ORIGINAL ARTICLE

Emergence and spread of moxifloxacin-resistant *Clostridium difficile* ribotype 231 in Sweden between 2006 and 2015

H. Harvala[1,2], E. Alm[1], T. Åkerlund[1] and K. Rizzardi[1]

1) *Public Health Agency of Sweden, Solna and* 2) *European Programme for Public Health Microbiology Training (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden*

Abstract

An aggregation of moxifloxacin-resistant *Clostridium difficile* ribotype 231 (RT231) isolates was first identified in the county of Stockholm in 2008, and by the end of 2015 isolates of RT231 had spread to 13 of 21 Swedish counties. We investigated the epidemiology of *C. difficile* RT231 in Sweden between 2006 and 2015 using whole genome sequencing (WGS) and evaluated whether its emergence could be associated with extended moxifloxacin use. We performed WGS and phylogenetic analysis of 51 *C. difficile* RT231 strains isolated in Sweden over a 10-year period. We also calculated the county-specific prescription rates for moxifloxacin between 2005 and 2015. Using WGS and detailed single nucleotide polymorphism analysis, we demonstrated three divergent sublineages of moxifloxacin-resistant *C. difficile* RT231 in Sweden from 2008 to 2015. A set of closely related RT231 was identified in hospitals located in the counties of Stockholm and Uppsala in 2008. Another set of RT231 isolates was found in four different counties in the Uppsala–Örebro Health Care Region. A gradual drop in moxifloxacin use in the county of Stockholm coincided with a reduction of RT231 in the area. However, RT231 continued to be frequent in surrounding counties including Uppsala, a county that also had the highest moxifloxacin prescription rates. We demonstrated frequent transmission of *C. difficile* RT231 within and between counties, indicating the importance of careful monitoring of hospitalized individuals infected with moxifloxacin-resistant *C. difficile* as well as the need for a strict moxifloxacin prescription policy.

Keywords: *Clostridium difficile*, epidemiology, moxifloxacin consumption, ribotype 231, whole genome sequencing
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Corresponding author: K. Rizzardi, Public Health Agency of Sweden, Solna, Sweden
E-mail: kristina.rizzardi@folkhalsomyndigheten.se

Introduction

Clostridium difficile, a Gram-positive and spore-forming anaerobic bacillus, is a leading cause of healthcare-associated diarrhoea in industrialized countries [1]. Transmission occurs primarily in healthcare facilities, where exposure to antibiotics and environmental contamination by *C. difficile* spores are common. Antibiotic treatment, advanced age (>65 years), comorbidities and hospitalization have been identified as major risk factors for *C. difficile* infection (CDI) [2]. However, antimicrobial therapy is the single most important risk factor for CDI. It leads to the disruption of the normal intestinal flora, creating favourable conditions for acquisition and proliferation of *C. difficile* [3]. Clindamycin, amoxicillin, third-generation cephalosporins and more recently fluoroquinolones have been shown to be associated with a particularly high risk of developing CDI [4].

The first whole genome sequence of *C. difficile* was obtained in 2006; sequencing revealed a large circular chromosome of 4 290 252 bp likely coding for 3776 proteins and an extracircular plasmid 7881 bp in size [5]. Several other complete or almost complete genomic sequences have since been obtained [6]. These studies have shown that the *C. difficile* genome contains a high proportion (over 10%) of mobile genetic elements including bacteriophages, repeat elements and genomic islands, as well as transposable and conjugative elements.

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Several typing methods have been used to study the epidemiology, genetic diversity and evolution of *C. difficile*. In Europe PCR ribotyping has been one of the most widely adopted methods, and over 600 *C. difficile* ribotypes have been identified. A particular subtype, *C. difficile* ribotype 027, has been associated with severe disease and hospital outbreaks in North America and Europe [7–9]. Global emergence of this moxifloxacin-resistant *C. difficile* type 027 prompted establishment of national surveillance programs in several European countries [10–12]. As a result of national surveillance, a clonal aggregation of a newly emerging, moxifloxacin-resistant *C. difficile* ribotype 231 (RT231) was uncovered in Sweden in 2008 [12]. This type was initially geographically limited to the Stockholm area, where over 70% of all moxifloxacin-resistant *C. difficile* strains were characterized as RT231 in 2008 (125/144) [12]. Despite an increasing awareness of CDI and enhanced infection control policies, RT231 has continued to spread in Sweden; by the end of the year 2015 it had been identified in 13 of 21 counties, suggesting interhospital transmission and possible local outbreaks [13].

Although no studies have shown whether moxifloxacin use implies higher risk for CDI than other commonly used antibiotics [14], the restriction of moxifloxacin use has been associated with a reduction in the number of CDIs in Northern Ireland and England [15,16]. Similarly, a study from Vienna demonstrated that enhanced antibiotic stewardship, including restriction of moxifloxacin use, was associated with a reduction in the number of CDI in population with a high rate of multidrug-resistant *C. difficile* [17].

We used whole genome sequencing (WGS) and phylogenetic analysis to investigate the spread of this newly emerging moxifloxacin-resistant *C. difficile* RT231 in Sweden between 2006 and 2015. We also evaluated the potential association between the extent of moxifloxacin prescription rates and the epidemiology of RT231.

Materials and Methods

Collection of *C. difficile* isolates

All 189 *C. difficile* RT231 isolates identified in Sweden between 2006 and 2015 were included in this study (Table 1). The strains collected between 2009 and 2015 were obtained through the national surveillance program for *C. difficile;* before 2009 samples were obtained via a nationwide surveillance study [12]. Date of sample and county of diagnostic laboratory were collected as previously described [12]. PCR ribotyping between 2006 and 2012 was performed by a gel-based method [12] and between 2013 and 2015 by capillary-gel electrophoresis [18]. Fifty-one of 189 *C. difficile* RT231 isolates were selected for WGS. All moxifloxacin-sensitive strains were included, and moxifloxacin-resistant isolates were selected to cover each isolation region per year to obtain a geographically and temporally representative sample collection. The number of patients or cases of CDI was not available.

Antimicrobial susceptibility assays

Isolates were grown on Mueller-Hinton fastidious agar, and minimum inhibitory concentration (MIC) values for moxifloxacin, erythromycin, clindamycin, metronidazole and vancomycin were determined using Etests (bioMérieux, Marcy l'Etoile, France) as described previously [12]. The epidemiologic cutoff breakpoints for resistance were as follows: metronidazole MIC >2 mg/L, vancomycin MIC >2 mg/L, erythromycin MIC >2 mg/L, clindamycin MIC >16 mg/L and moxifloxacin MIC >4 mg/L, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

TABLE 1. Total number of *Clostridium difficile* isolates collected in Sweden between 2006 and 2015, and numbers of *C. difficile* ribotype 231 (RT231) isolates included in study

Year	All *C. difficile* isolates	Moxifloxacin-resistant isolates	Moxifloxacin-resistant RT231	Moxifloxacin-sensitive RT231	RT231 isolates included in study (frequency of strains included per year)
2006	13	1	1	Unknown[a]	1 (1/1)
2007	22	6	6	Unknown[a]	1 (1/6)
2008	586	586	125	Unknown[a]	11 (11/125)
2009	393	57	8	1	6 (6/9)
2010	335	53	5	1	6 (6/6)
2011	418	59	8	0	6 (6/8)
2012	423	87	16	1	7 (7/17)
2013	459	60	8	0	4 (4/8)
2014	391	57	6	0	6 (6/6)
2015	413	43	3	0	3 (3/3)
Total	3446	1002	186	3	51 (51/189)

[a]In 2006 *C. difficile* typing was performed only for diagnostic purposes, and in 2007–2008 only moxifloxacin-resistant *C. difficile* strains were collected.