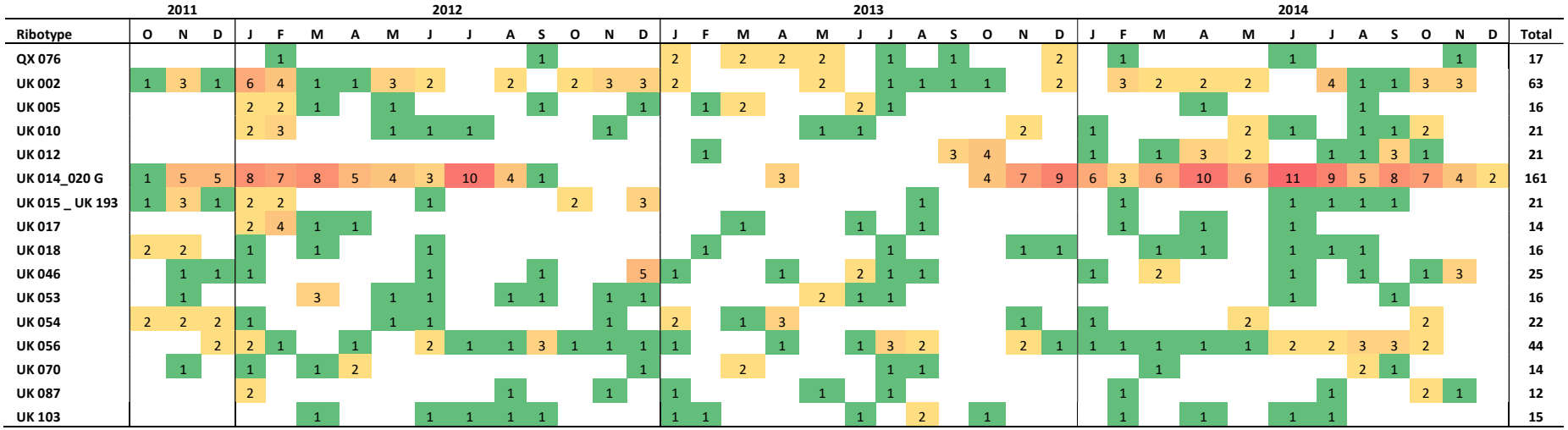
	2011			2012												2013												2014										
Ribotype	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	Total
QX 001	1	1	1		5	2		1								1	1	1	1				1					1	1				1							19
QX 014		1		1	2					1	1				1					2														2	2			1	14
QX 026								1		1	1											1						1	1					2	2		1		11
QX 076					1							1			1	2		3	2	2		2		1	1		2		1				1	1		1		1		23
UK 002	3	4	1	10	13	3	1	5	4	1	5	3	3	3	5	2	2	1		2		2	2	2	1		2	1	4	3	3	5	1	5	1	2	3	3		111
UK 005		1	1	2	2	1	1	1	1			2			1		1	2	3		2	1				1	1	1			1		1		1	1	1			30
UK 010				2	6	1		1	1	1	1			1			1			1	1					2		1				2	1		1	1	2		1	28
UK 012												1					1							4	5		1	2	4	1	4	3	2	3	2	5	2	3		43
UK 014/020 [G]	7	17	17	20	13	18	11	10	7	15	10	6		1				3	1	1		1		12	18	14	13	6	10	22	11	21	18	19	21	17	18	6	384
UK 015/UK 193	2	4	1	5	2				1	1			2		3			1				2						1				1	1	1	2				30
UK 017	1		1	2	4	2	1			1		1		1	1	1		1	2		2		1	1			1	1			1				1					28
UK 018	2	4	2	1	1	2		2	2			3			1	2	1	1	1			1	1	1			1	2	1		1	1		1	1	1			2		39
UK 046		2	2	2					1		1	2			5	1	1	1	1			2	2	3				1	1		2	1		2		1	1	2	3		41
UK 053		1			1	3		1	1	2	1	1		2	3			1		3	2	1								1			1			2	1			28
UK 054	6	2	4	1		1		1	1		1	3	1	1	1	2		2	5		1			1	2	1	1			1	3					3				45
UK 056	2	2	3	2	1	2	1		5	1	3	4	1	3	4	2	1	4	1			2	3	3		1	3	3	2	2	1	1	4	2	4	4	4	4		1	86
UK 064	2	2	1				1				1	1					1															1	1						11
UK 070	1	1		2	1	2	2								1			2		1	1	1	2							1		2		2	1	1		1	25
UK 087				2		1					1			1	1	1				2		1						2					1	1		2	1	1	18
UK 103				1		1			1	1	3	1				1	2				1		3		1		1		1	1	3		1	3	2				1	29
UK 244	3	1	1	1	3		1				1			1			1									1														14

Figure 16. Common ribotypes of *C. difficile* isolated from HI-CDI cases, Western Australia, 2011 – 2014.



	2011			2012											2013												2014											
Ribotype	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	Total
UK 002	1	1		3	6			1			3	3	1		2							1		1				1	1	1		3								29
UK 012												1												1	1		1				4			1	1	2	1	2	1	2		18
UK 014_020 G	5	8	5	4	3	6	4	4	3	5	4	3							1			1			7	6	4	5	3		8	3	7	5	6	11	5	7	3	136
UK 018		1	2					2	1			1			1	2			1				1	1														1		14
UK 046			1	1							1						1	1				1	1				1		1				1				1			11
UK 054	4		1			1						1	1		1			1	2		1				1	1	1				1						1			18
UK 056	2	2				2					1			1	3	1	1	3							1	1	2	1				3		2	1					27

Figure 17. Common ribotypes of *C. difficile* isolated from CAI cases, Western Australia, 2011 – 2014.



	2011			2012											2013												2014											
Ribotype	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	Total
QX 076					1							1				2		2	2	2		1		1			2		1			1						1		17
UK 002	1	3	1	6	4	1	1	3	2			2		2	3	3	2				2		1	1	1	1	2		3	2	2	2		4	1	1	3	3		63
UK 005				2	2	1		1				1			1		1	2				2	1								1				1					16
UK 010				2	3			1	1	1				1						1	1				2		1			2	1			1	1	2			21
UK 012																1								3	4			1		1	3	2		1	1	3	1			21
UK 014_020 G	1	5	5	8	7	8	5	4	3	10	4	1						3							4	7	9	6	3	6	10	6	11	9	5	8	7	4	2	161
UK 015 _ UK 193	1	3	1	2	2				1				2		3								1						1			1	1	1	1					21
UK 017				2	4	1	1											1				1	1						1		1									14
UK 018	2	2		1		1			1								1						1			1	1			1	1		1	1	1					16
UK 046		1	1	1					1			1			5	1			1			2	1	1				1		2			1		1		1	3		25
UK 053		1				3		1	1		1	1		1	1					2		1	1									1			1					16
UK 054	2	2	2	1				1	1					1		2		1	3							1		1				2					2			22
UK 056			2	2	1		1		2	1	1	3	1	1	1	1			1			1	3	2			2	1	1	1	1	1	2	2	3	3	2			44
UK 070		1		1		1	2								1			2					1	1						1					2	1				14
UK 087				2							1			1		1				1			1					1					1				2	1		12
UK 103						1			1	1	1	1				1	1					1		2		1			1		1			1	1					15

Figure 18. Common ribotypes of *C. difficile* isolated from HAI HCFO cases, Western Australia, 2011 – 2014.

5.6 DIVERSITY OF RIBOTYPES AMONG SUB-POPULATIONS

Within-group diversity analysis demonstrated a significantly higher diversity of ribotypes among HAI HCFO cases than CAI cases (Figure 19), which was expected given the relatively higher number of unique isolated present in this group. There were no significant differences observed by age group for the total dataset (Figure 20), although a decrease in diversity was observed among CAI cases 20-39 years and 40-59 years (Figure 21). Analysis of annual data demonstrates increasing diversity across the study period between 2011, and 2013, however there was a decline in 2014 (Figure 22). As shown in Table 10, this related to a dominance of the UK 014/020 group in this year, with this single strain accounting for 39% of all ribotyped cases.

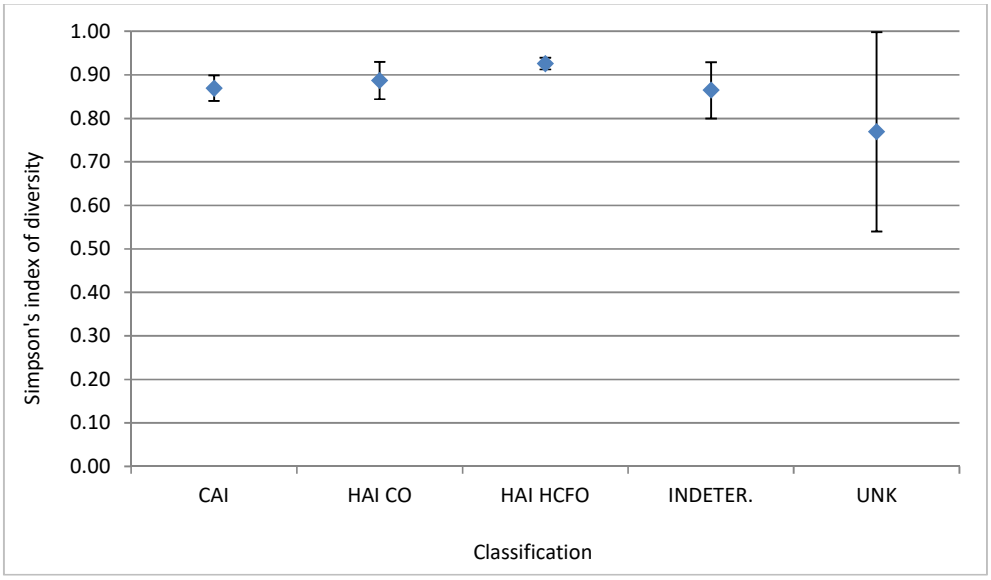


Figure 19. Diversity of ribotypes of *C, difficile*, by classification, Western Australia, 2011 – 2014.

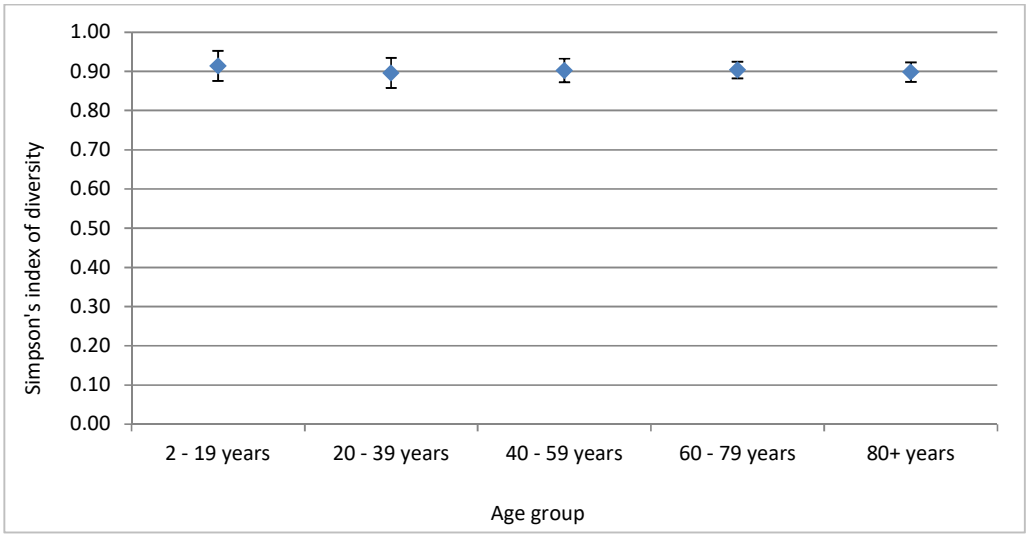


Figure 20. Diversity of ribotypes of *C. difficile*, by age group, Western Australia, 2011 – 2014.

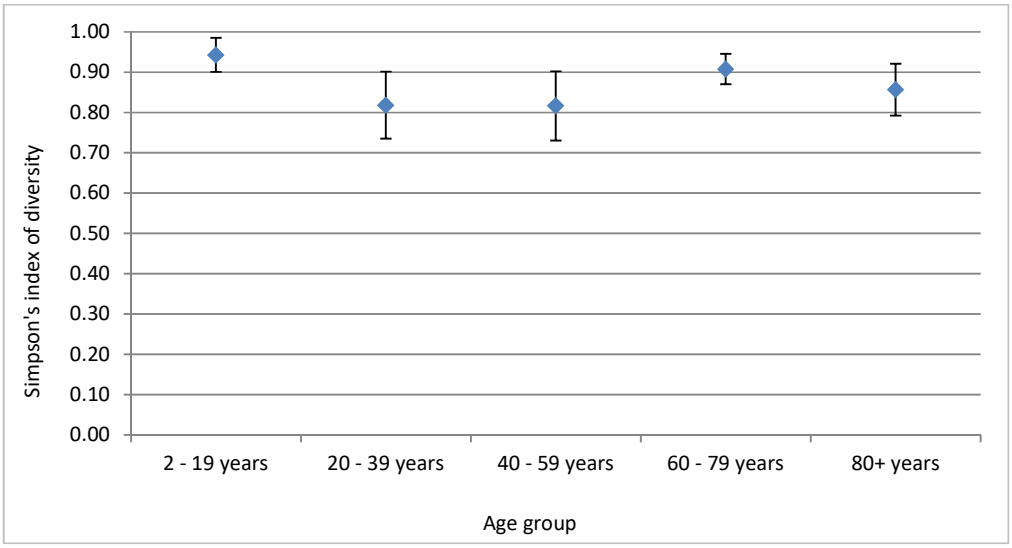


Figure 21. Diversity of ribotypes of *C. difficile* among CA-CDI cases, by age group, Western Australia, 2011 – 2014.

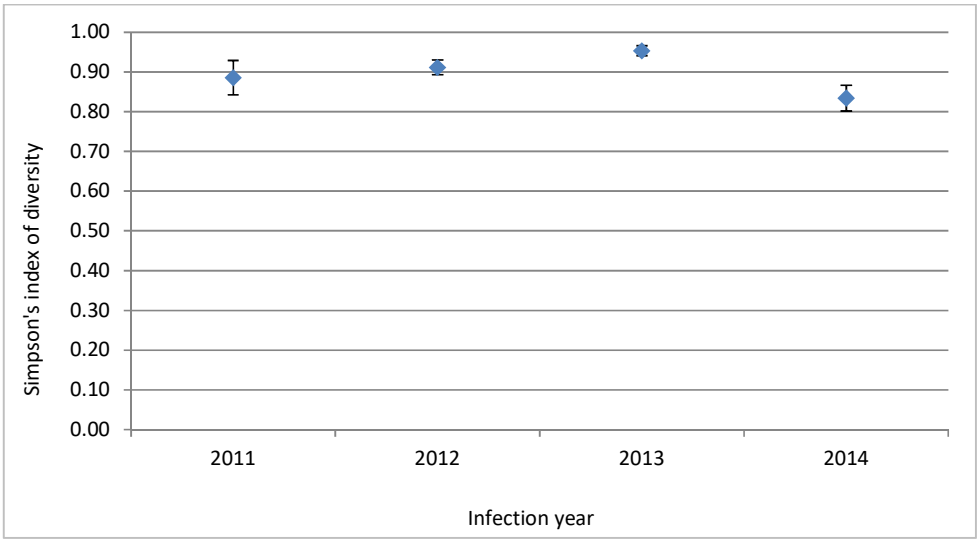


Figure 22. Diversity of ribotypes of *C. difficile*, by infection year, Western Australia, 2011 – 2014.

5.7 RECURRENT INFECTIONS (RELAPSE VS. REINFECTION)

Individuals may appear on the HISWA database more than once, provided at least 8 weeks had lapsed since their prior infection. In the dataset, 220 individuals had two or more presentations (recurrent infections) that met this criterion. Removing those for whom ribotyping was not available for at least two isolates, there were 70 individuals who had two (n=59) or three (n=11) episodes reported to HISWA with complete ribotyping data. Of these, 35 (50%) had a different ribotype for at least one of their repeat specimens, suggesting re-infection of a susceptible individual, rather than relapse with the original infecting strain.

6 ANTIMICROBIAL PRESCRIBING TRENDS

As Figure 23 shows, overall there was a slight increasing trend in prescriptions over the study period (R^2 = 0.0405). This increase was mainly associated with beta-lactams (R^2 = 0.074), with only marginal increases in cephalosporin prescriptions (R^2 = 0.017). Fluoroquinolone prescriptions showed a decreasing trend (R^2 = -0.0218), and macrolide prescriptions remain relatively stable for the study period (R^2 = 0.0002). There was no significant correlation between the total number of prescriptions and number of CA-CDI cases in each quarter (pwcorr = 0.2344, p > 0.4). There was also no significant correlation between beta-lactam

(pwcorr = 0.2867, p > 0.3) or cephalosporin (pwcorr = 0.0968, p > 0.7) prescriptions, and CA-CDI case numbers.

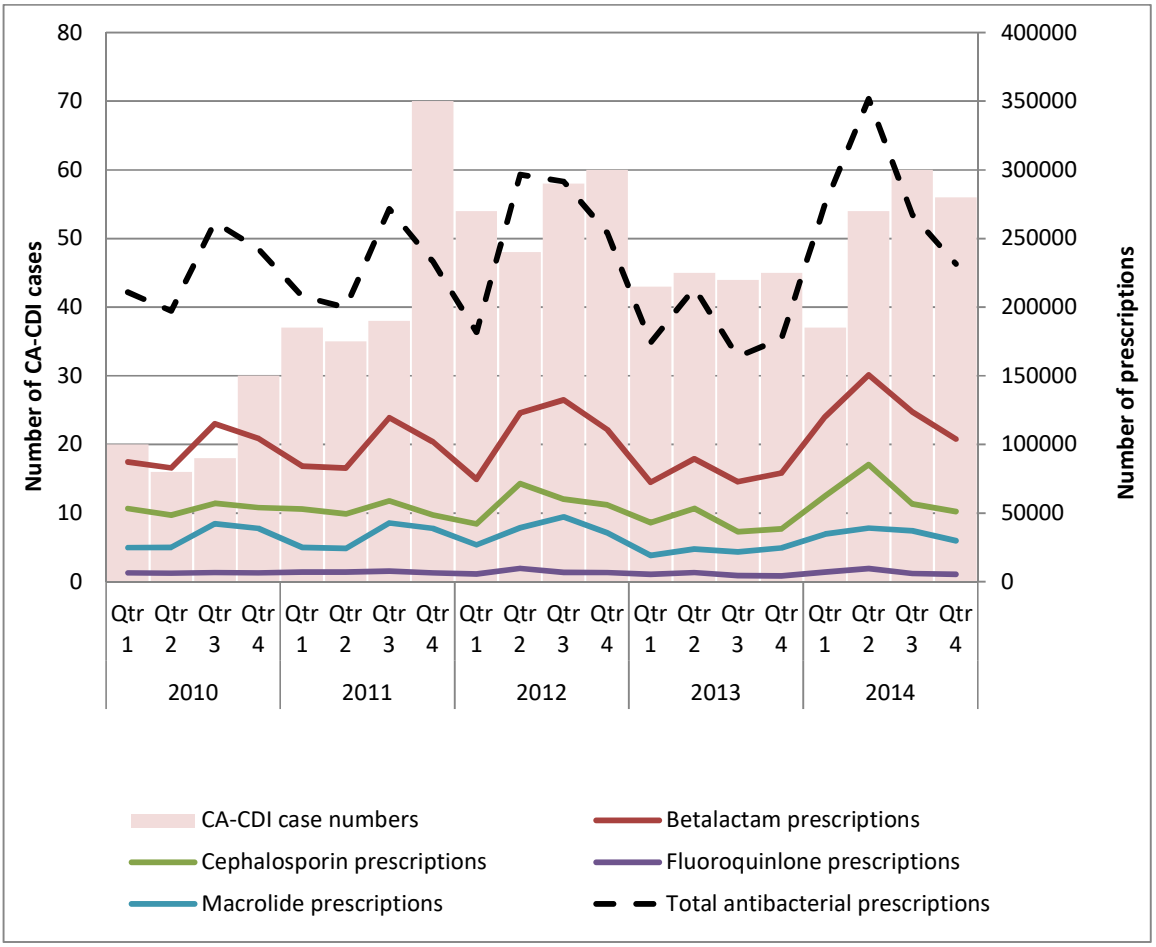


Figure 23. Prescriptions of antibacterials for systemic use and CA-CDI case numbers, Western Australia, 2010 – 2014.

CHAPTER 5 – DISCUSSION

1 INTRODUCTION

A substantial burden of CA infection found over the 5-year study period highlights the impact of infection that may be acquired outside of the hospital on HI-CDI rates in WA. The overall case numbers for both HA-CDI and CA-CDI cases showed an increasing burden on the healthcare system. Disease detected in the community (i.e. in patients attending a general practice) was not within the-scope of this project; however, it would not be unreasonable to assume that there was a growing incidence of CA-CDI outside of the hospital system. The trends in case counts, factors associated with CA-CDI and ribotyping data have implications for public health policy and action, and will be further explored below.

2 SIGNIFICANT RECENT DEVELOPMENTS IN THE LITERATURE

A comprehensive study of CDI in all Australian jurisdictions was published in 2014, although CA case data was not available for all geographical areas (302). From the data contributed by Tasmania, Victoria and Western Australia (including data from this dissertation), CA-CDI comprised 26% of all HI-CDI cases between 2010 and 2012 (302). Against the background of an overall increasing incidence of CDI in Australia, CA-CDI increased sharply in late-2011; the majority of jurisdictions observed a peak in their HI-CDI rates around this time, but it is unclear how much CA-CDI contributed to this peak overall.

In addition, two single-centre studies of CDI in New South Wales have recently been published (303, 304). The first, a retrospective observational study, determined 38/129 (29.4%) of cases were CA-CDI over a 12-month period (1 January – 31 December 2011). The second, another single year single centre study, yielded similar results, with 37/124 (29.8%) of cases determined to be CA-CDI. As single-year single-centre studies, however, it was not possible to establish how representative of the state these were, or whether there were any increases other in this region.

3 ENHANCED SURVEILLANCE

The differing characteristics of CA- and HA-CDI cases documented in the current study are suggestive of different risk factors for development of disease in these groups. Hospitalised patients who develop CDI exhibit the 'classic' profile for age (more elderly cases), along with co-morbidities which have resulted in the hospitalisation (11, 84). CDI more frequently occurs in younger populations in the community, often in people with no recent history of hospitalisation (which suggests no serious chronic co-morbidities) (30, 61, 80, 85-87).

The similar proportions of CA-CDI and HA-CDI detected per year across the 5-year period suggest that the increases in rates of HI-CDI observed in the HISWA dataset are likely attributable to overall increases in case numbers, rather than a growing incidence in one particular group. As HI-CDI cases are less likely to be representative of CA infections, it is difficult to determine if there are differential increases in the incidence CA and HA disease in the state as a whole based on the current study. A retrospective study of CA disease diagnosed in general practice may offer insights; however, lack of testing in this area may still result in an incomplete picture.

For the purposes of comparing CA-CDI and HA-CDI, HAI CO were deliberately excluded from the comparison. Grouping HAI CO with HAI HCFO cases is not appropriate for a number of reasons, which are discussed in section 3.2.

3.1 DIFFERENT DEMOGRAPHICS OF COMMUNITY- AND HEALTHCARE-ASSOCIATED CASES

The differing age and gender profiles of CA and HA cases indicates there are likely different risk factors for CA cases, and these should be further explored. While the risk factors for development of disease in hospitals are quite well understood, there is a clear need for more research into the risk factors for development of disease in the community, including host, medical and environmental exposures.

3.1.1 HEALTHCARE-ASSOCIATED INFECTIONS

While this study did not focus on HA-CDI, observations about local HA-CDI cases can be made based on the enhanced surveillance activities undertaken. The current study demonstrates a

significant increase in rates of HAI HCFO over the study period. The proportion of HAI HCFO remained stable over the 5- year reporting period, accounting for just over half of all cases reported. HAI HCFO cases were more frequently diagnosed at tertiary hospitals, which may reflect an overall population with more complicated diagnosis, and more comorbidities in these acute care facilities. HAI HCFO cases exhibited the 'classic' age profile, where the majority of cases occurred in patients >65 years old (OR 1.89, $p<0.001$).

While *C. difficile* has the potential to cause disease in people of all ages, infections in elderly patients are reported much more frequently than for other age groups (305, 306). Infections in patients with advancing age are also associated with increased attributable mortality (71, 306, 307), and this has been shown to be equivalent both in the community and healthcare facilities (308). The Canadian Nosocomial Infection Surveillance Program Study estimated the attributable mortality for CDI to be 3.5-times higher in patients older than 65 years (309), making this sub-group an important focus for research and interventions in the healthcare environment.

Endemic and epidemic (outbreak) infections are both a risk for hospitalised elderly patients, with special concerns surrounding colonisation and infection with multi-drug resistant organisms (MROs) (310). The incidence of infections that are a result of a healthcare intervention, or acquired during a stay in a healthcare facility, increases with advancing age (311, 312). A high rate of indwelling urinary catheter use in older patients, which can be unnecessary, means urinary tract infections are reportedly the most common HAI in this group (313, 314). A proportion of these infections is therefore potentially preventable, and this should be a concern when antibiotics are being prescribed frequently to treat preventable infections.

Antibiotic use is often an important (if not essential) precursor for CDI, and this also increases selective pressure for other MROs. Indeed, Sims et al. found the presence of indwelling catheters increased the odds of acute gastroenteritis by >2 (307). Although this study was not limited to *C. difficile*, it is clear from the results how choice of medical intervention can have serious implications for other adverse events. Treatment of preventable HAIs with antibiotics adds to the risk factors already present in these patients. Preventing HAIs in this group would

have a 2-fold effect, whereby the morbidity and mortality related to infection, along with risk factors for CDI and other MROs, are both reduced.

3.1.2 COMMUNITY-ASSOCIATED INFECTIONS

A generally younger age profile for CA-CDI was found in earlier studies (30, 86, 315). The HISWA enhanced surveillance data supported these findings, with a significantly lower median age among CA-CDI cases. CA-CDI was also more likely to be diagnosed at non-tertiary hospitals, which may indicate that people are using EDs at these hospitals as de facto primary care providers. It may be the case that people are more likely to present to smaller, outer metropolitan hospitals for complaints such as gastroenteritis than larger tertiary facilities. A lack of ED and outpatient data for the denominator makes this assertion difficult to evaluate. It is also reasonable to suspect that a much larger proportion of CA cases would be detected in general practice. This means current proportions of CA-CDI identified at hospitals are a considerable under-estimate. Conversely, this may also reflect a much lower number of HA-CDI cases being diagnosed at non-tertiary hospitals, resulting in a larger proportion of CA-CDI. However, this is unlikely given that testing for CDI is centralized for public non-tertiary hospitals. It is also possible that there is less clinical suspicion at smaller hospitals but again, this is unlikely.

The propensity for a higher proportion of CA-CDI in younger females warrants further investigation; and several risk factors that may be present in this group could explain these differences. The identification of neonates as a potential source of infection in the community (62, 96) may, at least partially, explain the higher rates in females in these age groups. A retrospective data linkage study matching cases in this group with the birth registry could help to determine if a child <2 years in the household is a risk factor for developing disease in the community, and this is currently underway.

Further, differences in antimicrobial prescribing trends could put this group at increased risk i.e. if women aged 20 – 29 were prescribed antimicrobials at a higher rate than the general population for conditions such as urinary tract infections. Antimicrobial prescribing data were not available to examine this hypothesis, however community-based studies investigating risk factors could ask for a medication history. Several studies have suggested the potential for

food to be a source of CDI, which could result in infection in the community (39, 41). If contaminated food does prove to be a significant risk factor, preparation of contaminated foods may, again, increase risk of disease in this group. Further research will be required to determine risk factors for this group.

For the purposes of CDI surveillance, the source of acquisition is particularly pertinent, although not necessarily easy to determine. From an infection prevention and control perspective, an organism acquired from outside the facility with disease precipitated by necessary treatment with antimicrobials within the facility may not reasonably be viewed as a preventable nosocomial infection. While the infection control implications to prevent spread are the same regardless of acquisition, the ability to prevent disease onset in a colonised individual shifts from an issue of environmental cleaning and hand hygiene to one of judicious use of antimicrobials.

In some cases, the appropriate treatment modality may have resulted in onset of disease acquired outside of the facility. In these instances, there is no 'fault' to be attributed to the facility; however healthcare epidemiologists should still be concerned with the sources outside of the hospital that lead to development of disease. Due to numerous questions surrounding the appropriate attribution of CDI to a particular facility, even amongst cases classified as HAI HCFO, strong caution is advised in using rates of CDI in hospitals as a measure of hospital performance.

3.2 LIMITATIONS OF CURRENT ENHANCED SURVEILLANCE DEFINITIONS

Current enhanced surveillance definitions attribute a case to a healthcare facility exposure if it occurred within 4 weeks of discharge, and provided the case was in hospital for a minimum of 48 h (HAI CO) (9, 53). There are several limitations with this definition. The necessary minimum 48 h stay as an inpatient prior to diagnosis for an infection to be considered potentially HA may not be necessary, as prolonged exposure to *C. difficile* is not required for infection, and potential sources of acquisition should be assessed in light of what is probable, possible or unlikely. Further evidence is needed to determine if this minimum 48 h stay may be resulting in misclassification of HAI CO cases as CAI cases.

Some patients frequently visit the hospital for day procedures (e.g. chemotherapy and dialysis) and, as a result, have not had a length of stay more than 48 h prior to diagnosis. With frequent contact with the hospital environment (which previous studies have shown can be contaminated with *C. difficile* spores) up to 3 – 4 times per week, ruling out the hospital as a source of acquisition may result in misclassification.

There are other circumstances under which application of the current enhanced surveillance definitions may be more likely to result in misclassification. One group is 'hospital in the home' (HITH) patients, who are provided hospital care via in-home medical visits, however are considered 'inpatients' of the hospital for this period for the reporting of bed days, and often in the reporting of other HA infections (i.e. central line associated bloodstream infections). While considered inpatients for reporting purposes, these cases reside in their home for the duration of their treatment, and therefore acquisition of the infection has occurred outside of the hospital setting. It is proposed that HITH patients not be considered inpatients for the purposes of surveillance, and infections occurring in these populations should be classified CAI.

The current study demonstrated that after a minimum of 8 weeks, 50% of cases with recurrent infections (with ribotyping data available) had different ribotypes reported. For these cases, it can be concluded that the recurrence was a result of reinfection, rather than relapse with the initial infecting organism. Without more discriminatory typing methods (i.e. whole genome sequencing) available for the remainder of cases, it is not possible to say with certainty if these represented true relapse or re-infection with the same strain. Current definitions do not take into account evidence of a reinfection post-discharge, and represent somewhat of an arbitrary cut-off in terms of attribution to a treating facility.

In addition, there is little evidence concerning the number of HAI HCFO cases who may have been colonised on admission to hospital, and only developed disease after being exposed to antimicrobials as part of their medical management. The rates of colonisation in 'healthy individuals' are relatively low (287, 288) (and likely to be zero), however people being admitted to hospital may not be representative of a normal, healthy population, and the rates of transient carriage in this group are not known locally. The work of Eyre and colleagues on over 1,200 cases of *C. difficile* over a 3.6-year period found that only 35% of cases were genetically

related to at least one previous case (143). These data support the hypothesis that asymptomatic colonisation on admission to a HCF has a significant impact on case numbers, even on those deemed HAI HCFO. As such further work on case definitions is required, as outlined in Chapter 7.

4 RIBOTYPING

The addition of ribotyping into such a large dataset with enhanced surveillance over an extended period has allowed a unique insight into changing prevalence of *C. difficile* strains over time among CA and HA infections. The ribotyping prevalence results show similarities to one other Australian jurisdiction (316), however differ from the most commonly reported isolates from a recent survey (301) in another Australian jurisdiction, suggesting there may be differences in common isolates within Australia in limited geographical locations/hospital sites. A more comprehensive, Australia-wide survey of 2010 isolates across 6 states and territories found the UK 014/020 to be the most prevalent ribotype in all jurisdictions (317). These results were replicated in another Australia-wide survey of isolated from 2012 (T. Riley, personal communication). There are likely to be differences in selecting cases based on hospitals as opposed to laboratories; the latter may be more representative of all patients within a geographic region and account for dominant strains that may be present at one particular facility, which may skew hospital-based surveys.

4.1 RIBOTYPE UK 014/020 GROUP

The UK 014/020 group has been identified in a previous European hospital-based study as the most prevalent ribotype (70), although overall this did not comprise such a large proportion of all cases as observed in the WA dataset. This group was also identified in general practice in Denmark as the most prevalent ribotype among CA-CDI cases, comprising 32% of all cases in an 18-month period (318). Further, prevalence surveys have shown UK 014/020 to be a common ribotype among animals (149), humans and the environment, suggesting either interspecies transmission and/or a common source (148).

Locally, a small South Australian study has found UK 014 to be the most common ribotype among a sample of CA-CDI and HA-CDI cases (316). In another recent Australian study, UK 014 was the most prevalent ribotype detected among Australian piglets (215). One of the interesting findings of this research was the near-disappearance of this ribotype group for almost a year between 2012 and 2013, in both HA and CA cases. The fact that this ribotype sharply declined and re-emerged during the same months suggests that CA and HA infections are not occurring in isolation of one another in two separate, closed systems, but rather there are links between the two. If CA and HA cases were occurring with minimal cross over, the disappearance of one ribotype in one setting should not be reflected in the other. While this does not confirm a directional flow of cases one way or the other, the evidence from the literature suggests similar ribotyping patterns are not reflective of hospital cases causing infection in the community, and rather this is likely the result of community-acquired strains being imported into hospitals (29), (143).

4.2 RIBOTYPE UK 244

The emergence of ribotype UK 244 in Australia, with a particular focus on Western Australia, for the period 2010 – 2012 has been described in a paper published in *Eurosurveillance* (146). The cases of this ribotype being detected in WA coincided with a cluster of cases identified in New South Wales by the WA State Reference Laboratory (146). At the same time Victoria reported 12 cases (originally thought to be ribotype UK 027 due to identification as such using the GeneXpert system), and a further retrospective ribotyping analysis performed in Queensland for the period 10 April – 15 June 2012 revealed 7 UK 244 cases, making this the third most prominent ribotype in that state (301).

A retrospective cohort study on the 12 Victorian UK 244 cases revealed that this ribotype was associated with more severe disease and higher mortality (145). These results were confirmed in a New Zealand based study of UK 244 cases, which found a higher odds of severe disease among cases than controls infected with other ribotypes (319). The enhanced analysis on WA cases for severe disease did not find a large proportion of cases with markers of severe disease (2/11, 18%), although the sample size was relatively small. This ribotype signifies an important

introduction of a strain similar to UK 027, with the potential to cause severe disease, particularly in hospitalised populations with existing comorbidities.

4.3 Ribotype UK 056

As previously noted, ribotype UK 056 has been detected in production animals in Australia (211, 212). This ribotype is uncommon among non-Australian production animals (Table 3), and the consistently high prevalence among human isolates is of interest. Non-detection of ribotype UK 078 among calves in this same study, along with the lack of UK 078 among human isolates, is also a significant finding. In regions where UK 078 is prevalent among production animals, this ribotype is also found in food and humans (Table 3 and Table 4).

While there were no Australian studies that detected UK 056 in meat samples (no Australian meat studies were found in the literature review), evidence that this strain is prevalent in food could further support a foodborne infection hypothesis. The similar proportions of HA-CDI among UK 056 cases as compared to other HI-CDI suggests that exposure to contaminated food can occur inside and outside a healthcare facility. If hospital foods are sourced from local produce, there is no reason to suspect that this would not be the case.

4.4 Ribotype UK 012

The emergence of 'hypervirulent' ribotype UK 027 which caused outbreaks of severe disease in Europe and North America has highlighted the potential for differential strains of *C. difficile* to emerge and have a significant impact on healthcare systems. Ribotype UK 012 was virtually unknown in WA prior to late 2012, but has become the equal-second most prevalence strain over the study period (Table 8). The initial detection of this strain was a CA infection at a non-tertiary hospital in September 2012. One further case was reported in February 2013, and following this there were no further cases reported for a 6-month period, before a cluster of 9 cases in September and October of the same year (2 CAI and 7 HAI), all reported at various tertiary facilities.

Enhanced surveillance of UK 012 cases demonstrated a higher proportion of CA infection compared to the other ribotypes. Coupled with evidence that this strain was not established in WA metropolitan hospitals prior to 2013, this emergence may have occurred via

introduction in the community. While HI-CDI surveillance will identify a proportion of CA cases, this method of surveillance will underestimate the true prevalence of CA cases, who may either present for treatment outside of the hospital environment, or fail to seek medical attention due to mild, self-limiting disease. As such it is not possible to elucidate the prevalence of UK 012 in the community, or the likely time of introduction of UK 012 to the WA population.

The enhanced surveillance data support the potential for an emerging ribotype to rapidly become a highly prevalent strain among an established patient population. In major outbreaks of CDI during 2000 – 2003, UK 027 accounted for almost half of all ribotypes; prior to this the strain was relatively unknown, accounting for less than 0.1% of all isolates from 1984 – 1993 (320). While the assessments for severity suggest that ribotype UK 012 is not of particular significance in terms of the potential to cause severe disease (0/43 cases were classified as severe disease), its rapid ascension to the second most prevalent ribotype on WA in a 2-year window demonstrates the need for continued surveillance in order to determine if any 'hypervirulent' strains are establishing themselves in the community or healthcare environments.

The enhanced surveillance data supports the possibility that this may have been a community-based introduction, although more robust sentinel community surveillance would be required to support this assertion. In the case of UK 027, a French research team identified numerous cases at a large hospital were most likely acquired in the community, with onset in hospital after receiving antimicrobial therapy (321). These findings are concerning, as they highlight the potential for community-acquired disease to develop in hospitalised patients. Monitoring emerging ribotypes in the community would support ongoing healthcare surveillance systems, and allow identification of introduced strains of interest into the local population.

4.5 DIVERSITY OF RIBOTYPES IN THE WA POPULATION

Ribotyping data demonstrated a large diversity among cases, particularly among HA-CDI cases. An advantage of this diversity is the ability to rule out case-to-case transmission in hospitals among those who have reported a unique isolate. This does, however, present a challenge in determining the source of introduction of these strains for HA-CDI cases. One possibility is that

these cases were colonised with the organism on admission to hospital, and some alteration to host risk factors (e.g. administration of antimicrobial therapy) resulted in the development of symptomatic disease. For these cases, place of acquisition is different to the place of disease development, which may be a complicating factor when attempting to attribute 'preventable' HAI to a treating facility. The data from this study provide evidence to support this hypothesis, with a high level of diversity among HA cases. Evidence of a high level of diversity among CDI cases (including hospitalised cases) using highly discriminatory techniques (i.e. whole genome sequencing) has previously been shown (143), and further supports the need for mechanisms to understand underlying sources of infection.

4.6 RELAPSE VS REINFECTION

Relapse after a first episode of CDI within 2 months occurs in approximately 15 – 35% of cases (322). A substantial proportion (50%) of patients who had multiple specimens reported to HISWA (>8 weeks apart) were infected with different ribotypes on the second (or third) specimen. For this group of patients, relapse can be ruled out, as the susceptible case must have been reinfected and developed symptomatic disease. Previous, single centre studies have demonstrated that between 38% and 56% of recurrences of CDI were due to reinfection (323-325). In a multi-centre study (similar to the current project) Barbut and colleagues found a similar proportion (48.4%) were due to reinfection rather than relapse (322). Local data on reinfection vs relapse was only available for a small proportion of cases, however this trend warrants further investigation.

Current case definitions define a recurrence as occurring within 8 weeks, and enhanced surveillance definitions currently ascribe HAI CO to any facility if a case occurs within 4 weeks of discharge. In order to determine if both of these definitions are accurately reflecting case activity, a review of variable ribotypes among individuals should be conducted. Although testing for cure is not recommended, laboratory data demonstrates that many cases have repeat specimens taken within 8 weeks of their initial diagnosis. Identifying individuals with repeat specimens collected at varying time points following the initial diagnosis would allow determination as to whether people are becoming reinfected within 8 weeks (and how

commonly), and whether HAI CO cases occurring within 4 weeks are likely to be due to acquisition outside of the admitting facility.

5 ANTIMICROBIAL PRESCRIBING TRENDS

Changing or increasing antimicrobial prescribing trends as a potential driver behind increasing disease warrant further investigation. Certain agents have been demonstrated to increase to risk of CDI (92, 93), and outbreaks of fluoroquinolone-resistant strains have occurred in countries with less-restrictive prescribing policies in this class of antibiotics (134). Pharmaceutical Benefits Scheme (PBS) prescribing data for the study period showed a slight upward trend in the number of prescriptions of antibacterials for systemic use. These data do not, however, account for the increase in numbers of CA infections observed during the study period, with no significant correlation between prescribing trends and CA case numbers. An increase in antimicrobial use in winter/spring months due to seasonal influenza and respiratory tract infections is expected. The results, however, did not demonstrate an increased number of cases during this period each year which may be attributed to this rise. Recent efforts in antimicrobial stewardship aimed at promoting judicious use of these drugs in primary care may be helping to curb prescription numbers in the community (326, 327).

A major limitation of the available data is that this is this is not limited to just prescriptions in general practice, but rather includes all prescriptions. The possibility that the prescribing rates increased in the community but decreased in hospitals over the study period, leading to overall stable rates, must be considered. There is, however, no evidence that supports this hypothesis, and based on the data obtained from the PBS, an increase in the use antimicrobials is not a primary driver behind increasing rates of CDI in the community. Future work determining prescribing rates within the community setting is required to strengthen the evidence base in this area.

Antimicrobial stewardship policies are currently in place among WA hospitals as part of facility accreditation and this is a clinical care standard for the Australian Commission on Safety and Quality in Healthcare (328). Such policies restrict the use of broad-spectrum antimicrobials,

with a view to preventing bacterial resistance. The result has been a limiting of the use of certain classes of antimicrobials e.g. fluoroquinolones, which has important implications for CDI. The 'hypervirulent' UK 027 is fluoroquinolone resistant, and the lack of restrictions on the use of these drugs overseas may have been a contributing factor in the proliferation of this ribotype. Ensuring that prescribers remain vigilant in hospital, community and veterinary settings remains an essential component of disease prevention and control strategies for CDI in the future.

6 OTHER POTENTIAL RISK FACTORS

Available data suggest that an increase in antimicrobial prescribing is not a major driving force for an increase in CA-CDI. If anything, there is increasing awareness among providers about the need for judicious prescribing practices in the community, to limit the spread of antimicrobial resistance. Without evidence that there is a larger proportion of people in the community who are susceptible to infection, the alternative hypothesis is that there is an increased prevalence of the organism in the homes or environment of these cases, which is resulting in an increase in disease. Despite several studies reporting the presence of *C. difficile* in the environment (160, 161, 168), no current studies available that support an *increase* in environmental contamination, so at this stage this remains an untested hypothesis.

There is also a possibility that there has been no material change the presence of *C. difficile* spores in the community, be it in homes, on food or via other reservoirs, which means that there has been some other alteration in host susceptibility that has driven an increase in recent years. The recent surge in popularity of alcohol-based hand rubs (ABHRs) as a means of hand hygiene in the home, coupled with evidence that these products are ineffective at removing *C. difficile* spores from hands (329), may be a factor associated with this increase in the community. A hospital-based study testing this hypothesis rejected the use of ABHRs as a driving factor for increased hospital rates of CDI (330). A community-based survey looking at risk factors in the home should take into account the use of these products as a primary mechanism for hand hygiene as a potential facilitator for development of disease.

7 LIMITATIONS

This project had a number of limitations. Full hospitalisation history was only available for admissions and presentations to public facilities. Discharge summaries were reviewed for evidence of direct transfer or recent hospitalization at a private facility, and where evidence of prior hospitalisation was available; the case was classified accordingly (including 'unknown' if hospitalisation dates could not be confirmed). However, there is a possibility that details of hospitalisation at a private facility within the 12 weeks prior to diagnosis may not have been recorded in the patient notes, and subsequently a patient may have been misclassified. This misclassification would result in HAI CO or indeterminate cases being incorrectly classified as CAI. The impact of this is not known, however the overall low rate of HAI CO cases in the dataset (based on previous admissions to public hospitals) suggests that this would not substantially alter the current classifications.

Per the international enhanced surveillance definition "*An HCF is defined as any acute care, long-term care, long-term acute care, or other facility in which skilled nursing care is provided and patients are admitted at least overnight* (53)." This means that a patient transferred out of an acute care facility and into a long-term care facility who develops symptomatic CDI within 4 weeks would be classified as HAI CO (attributed to the acute facility) rather than HAI HCFO (attributed to the long term care facility). This is relevant for tracking HAI HCFO as part of post-discharge surveillance for an individual facility; however, in the context of aggregated state surveillance this becomes less relevant, particularly when HAI HCFO and HAI CO are grouped together as 'HAI'.

The unavailability of ribotyping data over the full 5-year study period, and also the incomplete ribotyping data for the period 01 Oct 2011 – 31 Dec 2014 means a complete picture for our population was not available. There is a possibility that the ~40% of cases with missing ribotyping data could represent a significant proportion of unique isolates, or could skew the proportion presented in the results of this study. On a site-by-site basis, the range of complete ribotyping data was 44.8% - 67.2%, with an average of 61.7% typed at the three, largest tertiary hospitals. The proportion of non-ribotyped cases does vary across year by site, however these missing cases are not grouped in a particular pattern, and there is no reason to suspect that

the missing cases would result in differential misclassification of the overall proportions of ribotypes throughout the study period.

The final limitation is the inclusion of only metropolitan, public hospital cases as part of the study group. The major reason behind this restriction was data privacy around gathering information from private hospital cases, and the unavailability of a centralised patient system for country cases, which would have allowed for hospitalisation history review. Private hospitals represent the smallest proportion of HI-CDI cases out of all hospital groups, and country hospitals traditionally had the second lowest rates (although this has increased in recent times). The exclusion of these cases precludes an analysis of the entire state, including determination on whether certain geographical areas have unique ribotypes of interest and the burden of CA-CDI in hospitals outside of the metropolitan area. Future work with country hospitals to commence enhanced surveillance, and to include ribotyping, would allow for a more complete understanding of CA-CDI in this region.

8 SUMMARY OF MAJOR FINDINGS

All research questions, aims and objectives outlined in Chapter 3 were addressed in this research project. A summary of the major findings is outlined in Table 12. Overall, a higher proportion of CA-CDI was evident in 2014, as compared to the baseline year (2010). CA-CDI cases overall comprised approximately 30% of all HI-CDI, and were younger than HA-CDI cases. These findings are in keeping with the international literature. A higher proportion of CA-CDI in females aged 20 – 39 was a key finding, which warrants further investigations.

The documentation of emerging and potentially serious ribotypes demonstrates the ability for previously unknown strains to be introduced to, and amplify within, local populations. There is evidence that prominent strains in local animal populations are also found in human cases. Food surveys are required to determine if this is a possible link between the two. Ongoing monitoring of the circulating ribotypes is an essential part of statewide surveillance.

Table 12. Major findings of this research.

Aim	Objective	Significant findings
Question 1 What is the incidence of CA-CDI in WA, and is it increasing?		
Aim 1 To determine the incidence of CA-CDI cases, as a proportion of HI-CDI cases, reported to HISWA between 1 January 2010 and 31 December 2014	**Objective 1.1** Apply internationally accepted enhanced surveillance definitions to HI-CDI cases, to determine if they are CA-CDI or HA-CDI cases	Of 2,962 HI-CDI cases reviewed. 29.2% were CAI and 62.9% were HA-CDI (52.2% healthcare facility onset and 10.7% community onset). These findings are in keeping with other hospital-based CAI prevalence studies in the published literature.
	Objective 1.2 Describe the characteristics of HI-CDI cases for the study period	There was a significant increase in rates of HI-CDI at all hospital groups over the study period. Of the 2.962 cases reviewed, 55.4% of cases were female, with 84.1% diagnosed at a tertiary hospital. The median age was 67.15 years, and this decreased over the study period, which is likely related to the increase in CA-CDI case numbers.
	Objective 1.3 Determine evidence for increasing proportions of CA-CDI among HI-CDI cases, using trend analysis	Trend analysis did not find a significantly increasing proportion of CA-CDI cases over the study period relative to the total number of HI-CDI cases. The data did show a significant increase in proportions of CAI between 2010 and 2011 (24.7% to 31.1%, p=0.037); however, the proportion then remained relatively stable (no significant variations) across the rest of the reporting period (hence no significant trend). There was, however, more than double the case numbers of CA-CDI reported in 2014 (205), as compared to 2010 (84). This represents a 144% increase between 2010 and 2014, whereas HA-CDI (healthcare facility onset) case numbers increased by 70% over the same period. When comparing 2014 to 2010 (assuming 2010 as a baseline year), there was a borderline non-significant increase in the

		proportion of cases between the first and last year of the study period (p=0.061). Ongoing surveillance could determine and future year-on-year or longitudinal increases. As a hospital-based study, a growing proportion of CA-CDI outside of hospitals was not within scope.
	Objective 1.4 Describe patient characteristics of CA-CDI cases	CA-CDI cases were younger, and more likely to be diagnosed at a non-tertiary hospital. Further, females aged 20-39 years were almost twice as likely to be diagnosed with CA-CDI than HA-CDI, (OR 1.91, p = 0.006). This group warrants further investigation.
Question 2 Which ribotypes are prevalent among CA-CDI and HA-CDI cases?		
Aim 2 To determine common ribotypes among HISWA cases between 11 October 2011 and 31 December 2014	**Objective 2.1** Integrate available ribotyping data into the HISWA dataset	Routine ribotyping commenced on 1 October 2011. Of 2,252 eligible specimens, ribotyping data was available for 1,358 (60.3%) cases.
	Objective 2.2 Describe common ribotypes among HI-CDI cases, and within population sub-groups, including analysis of strain diversity	The most commonly ribotyped reported across the study was the UK 014/020 group, comprising a total 28.3% of all cases. The proportion of this ribotype fell to 16% in 2013, before increasing to a high of 39% in 2014. This group was associated with the highest proportion of cases among both CA-CDI and HA-CDI cases. This ribotype is common in Europe, among both humans and animals, and has also been found locally in Australian piglets. Other common ribotypes reported in WA HI-CDI cases include UK 002, UK 056, UK 054 and UK 012, although these make up significantly smaller proportions of cases (range 3.2% - 8.2%). The simultaneous disappearance of the UK 014/020 group in both CA and HA cases for ~1 year suggests that the hospital and community cases are linked; lack of international evidence

		of transmission from discharged symptomatic patients as a significant reservoir in the community suggests importation from the community to the hospital may be occurring frequently. There was higher diversity among HA-CDI cases than CA-CDI cases, with 69 unique ribotypes occurring only among HAI HCFO cases. There were no significant variations in diversity by any other subgroup, including age group, diagnosing hospital and reporting year.
	Objective 2.3 Document emerging strains and perform analysis for severe disease on ribotypes of interest	Three strains of particular interest were documented over the study period; UK 056, UK 244 and UK 012. UK 056 has been previously detected in Western Australian ovine species, and was detected in 7.7% of <7-day-old calves in another recent Western Australian study. Overall, ribotype UK 056 was the third most prevalent ribotype in WA, comprising 6.3% of ribotyped samples. Just over half (51.2%) of UK 056 cases were determined to be HAI HCFO, with 31.4% classified as CAI. UK 244 has previously been documented to cause severe disease. UK 244 was the 19th most prominent ribotype, with 14 detections accounting for 1% of cases. The majority of detections in the HISWA dataset occurred in 2011 – 2012, with no detections in 2014. UK 244 was significantly more likely to be diagnosed in among CAI cases compared with HAI HCFO cases (p=0.0262), with CAI accounting for 57% of cases. 2/14 (14%) of cases were determined to be severe disease, 1 CAI case and 1 HAI HCFO case.

		UK 012 is of interest due to its emergence from a previously uncommon ribotype in Western Australia to become one of the most prevalent ribotypes. Prior to 2013, there was only one documented cases of UK 012 in the HISWA dataset. Between 01 October 2011 and 31 December 2014, 43 cases of UK 012 were detected in metropolitan hospitals via HISWA. From a previously unknown strain, UK 012 was the equal second most prevalent in 2014. 18/43 (42%) of UK 012 cases CA-CDI and 24/43 (56%) were HA-CDI (21 with hospital onset and 3 with community onset). UK 012 cases were more likely to be CA-CDI (OR 1.75, CI_{95} 0.94 – 3.24) and female (OR 1.81, CI_{95} 0.95 – 3.46) compared to other CDI cases, although these differences were not statistically significant (p = 0.077 and 0.071, respectively). None of the 43 cases were deemed to severe disease, based on assessment against these criteria.

9 CONCLUSIONS

This project was the largest of its kind undertaken in Australia, and provides a comprehensive review and analysis of CA-CDI in one state. Aside from some evidence that different ribotypes may be circulating in different geographic regions, similar policies around antimicrobial stewardship, importation of food products and use of antibiotics in veterinary practice suggests that prevalence data are likely to be representative of other Australian jurisdictions. The findings of the current study are also supported by the international literature, with CA-CDI affecting a younger cohort, who may have different risk factors to 'traditional' HA-CDI cases, and who represent approximately one third of CDI cases detected by healthcare facilities.

A comprehensive review of the literature has highlighted mechanisms via which exposure to *C. difficile* may be occurring outside of the hospital environment. Contaminated wastewater can cause widespread dissemination into natural waterways, or onto agricultural products via treated biosolids (63, 167). This demonstrates a plausible means by which waste products of either humans or animals can contaminate the environment and food. Further, similar strains found in production animals, food and humans in the same regions does point to the potential for CDI to be a foodborne disease, although the role of this potential pathway for disease transmission requires further research.

The findings of the current study support a more methodical approach to collecting and assessing evidence of increasing CA-CDI. Continuing enhanced surveillance activities will allow the state to monitor variations in trends over time. Further, education of primary care providers regarding risk factors for patients in the community will facilitate early identification and treatment of at-risk cases outside of hospitals. The potential for severe disease to occur among CA cases highlights the need for practitioners to be aware of this disease in what may have traditionally been considered 'low-risk' patients.

Alongside ongoing surveillance and education, WA is in a position to address some research questions that have arisen from both the literature, and this project. Current enhanced surveillance definitions require review in light of evidence that these may not be adequately

classifying all cases correctly. Misclassification of CA-CDI and HA-CDI may be adding to the already difficult task of correctly identifying patient populations at risk. Further longitudinal surveillance in animal populations and food sources, along with the environment is required to determine the baseline prevalence of this organism in these reservoirs, and determine any evidence for increases. Further, highly discriminatory typing techniques can determine links between cases in humans and potential sources. Finally, reviews of existing cases, along with future prospective studies are required to add to the body of knowledge around host risk factors for development of disease.

The data from this study support the validity and effectiveness of the comprehensive surveillance system that WA currently has in place for monitoring CDI. The overarching public health mandate to understand this organism, and to prevent and control disease, both inside and outside of hospitals is clear. A robust surveillance system underpins any efforts into reducing the burden of this infection in the WA community. The results of this study should be used to support other research within the state, with the ultimate aim to capitalise on existing local strengths in data linkage, case surveillance and laboratory methods. This will ensure the application of appropriate public health policy, and contribute to the global understanding of this important disease.

CHAPTER 6 – RECOMMENDATIONS FOR PUBLIC HEALTH ACTION

1 INTRODUCTION

As a result of this project, a number of public health actions are recommended. These include continued surveillance to maintain an understanding of local epidemiology, further research to fill gaps in knowledge that remain, and future research that builds on the findings of this project. While this project has provided a foundation of descriptive local epidemiology, further work is required to ensure that appropriate surveillance and disease control measures are implemented locally. The following recommendations for the Western Australian Multi Resistant Organism (WAMRO) Expert Advisory Group are based on current evidence in the international literature, and on outcomes from the research in CA-CDI in WA.

2 CONTINUED ENHANCED SURVEILLANCE

This project involved a large-scale, centralised review of almost 3,000 HI-CDI cases diagnosed at public hospitals in the metropolitan area of Perth, Western Australia. As reporting of HI-CDI will continue to be mandatory in WA, ongoing enhanced surveillance is recommended to monitor changes in the local epidemiology of this disease. Hospitals in country regions of WA, who report a smaller proportion of cases to HISWA, will need to conduct their own enhanced surveillance of cases, until such time that all public hospital case histories are available centrally. Inclusion of country hospital data will allow a more complete picture of the epidemiology of CDI in WA.

Review of private hospital cases may prove to be more difficult; with privacy laws restricting the flow of information between public and private facilities it may be difficult for private hospital infection control staff to determine if a private patient has been hospitalised recently in the public system. Similarly, WA Department of Health staff do not have full access to either public hospitalisation histories of private patients (due to use of different medical record number systems) or private hospitalisation histories (due to privacy laws). Collecting details of a recent hospitalisation on admission documents would allow a case classification to be determined, however the decision to adopt this practice would have to be made by each facility.

At this stage, the patient populations, hospital structures and reasons for presentation/admission to private hospitals in WA would lend themselves towards a likelihood that the majority of cases diagnosed at these facilities would be HA. There are currently four metropolitan facilities which HISWA classifies as private for the purposes of reporting that have emergency departments (compared to seven metropolitan public facilities), so CA cases presenting with gastroenteritis for treatment would be less common than observed at public facilities. Conversely, a large proportion of presentations to private facilities are planned admissions (i.e. for surgery), so it is less likely that patients would be symptomatic on admission and test positive for CDI within 48 hours.

While it is currently only possible to make an estimate of the proportions of CAIs in these patients, the growing incidence of HI-CDI in this group observed over the study period should be of concern for hospital administrators, particularly as insurance companies are moving towards models where they will not cover expenses related to healthcare-associated infections (331). While there is no indication that this is currently affecting CDI cases, understanding the proportion of potentially preventable cases, and taking appropriate action to reduce numbers of these cases is just as important in private facilities as it is in public facilities.

The Communicable Disease Control Directorate should continue to undertake enhanced surveillance of HI-CDI cases reported to HISWA. This information can be integrated with the current HISWA dataset, and reported back to individual facilities to inform infection control staff about the relative proportions of HAI and CAI. Continued, prospective determination of case classifications, and integrated ribotyping data, will allow public health authorities to monitor local epidemiology and link enhanced surveillance to other datasets, in order to carry out future research projects, as outlined in recommendation 5.

3 REVIEW OF CURRENT ENHANCED SURVEILLANCE DEFINITIONS

The current definitions being used to classify HA and CA disease were adopted by ESCMID and CDC in 2006/2007, and were designated as 'interim' case definitions for standardized surveillance (9, 53). As noted, there have been no updates to these case definitions since their

release, and the most recent surveillance protocol (released November 2015) still uses these same case definitions (54).

There are currently limitations with the enhanced surveillance definitions. This includes potential misclassification of HAI HCFO as CAI (with frequent inpatient contact <48 hours, such as dialysis patients), and potential misclassification of CAI as HAI CO, as the 4-week attribution back to a treating facility may be too long. For the purposes of the current research, HAI CO was excluded from the analysis (rather than being grouped with HAI HCFO) to limit the potential impact of any misclassification.

There is a large collection of *C. difficile* isolates available at PathWest, including in some instances multiple repeat specimens for the same patient. This repository of isolates, which have not been ribotyped due to their occurrence within 8 weeks of an index case, should undergo molecular testing to determine ribotypes. The results of this study would confirm if repeat specimens collected within 4 weeks are being appropriately attributed to a healthcare facility, or if this definition requires review.

It is not currently recommended that facilities screen asymptomatic patients on admission for the presence of *C. difficile* (158), although the potential to reduce HA-CDI using this method has been suggested (332). A targeted, prospective survey designed to determine the proportions of admitted patients who are asymptomatic carriers, as well as who goes on to develop disease, could further inform infection control professionals about the proportions of disease that may truly be hospital-acquired. This information will assist in the development of prevention and control policies.

It is a recommendation that WA Health partners with appropriate research groups to review if current enhanced surveillance definitions are accurately reflecting burdens of CAI and HAI. Although any surveillance definitions will have limitations it is important that, within reason, cases are appropriately reviewed and classified so they reflect the true nature and scope of the problem. This will allow appropriate policy development by infection prevention and control personnel, and accurate reporting of local epidemiology.

4 LONGITUDINAL FOOD PREVALENCE SURVEYS

One of the major limitations of the current evidence surrounding *C. difficile* in food is that all prevalence surveys reviewed involved one-off sampling of particular food product(s) within a certain region. The presence of *C. difficile* in food is of public health interest, however it is not possible to objectively state whether a finding in a single study is significant. In addition, the variety of enrichment and culture techniques used by different groups leaves open the possibility that some prevalence surveys were unable to detect *C. difficile* even when it was present, whereas others with more sensitive methods detected the organism from the maximum number of samples (333).

In order to establish if there is an increasing prevalence of *C. difficile* in food products which may be contributing to an increase in CA-CDI, longitudinal food studies must be undertaken. These need to involve systematic sampling of selected imported and local food products from a variety of sources (e.g. supermarkets, farmers' markets, organic food stores, hospitals, long term care facilities), with the same culture methodology used. Based on the available evidence, these surveys should primarily focus testing of meat and vegetable products, but can be extended to other products of interest if resources are available. Any positive samples should undergo ribotyping and whole genome sequencing, to determine similarities between food, animal and human strains, and to determine if there has been a potential introduction to humans via the food chain.

Undertaking these surveys will allow local public health authorities to:

- identify potential sources of *C. difficile* in food
- monitor potential increases in *C. difficile* in food products and any associated increases in human disease, and
- identify any introduced ribotypes from imported food, and determine if any cases in humans follow this introduction.

A further recommendation is that WA Health undertakes surveys, at a minimum once per year, to establish local prevalence and ribotyping patterns of *C. difficile* in food. The results should be reviewed with other epidemiological data, to determine if food has any likely role in local

transmission. Public health authorities should work with veterinarians and key agricultural industry stakeholders to discuss the outcomes of these surveys, and work collaboratively to limit the potential for susceptible individuals in the community to develop disease via the food chain. This includes reducing the potential for an introduction to local populations via imported food products.

5 SENTINEL COMMUNITY SURVEILLANCE FOR EMERGING RIBOTYPES

It has been noted in this study that hospital-based surveillance of CA-CDI is not representative of the full scope of the issue. Using HI-CDI cases as the sole source of surveillance will likely under-estimate the burden and over-estimate the severity of disease, and further will not allow identification of emerging ribotypes in the community that may occur via local routes, or through importation. In order to establish what is happening in the community, general practice based surveillance is required.

A counterpoint to this recommendation is current guidelines that do not recommend routine testing for acute episodes of diarrhoeal illness (290), which may still limit the spectrum of disease detected to severe episodes of diarrhoea in recently hospitalised patients. It is not within the scope of this project to make recommendations about current testing practices in the community, however it is essential that information within this arena be made available to those conducting state-wide surveillance, to provide a more complete understanding of CA-CDI.

The most cost effective method of surveillance in the community, without large scale changes to recommended testing practices for diarrhoeal illness, is to establish a sentinel surveillance network of general practitioners. Diarrhoeal samples from these practitioners could routinely be tested for *C. difficile,* with the samples routinely ribotyped. The network would need to include a representative sample of practitioners from across the state, and some baseline case characteristics would need to be collected at the time of specimen requisition, including details of any recent hospitalisations. Such networks have previously been established in WA for surveillance and vaccine-preventable diseases, and have proven effective (334).

It is a recommendation that WA Health explore options for either collaborating with existing sentinel GP networks, or establishing a new network for ongoing surveillance of CA-CDI outside of hospitals in WA. In order for this to be a cost-effective solution with maximum public health benefits, a new network should encompass surveillance of other multi-resistant organisms of concern which may be emerging in the WA community. This may include gathering evidence for changing patterns of resistance, emerging organisms or molecular types of interest, and important of novel pathogens in returning travelers.

6 FURTHER RESEARCH INTO RISK FACTORS AND OUTCOMES FOR CDI CASES

CA-CDI represents approximately one third of CDI cases diagnosed at metropolitan public hospitals in WA. Analysis of the data has highlighted some groups of further interest, particularly among CA-CDI cases. There are several research questions that have arisen at the conclusion of this project.

The finding that CAI occurs among women aged 20 – 39 at almost twice the rate of HAI is significant, and warrants further investigation. Exposure to children <2 years of age is a risk factor for CAI, and further research is required to confirm these findings locally. A data linkage project could be undertaken relatively easily to determine if CA cases had substantial contact with a child under 2 years of age immediately prior to their diagnosis. This could be achieved by linking cases of CAI and HAI to the birth register.

Other risk factors for CAI have to be confirmed. Younger women only make up a relatively small proportion of all CAI cases detected over the study period, and other host or environmental factors are playing a role in the development of disease. Investigations into potential risk factors for disease for CAI should be undertaken, with a general practice based study likely the best source for case determination. If the recommendation for sentinel surveillance of CAI at general practice is adopted, this is the ideal scenario under which case recruitment could occur.

There is also evidence in the literature of a growing severity of disease associated with CDI. Reviewing the outcomes of disease was outside of the scope for this research, with the

exception of a number of ribotypes of interest. However, establishing if there is an increase in severe outcomes for disease occurring both in hospitals and the community could determine if this increase is occurring locally, and allow more accurate projections of the burden of CDI in WA into the future. There are a number of options for determining increases in severity. For cases collected in the HISWA database, a review against accepted criteria or markers of severe disease could be undertaken retrospectively. This collection only commenced in 2010 however, and most evidence of increasing severity in the literature appears to pre-date this time.

Other methods could be employed to determine increasing severity, and these would need to use linked data and diagnostic codes to match confirmed CDI cases to severe outcomes i.e. colectomy and death. There are limitations on each method, and a determination on the best approach would need to be made in the context of available resources and suitability of each method to accurately answer the research question.

WA Health should support research into risk factors and outcome for local CDI cases, in order to contribute to the body of knowledge and support evidence-based public health policy.

7 EDUCATION OF PRIMARY CARE PROVIDERS ON CDI AS A COMMUNITY DISEASE

Evidence available from both Australian and international authors suggests CA-CDI is almost certainly underdiagnosed (64, 71). This is owing to the relatively recent emergence of this disease in the community, the presence among individuals lacking traditional risk factors, and clinical management guidelines concerning diarrhoea in the community. Following on from work to establish a better profile for risk factors among people living in the community, communication with primary care providers is essential to ensure that this disease is 'on the radar' of those seeing diarrhoeal patients with no recent travel or hospitalisation history.

Gastroenteritis from all causes is the sixth most common new problem managed by Australian GPs, seen at a rate of 1.1 cases per 100 patient encounters (335). At this rate the typical GP could expect to see over 100 cases of new diarrhoeal illness per year. The proportion of these

cases with CA-CDI is unknown. It is a recommendation that WA Health commits to disseminating findings of any local research to key primary care stakeholders, to ensure that this disease in the community is recognised among those who are in a position to diagnose and treat in a timely manner. This includes providing a risk profile of individuals who may benefit from further investigations, in order to receive prompt, appropriate therapy.

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APPENDICES

APPENDIX 1 – RIBOTYPES FROM ENHANCED SURVEILLANCE CASES

Ribotype	CAI	HAI CO	HAI HCFO	INDETER.	UNK	Grand Total
UK 014/020 [G]	135	49	161	32	7	384
UK 002	29	11	63	8	0	111
UK 056	27	11	44	4	0	86
UK 054	18	5	22	0	0	45
UK 012	18	3	21	1	0	43
UK 046	11	3	26	1	0	41
UK 018	14	5	16	4	0	39
UK 005	9	5	16	0	0	30
UK 015/UK 193	5	4	21	0	0	30
UK 103	9	3	15	1	1	29
UK 010	2	4	21	1	0	28
UK 017	10	1	14	3	0	28
UK 053	3	4	16	4	1	28
UK 070	3	5	14	3	0	25
QX 076	3	1	17	2	0	23
QX 001	5	4	7	3	0	19
UK 087	4	1	12	1	0	18
QX 014	4	2	7	1	0	14
UK 244	8	2	4	0	0	14
QX 026	1	1	9	0	0	11
UK 064	3	1	5	1	1	11
QX 024	1	1	5	2	0	9
QX 033	4	1	4	0	0	9
QX 150	2	1	5	1	0	9
UK 247	2	0	4	2	0	8
UK 251	1	2	4	1	0	8
QX 013	1	0	4	2	0	7
QX 005	0	0	5	1	0	6
QX 029	1	1	3	1	0	6
QX 051	1	0	4	0	0	5
QX 064	1	2	2	0	0	5
QX 086	2	0	3	0	0	5
QX 256	0	0	5	0	0	5
QX 399	1	0	3	1	0	5
UK 001	1	1	3	0	0	5
UK 001/UK 271	2	0	3	0	0	5
UK 003	3	2	0	0	0	5
UK 081	2	0	3	0	0	5
UK 137	1	0	3	1	0	5
QX 068	2	0	1	1	0	4
QX 069	0	0	4	0	0	4
QX 077	2	0	1	1	0	4
QX 142	0	2	2	0	0	4
QX 199	0	0	4	0	0	4
UK 049	0	1	2	1	0	4
UK 051	0	1	2	1	0	4
UK 106	2	1	1	0	0	4
UK 237	0	0	4	0	0	4
QX 058	1	1	1	0	0	3
QX 113	1	0	1	1	0	3
QX 244	1	0	2	0	0	3

QX 273	0	0	3	0	0	3
UK 131	0	0	3	0	0	3
QX 011	0	0	2	0	0	2
QX 015	0	0	2	0	0	2
QX 035	1	0	1	0	0	2
QX 039	0	0	2	0	0	2
QX 060	2	0	0	0	0	2
QX 087	2	0	0	0	0	2
QX 097	0	1	1	0	0	2
QX 100	2	0	0	0	0	2
QX 102	0	0	2	0	0	2
QX 121	1	0	1	0	0	2
QX 135	1	0	1	0	0	2
QX 138	2	0	0	0	0	2
QX 148	1	0	1	0	0	2
QX 151	1	0	1	0	0	2
QX 221	1	0	1	0	0	2
QX 224	0	1	1	0	0	2
QX 353	0	1	1	0	0	2
QX 408	1	0	1	0	0	2
QX 439	1	1	0	0	0	2
UK 009	0	0	2	0	0	2
UK 015	0	0	1	1	0	2
UK 029	0	1	1	0	0	2
UK 095	1	0	1	0	0	2
UK 280	0	1	1	0	0	2
QX 017	1	0	0	0	0	1
QX 025	0	0	1	0	0	1
QX 028	0	0	1	0	0	1
QX 032	0	0	0	0	1	1
QX 049	0	0	1	0	0	1
QX 050	0	1	0	0	0	1
QX 075	0	0	1	0	0	1
QX 078	1	0	0	0	0	1
QX 081	0	0	1	0	0	1
QX 098	0	0	1	0	0	1
QX 099	1	0	0	0	0	1
QX 103	1	0	0	0	0	1
QX 118	1	0	0	0	0	1
QX 119	1	0	0	0	0	1
QX 127	0	0	1	0	0	1
QX 137	0	1	0	0	0	1
QX 141	0	0	1	0	0	1
QX 157	1	0	0	0	0	1
QX 161	1	0	0	0	0	1
QX 166	1	0	0	0	0	1
QX 169	0	1	0	0	0	1
QX 170	1	0	0	0	0	1
QX 175	0	0	1	0	0	1
QX 176	0	0	1	0	0	1
QX 180	0	0	1	0	0	1
QX 183	0	0	1	0	0	1

QX 188	0	0	0	0	1	1
QX 193	0	1	0	0	0	1
QX 197	0	0	1	0	0	1
QX 203	0	0	1	0	0	1
QX 209	0	0	1	0	0	1
QX 210	0	0	1	0	0	1
QX 214	0	0	1	0	0	1
QX 216	1	0	0	0	0	1
QX 225	0	0	1	0	0	1
QX 228	1	0	0	0	0	1
QX 230	0	0	1	0	0	1
QX 231	1	0	0	0	0	1
QX 234	0	1	0	0	0	1
QX 235	0	0	0	1	0	1
QX 276	0	0	1	0	0	1
QX 277	0	0	1	0	0	1
QX 278	1	0	0	0	0	1
QX 305	0	0	1	0	0	1
QX 323	0	0	1	0	0	1
QX 326	0	0	1	0	0	1
QX 328	0	0	1	0	0	1
QX 356	0	0	1	0	0	1
QX 360	0	0	1	0	0	1
QX 361	0	0	1	0	0	1
QX 369	1	0	0	0	0	1
QX 386	0	0	1	0	0	1
QX 388	0	1	0	0	0	1
QX 398	0	0	1	0	0	1
QX 409	0	0	1	0	0	1
QX 413	1	0	0	0	0	1
QX 414	0	0	1	0	0	1
QX 417	0	1	0	0	0	1
QX 421	0	0	1	0	0	1
QX 422	1	0	0	0	0	1
QX 423	1	0	0	0	0	1
QX 424	0	0	1	0	0	1
QX 427	0	0	1	0	0	1
QX 428	1	0	0	0	0	1
QX 430	0	0	1	0	0	1
QX 433	1	0	0	0	0	1
QX 434	0	0	0	1	0	1
QX 435	0	1	0	0	0	1
QX 442	0	0	0	0	1	1
QX 453	0	0	1	0	0	1
QX 474	0	0	1	0	0	1
QX 476	1	0	0	0	0	1
QX 481	1	0	0	0	0	1
QX 488	0	0	1	0	0	1
QX 490	1	0	0	0	0	1
QX 492	0	0	1	0	0	1
QX 493	0	0	1	0	0	1
QX 497	1	0	0	0	0	1

QX 500	0	0	1	0	0	1
QX 502	0	0	0	0	1	1
UK 039	0	0	1	0	0	1
UK 052	1	0	0	0	0	1
UK 056/UK 255	1	0	0	0	0	1
UK 063	0	0	1	0	0	1
UK 070/QX 014	1	0	0	0	0	1
UK 075	0	0	1	0	0	1
UK 101	0	0	1	0	0	1
UK 127	0	0	0	1	0	1
UK 193	0	0	1	0	0	1
UK 256	0	0	1	0	0	1
Grand Total	**400**	**156**	**697**	**91**	**14**	**1358**

APPENDIX 2 – ANTIBACTERIALS FOR SYSTEMIC USE – FULL LIST

J01 - ANTIBACTERIALS FOR SYSTEMIC USE			
ATC	**ITEM TYPE**	**CODE**	**FORM AND STRENGTH**
GENERAL ANTIINFECTIVES FOR SYSTEMIC USE			
ANTIBACTERIALS FOR SYSTEMIC USE			
TETRACYCLINES			
TETRACYCLINES			
J01AA02	DOXYCYCLINE		
	P	2702F	Tablet 100mg (HCl salt after 1 Oct 2007) 28
	P	2703G	Capsule 100mg (as HCl salt after 1 Oct 2007)
	P	2707L	Capsule 50mg (as HCl salt after 1 Oct 2007)
	P	2708M	Capsule 100mg (as HCl salt after 1 Oct 2007)
	P	2709N	Tablet 100mg (as HCl salt after 1 Oct 2007)
	P	2711Q	Tablet 50mg (as HCl salt after 1 Oct 2007) 25
	P	2714 W	Tablet 100mg (as HCl salt after 1 Oct 2007) 21
	P	2715X	Capsule 100mg (as HCl salt after 1 Oct 2007)
	P	3321T	Tablet 100mg
	P	3322 W	Capsule 100mg
	P	5082L	Tablet 100mg (monohydrate) 7
	P	9105F	Tablet 100mg (monohydrate) 7
	P	9106G	Tablet 50mg (as monohydrate) 25
	P	9107H	Tablet 100mg (monohydrate) 28
	P	9108J	Tablet 100mg (as monohydrate) 21
J01AA08	MINOCYCLINE		
	P	1616C	Tablet 50mg
	P	3037 W	Capsule 100mg
BETA-LACTAM ANTIBACTERIALS,PENICILLINS			
PENICILLINS WITH EXTENDED SPECTRUM			
J01CA04	AMOXYCILLIN		
	P	1878 W	Sachet containing oral powder 3g
	P	1884E	Capsule 250mg
	P	1886G	Powder for syrup 125mg per 5mL 100mL
	P	1887H	Powder for syrup 250mg per 5mL 100mL
	P	1888J	Powder paediatric oral drops 100mg per mL 20mL
	P	1889K	Capsule 500mg
	P	3300Q	Capsule 500mg
	P	3301R	Capsule 250mg
	P	3302T	Powder for syrup 125mg per 5mL 100mL
	P	3309E	Sachet containing oral powder 3g
	P	3393N	Powder for syrup 250mg per 5mL 100mL
	P	5225B	Powder for oral suspension 500mg per 5mL 100mL
	P	8581P	Tablet 1g 14

	P	8705E	Powder for oral suspension 500mg per 5mL 100mL
	P	9714G	Powder paediatric oral drops 100mg per mL 20mL
J01CA01	AMPICILLIN		
	P	2977Q	Injection 1g (solvent required)
BETA-LACTAMASE SENSITIVE PENICILLINS			
J01CE08	BENZATHINE PENICILLIN		
	P	2267H	Injection 900mg in 2.3mL cartridge-needle unit
J01CE10	BENZATHINE PHENOXYMETHYLPENICILLIN		
	P	5012T	Oral suspension 150mg per 5mL 100mL
	P	9143F	Oral suspension 150mg per 5mL 100mL
J01CE01	BENZYLPENICILLIN		
	P	1775K	Injection 600mg (solvent required)
	P	2647H	Injection 3g (solvent required)
	P	3486L	Injection 600mg (with sterilised water)
	P	3487M	Injection 3g (solvent supplied)
J01CE02	PHENOXYMETHYLPENICILLIN		
	P	1703P	Tablet 250mg
	P	1705R	Capsule 250mg
	P	1787C	Tablet 250mg
	P	1789E	Capsule 250mg
	P	2965C	Capsule 500mg
	P	3028J	Tablet 500mg
	P	3360 W	Tablet 250mg
	P	3361X	Tablet 500mg
	P	3363B	Capsule 250mg
	P	3364C	Capsule 500mg
J01CE09	PROCAINE PENICILLIN		
	P	1794K	Injection 1.5g
	P	3485K	Injection 1.5g
BETA-LACTAMASE RESISTANT PENICILLINS			
J01CF01	DICLOXACILLIN		
	P	8121K	Capsule 250mg
	P	8122L	Capsule 500mg
J01CF05	FLUCLOXACILLIN		
	P	1524F	Injection 500mg (solvent required)
	P	1525G	Injection 1g (solvent required)
	P	1526H	Capsule 250mg
	P	1527J	Capsule 500mg
	A	20436	Powder for syrup 250mg per 5mL 100mL
	P	5091Y	Capsule 500mg
	P	9149M	Powder for oral liquid 125mg per 5mL 100mL
	P	9150N	Powder for oral liquid 250mg per 5mL 100mL

COMBINATIONS OF PENICILLINS, INCL.BETA-LACTAMASE INHIBITORS			
J01CR02	AMOXYCILLIN with CLAVULANIC ACID		
	P	1891M	Tablet 500mg-125mg
	P	1892N	Powder for syrup 125mg-31.25mg per 5mL 75mL
	P	5006L	Tablet 875mg-125mg
	P	5008N	Tablet 500mg-125mg
	P	5011R	Powder for syrup 400mg-57mg per 5mL 50mL
	P	8254K	Tablet 875mg-125mg
	P	8319 W	Powder for syrup 400mg-57mg per 5mL 50mL
J01CR03	TICARCILLIN with CLAVULANIC ACID		
	P	2179Q	Injection 3g-100mg (solvent required)
	P	6884H	Injection 3g-100mg 10mL and NS
OTHER BETA-LACTAM ANTIBACTERIALS			
FIRST-GENERATION CEPHALOSPORINS			
J01DB01	CEFALEXIN		
	P	3058Y	Capsule 250mg
	P	3094 W	Granules for syrup 125mg per 5mL 100mL
	P	3095X	Granules for syrup 250mg per 5mL 100mL
	P	3119E	Capsule 500mg
	P	3317N	Capsule 250mg
	P	3318P	Capsule 500mg
	P	3320R	Granules for syrup 250mg per 5mL 100mL
J01DB03	CEFALOTHIN		
	P	2964B	Injection 1g (solvent required)
J01DB04	CEPHAZOLIN		
	P	1257E	Injection 1g (solvent required)
	P	9326 W	Powder for injection 2g 10
SECOND-GENERATION CEPHALOSPORINS			
J01DC04	CEFACLOR		
	P	1169M	Tablet 375mg (sustained release)
	P	2460L	Powder for oral susp 125mg per 5mL 100mL
	P	2461M	Powder for oral susp 250mg per 5mL 75mL
	P	5045M	Tablet 375mg (sustained release)
	P	5047P	Powder for oral susp 250mg per 5mL 75mL
J01DC02	CEFUROXIME		
	P	8292K	Tablet 250mg 14
THIRD-GENERATION CEPHALOSPORINS			
J01DD01	CEFOTAXIME		
	P	1085D	Injection 1g (solvent required)
	P	1086E	Injection 2g (solvent required)
J01DD04	CEFTRIAXONE		
	P	1784X	Injection 1g (solvent required)

	P	1785Y	Injection 2g (solvent required)
	A	18777	Injection 1g (solvent required)
	P	9058R	Injection 500mg (solvent required)
FOUTH-GENERATION CEPHALOSPORINS			
J01DE01	CEFEPIME		
	P	8315P	Injection 1g (solvent required)
	P	8316Q	Injection 2g (solvent required)
SULFONAMIDES AND TRIMETHOPRIM TRIMETHOPRIM AND DERIVATIVES			
J01EA01	TRIMETHOPRIM		
	P	2922T	Tablet 300mg
COMBINATIONS OF SULFONAMIDES AND TRIMETHOPRIM, INCL. DERIVATIVES			
J01EE01	TRIMETHOPRIM with SULPHAMETHOXAZOLE		
	P	2949F	Tablet 80mg-400mg
	P	2951H	Tablet 160mg-800mg
	P	3103H	Oral suspension 40mg-200mg per 5mL 100mL
	P	3390K	Tablet 160mg-800mg
MACROLIDES, LINCOSAMIDES AND STREPTOGRAMINS MACROLIDES			
J01FA10	AZITHROMYCIN		
	A	19032	Tablet 500mg 3
	A	20776	Tablet 500mg 3
	P	4115N	Tablet 500mg
	P	6221K	Tablet 600mg 16
	P	8200N	Tablet 500mg
	P	8201P	Powder for oral suspension 200mg per 5mL 15mL
	P	8336R	Tablet 500mg 2
J01FA09	CLARITHROMYCIN		
	P	6151R	Tablet 250mg 100
	P	6152T	Tablet 500mg 100
	P	8318T	Tablet 250mg 14
	P	9192T	Powder for oral liquid 250mg per 5mL 50mL
J01FA01	ERYTHROMYCIN		
	P	1397M	I.V. infusion 1g (base)
	P	1404X	Capsule 250mg
	P	2424N	Granules for paediatric oral susp 200mg
	P	2428T	Granules for oral susp 400mg (base) per 5mL 10
	P	2750R	Tablet 400mg (base)
	P	3325B	Capsule 250mg
	P	3334L	Granules for paediatric oral suspension 200mg
	P	3336N	Tablet 400mg (base)
J01FA06	ROXITHROMYCIN		
	P	1760P	Tablet 150mg 10

	P	5260 W	Tablet 150mg 10
	P	5261X	Tablet 300mg 5
	P	8016X	Tablet 300mg 5
	P	8129 W	Tablet for oral suspension 50mg 10
LINCOSAMIDES			
J01FF01	CLINDAMYCIN		
	P	3138E	Capsule 150mg
	P	5057E	Capsule 150mg
J01FF02	LINCOMYCIN		
	P	2530E	Injection 600mg in 2mL
AMINOGLYCOSIDE ANTIBACTERIALS			
OTHER AMINOGLYCOSIDES			
J01GB03	GENTAMICIN SULPHATE		
	P	2824P	Injection 80mg (base) in 2mL
J01GB01	TOBRAMYCIN		
	P	1356J	Injection 80mg (base)
	P	8872Y	Injection 80mg (base) in 2mL (without preserv)
QUINOLONE ANTIBACTERIALS			
FLUOROQUINOLONES			
J01MA02	CIPROFLOXACIN		
	P	1208N	Tablet 250mg
	P	1209P	Tablet 500mg
	P	1210Q	Tablet 750mg
	P	1311B	Tablet 250mg
	A	21046	Tablet 250mg 2
J01MA14	MOXIFLOXACIN		
	A	20350	Tablet 400mg 5
J01MA06	NORFLOXACIN		
	A	13660	Tablet 400mg 6
	P	3010K	Tablet 400mg
OTHER ANTIBACTERIALS			
GLYCOPEPTIDE ANTIBACTERIALS			
J01XA01	VANCOMYCIN		
	P	2269K	Powder for injection 1gm (1000000iu) 1
	P	2270L	Powder for injection 1gm (1000000iu) 3
	P	3130R	Injection 500mg (500000iu) (solvent required)
	P	3131T	Injection 500mg (500000iu) (solvent required)
STEROID ANTIBACTERIALS			
J01XC01	FUSIDIC ACID		
	P	2312Q	Tablet (sodium salt) 250mg (enteric coated)
IMIDAZOLE DERIVATIVES			
J01XD01	METRONIDAZOLE		
	P	1638F	I.V. infusion 500mg in 100mL
NITROFURAN DERIVATIVES			
J01XE01	NITROFURANTOIN		
	P	1692C	Capsule 50mg

	P	1693D	Capsule 100mg
OTHER ANTIBACTERIALS			
J01XX05	HEXAMINE HIPPURATE		
	P	3124K	Tablet 1g

Conference presentations

Tracey L, McCann R, Armstrong P and Riley TV. Enhanced surveillance for community-associated *Clostridium difficile* infection in Western Australia. 15th Asia Pacific Congress of Clinical Microbiology and Infection, Malaysia, November 2014 (poster).