Increased multi-drug resistant *Clostridium difficile* is driven by the prevalence of 027 in nursing homes

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ABSTRACT

8,109 consecutive fecal samples from inpatient (IP), outpatient (OP), and nursing home patients (NH) were submitted to a southwestern Virginia hospital laboratory and anonymous, unlinked, excess material from each was screened for the presence of *C. difficile*. 2077 isolates were recovered and PCR ribotyped. The minimum inhibitory concentrations (MIC) of metronidazole (MZ), vancomycin (VA), rifampicin (RIF), moxifloxacin (MOX), clindamycin (CL), and erythromycin (ERM) were measured using the Etest. Ribotypes and antibiotic susceptibilities were analyzed by prevalence and by patient populations. ARL 027, 014, and 053 comprised the top three ribotypes, respectively. MIC50, MIC90, and geometric mean were calculated and multi-drug resistance (MDR) phenotypes were identified, ranging from sensitive isolates (MDR-0) to strains resistant to 4 drugs (MDR-4). NH had the highest rate of *C. difficile* (48.5%), while IP and OP were in the 20-25% range, a standard expectation of *C. difficile* prevalence. OP had the highest Shannon-Weiner diversity index (SWDI) of 3.338, the lowest prevalence of 027 (18%), the highest prevalence of 014 (13%), a very low prevalence of 053 (2%), and was dominated by MDR-0 isolates (63%). NH had the lowest SWDI (2.086), the highest prevalence of 027 (53%), the lowest prevalence of 014 (8%), and was mostly phenotype MDR-4 (38%). IP resembled an average of the OP and NH. Its SWDI was 2.853, the prevalence of 027, 014, and 053 was 33%, 10%, and 11%, respectively, and IP had a larger mix of MDR types, although it was mostly MDR-0 (41%). The MIC50 and MIC90 of NH and ARL 027 were greater than or equivalent to the other groups for each of the antibiotics tested. NH had a significantly higher MIC geometric mean versus IP and OP for MOX, RIF, ERM, and CL (all were p=≤0.05) and also versus OP for VA (p=0.0058) and for MZ (p=0.0153). ARL 027 had a significantly higher MIC geometric mean versus 014 and 053 for MOX, VA, RIF, ERM, and for CL (all were p=≤0.05) and also versus 014 for MZ (p=≤0.001). Increased multi-drug resistance is driven by the high prevalence of 027 which in turn is influenced by its dominance within the NH community.

BACKGROUND

The epidemiology of *C. difficile* can be very fluid. As different factors impact the ecology of *C. difficile*, previously dominant strains are displaced - but not totally replaced - by fitter strains, better adapted to the current selective pressures. Selection pressures are exerted by antibiotics, enriching the fitter (resistant) ribotypes at the cost of the less fit (susceptible) ribotypes. Clindamycin (Johnson et al.,1999) and cephalosporins (Bignard, 1998) resistance have both been linked with outbreaks. Outbreaks featuring fluoroquinolone resistant 017 isolates have occurred in Ireland (Drudy et al., 2007) and Korea (Kim et al., 2008); the fluoroquinolone resistant 027 epidemic remains a problem especially in parts of the USA. The accumulation of resistance to an antibiotic or to several antibiotics is thus both undesirable and, in a clinical ecosystem perhaps inevitable.

AIMS

- To survey, by ribotype and by patient location and over an extended period, the minimum inhibitory concentrations of several antimicrobial agents active against *C. difficile*.
- This will provide a baseline against which newly appearing resistance and newly emerging ribotypes can be monitored.

MATERIALS AND METHODS

•Feces: From March 2012 to August 2013 excess material from 8109 sequential, anonymous and unlinked fecal samples, submitted within a local health care system for routine *C. difficile* testing, were shipped to TechLab. No samples were excluded on the basis of consistency or treatment or because they were "repeat" samples. Samples were from inpatients, outpatients, and nursing home patients. The study was approved by TechLab IRBs. The data collected at TechLab and reported in this poster was not part of any patient's diagnosis.
•Bacterial and toxigenic culture: We used alcohol shock and culture on CCFA to isolate *C. difficile*. Presumptive identifications were confirmed (Carman et al., 2012) a positive glutamate dehydrogenase (GDH) reaction in the *C. DIFF QUIK CHEK*® and various PCR tests.
•PCR analysis. Isolates were probed by PCR for toxin genes (*tcdA* and *tcdB*), for the binary toxin (CDT) locus (*cdtR, cdtA,* and *cdtB*) and for the glutamate dehydrogenase gene (*gluD*; Carman et al., 2009)
•Ribotyping: Our PCR ribotyping methods were those of the Anaerobe Reference Laboratory, Cardiff, Wales (Stubbs et al., 1999).
•Minimum inhibitory concentrations (MIC): The MICs of moxifloxacin, vancomycin, metronidazole, rifampicin, erythromycin and clindamycin were measured using Etest strips (AB Biodisk N.S. Inc., Piscataway, NJ).

IMPACT OF MOXIFLOXACIN (MOX) RESISTANCE ON ISOLATION RATES

Table 1. Ribotype frequency in SW Virginia and moxifloxacin resistance, 2011-2013

Left: By individual ribotype

Rank	Ribotype	% of 2077	% MOX res
1	027	33	98
2	014	10	11
3	053	8	99
4	010	4	11
5	039	4	69
6	009	4	10
7	106	3	9
8	002	3	0
9	056	3	2
10	126	2	5
11	005	2	0
12	015	2	8
13	054	1	7
14	012	1	4
15	001	1	42
16	057	1	5
17	103	1	5
18	032	0.9	0
19	046	0.8	24
20	017	0.8	29
21	244	0.8	0
22	251	0.8	6
23	019	0.6	0
24	137	0.6	0
25	240	0.6	0
26	116	0.6	0
	22 other A+B+CDT+	0.02	<1
	35 other A+B+CDT-	0.05	<1
	20 other non-tox	0.03	<1
	Total 142 ribotypes		50

Right: By toxin phenotype

Phenotype & Ribotype	n	% of 2077	% MOX res
039	84	4	68
010	83	4	11
22 other nontox	167	8	14
027	684	33	98
126	43	2	5
26 other CDT+	101	5	9
053	166	8	99
014	210	10	11
46 other A+B+	522	25	8
A-B+CDT- (all 017)	17	1	29

Key to cell color & toxin phenotype

- Non-toxigenic = grey
- A+B+CDT- = clear
- A-B+CDT- = yellow
- A+B+CDT+ = blue

DISCUSSION

• 027 was the fittest and most abundant ribotype in the SW Virginia clinical ecosystem between 2011 and 2013. 014 and 053 ranked 2 and 3. These three ribotypes were >50% of all isolates. MOX resistance was common (Table 1 Left).

• Toxin was not essential for successful colonization. Non-toxigenic ribotypes ranked 4, 5, and 6 and were 12% of all isolates recovered. MOX resistance was common (Table 1 Left).

• MOX resistance was most common in the commoner ribotypes. Levels were >10% in those ribotypes occurring as ~5% or more of all isolates (Table 1 Left). The frequency of resistance within a ribotype was predictive of its ranking, though because other factors had significant roles, this is clearest when considering toxin phenotypes. Accordingly, the most abundant non-toxigenic ribotypes were also the most resistant non-toxigenic ribotypes. The most common of the Other (A+B+) ribotypes had the highest frequencies of MOX resistance among A+B+ ribotypes, and so on (Table 1 Right).

• Only 014 and 010 were more common than their MOX resistance levels suggested (Table 1 Right). This overrepresentation may have been a legacy from a pre-fluoroquinolone level or 014 and 010 might be genuinely aggressive colonizers, successful without the benefits of widespread MOX resistance and not necessarily because of toxin production. Sambol et al (2001) say 014 is only weakly virulent and is recovered more often from asymptomatic carriers than from diarrheic patients. Since 2009, samples tested at TechLab containing 014 but no toxin have outnumbered those containing 014 and toxin by 2.3-fold (Not shown).

• Below a 1% incidence (~20 isolates/ribotype; Table 1 Left) resistance alone may be insufficient to elevate uncommon but resistant ribotypes (e.g. 001, 017 and 046) into the Top 10, at least not when pitted against MOX resistant 027, 053 and 014.

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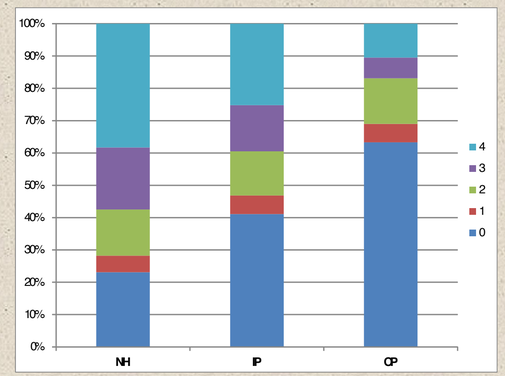
MICs AND MULTI-DRUG RESISTANC BY PATIENT LOCATION

Table 2. Minimum inhibitory concentrations (MIC) of six compounds against *Clostridium difficile* isolates separated by patient location

	NH		IP		OP	
	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
MOX	32	32	6	32	3	32
VANC	1.5	4	1.5	3	1.5	3
METRO	0.5	1	0.4	1	0.4	0.8
RIF	0.003	32	0.002	32	0.002	32
ERM	256	256	256	256	0.8	256
CLIND	256	256	3	256	2	256

Figure 1. Relative incidence of 0, 1, 2, 3,or 4 drug resistances among *Clostridium difficile* isolates separated by patient location



•No isolate was resistant to metronidazole or to vancomycin.

• MIC50% levels for MOX, ERM and CLIN were higher in nursing home than outpatient isolates. Inpatient isolates fell in between (Table 2).

•Resistance to 4 drugs was most common (40%) among nursing home isolates and least common (5%) among outpatient isolates. Fully susceptible isolates (0 resistance) followed the reverse trend, being most common in outpatients (65%) and least common in nursing homes (25%; Fig 1).

•The role of ribotype in the distribution of multi-drug resistance was assessed next,

Table 3. Ribotype incidence in nursing home, in and outpatients

Ribotype	% NH	% IP	% OP
001	0	1	2
002	2	2	5
009	2	4	6
010	1	5	6
014	8	10	13
015	0	2	3
027	52	33	17
039	4	4	4
053	12	11	2
056	2	2	5
106	1	2	5
126	2	3	3
Other A+B+CDT-	11	19	25
Other nontox	2	5	5
Total (n)	568	806	687
Number of ribotypes	49	67	82

•Table 3 shows an uneven distribution of ribotypes between locations..

•027 was common everywhere but especially in nursing homes, 053 was largely absent from outpatients. 014 was most frequently from outpatients.

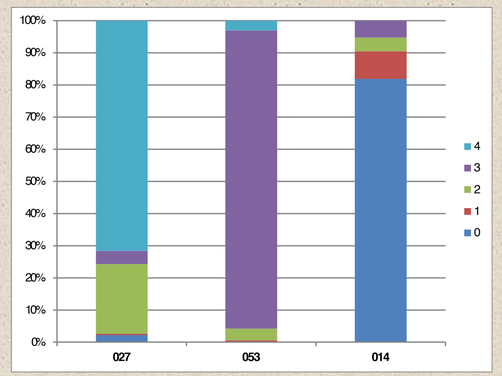
•When 027 was common, non-toxigenic and 014 isolates were less frequent and vice versa.

MICs AND MULTI-DRUG RESISTANCE BY RIBOTYPE

Table 4. Minimum inhibitory concentrations (MIC) of six compounds against three common *Clostridium difficile* ribotypes

	ARL 027		ARL 014		ARL 053	
	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
MOX	32	32	3	32	32	32
VANC	2	4	1	2	1	2
METRO	0.5	1.5	0.25	0.75	0.5	1
RIF	32	32	0.002	0.003	0.002	0.003
ERM	256	256	0.75	256	256	256
CLIND	256	256	1.5	3	256	256

Figure 2. Relative incidence of 0, 1, 2, 3,or 4 drug resistances among *Clostridium difficile* ribotypes 027, 053 and 014



•Four drug resistance was common (>70% of isolates) in ribotype 027. <5% of isolates were susceptible to all compounds tested.

•Three drug resistance was common in 053 (>95% of isolates). No 053 isolate was fully susceptible.

•Over 80% of 014 isolates were fully susceptible. About 5% were resistant to 3 drugs. None was resistant to four.

CONCLUSIONS

•No vancomycin or metronidazole resistance was seen.
•Fluoroquinolone resistance was most frequent among the most abundant ribotypes suggesting resistance conferred a selective advantage in this ecosystem.
•MOX resistance was most closely associated with 027 and 053 isolates and with patients in nursing homes.
•Multi-drug resistant 027 were common in nursing homes.
•Susceptible 014 isolates were most common among out patients.

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