Antibiotic resistance in *Clostridium difficile* – recent advances

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New traits of antibiotic resistance in *C. difficile* clinical isolates:

- resistance to multiple antibiotics
- reduced susceptibility to antibiotics used for treatment
- rapid spreading and persistence of resistance

Antibiotics and CDI

- Antibiotic treatment is the main risk for CDI
- *C. difficile* infection (CDI) occurs when the intestinal microflora is altered or disrupted, allowing *C. difficile* to colonize the gut and produce toxins
- Resistance to a wide range of antibiotics allow *C. difficile* to colonize and infect in the presence of drugs

- The antibiotics more frequently implicated in causing CDI include:

Cephalosporins (intrinsic)

Clindamycin (historical)

Fluoroquinolones (recent - associated to emergence and spread of RT027)

Phenotypic traits of *C. difficile* clinical isolates

AAC Journals.ASM.org

Antimicrobial-Resistant Strains of *Clostridium difficile* from North America

Fred C. Tenover, Isabella A. Tickler, and David H. Persing

% of resistance		% of the most frequent RTs	
CLI	41.50	027	49.00
MXF	38.00	002	10.4
RIF	7.90	106	10.4
MTZ	0.0	078	8.6
		053	7.4

AAC 2012. 56: 2929 -2932

Pan-European longitudinal surveillance of antibiotic resistance among prevalent *Clostridium difficile* ribotypes

J. Freeman[1], J. Vernon[2], K. Morris[1], S. Nicholson[2], S. Todhunter[2], C. Longshaw[3] and M. H. Wilcox[1,2] and the Pan-European Longitudinal Surveillance of Antibiotic Resistance among Prevalent *Clostridium difficile* Ribotypes' Study Group

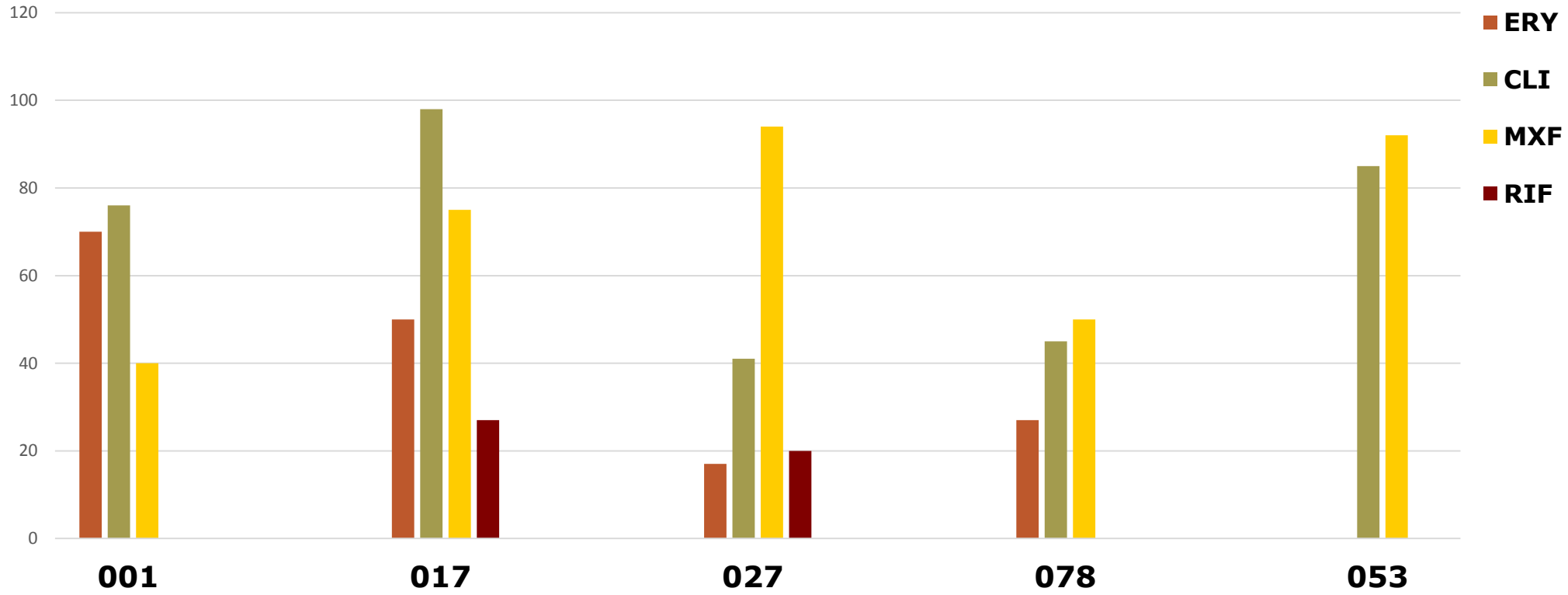
% of resistance		% of the most frequent RTs	
CLI	49.62	027	12.5
MXF	39.99	001/072	9.0
RIF	13. 40	014	8.1
IMP	7. 41	078	8.1
CHL	3.70	020	4.2
MTZ	0.11		
VAN	0.87		
FDX	0.0		
TIG	0.0		

Clin Microbiol Infect 2015. 21:248.e9-248.e16

Resistance to CLI and MXF is a common trait of epidemic strains but…

Percentage of resistance

(based on 10 studies published between 2012 and 2014 - 486 *C. difficile* isolates)



…an increasing percentage of resistance to RIF has been also observed in some RTs

High level of resistance to RIF has been observed in 17/22 EU countries.
In particular:

	% of resistant strains	
Czech Republic	63.64	(RT017, RT027, RT176, RT001/072)
Italy	62.37	(RT018, RT356)
Denmark	56.52	(RT027)
Hungary	58.67	(RT027)

Clin Microbiol Infect 2015. 21:248.e9-248.e16

C. difficile: a multi-drug resistant pathogen

J Antimicrob Chemother 2011; **66**: 2227–2234
doi:10.1093/jac/dkr292 Advance Access publication 19 July 2011

Journal of Antimicrobial Chemotherapy

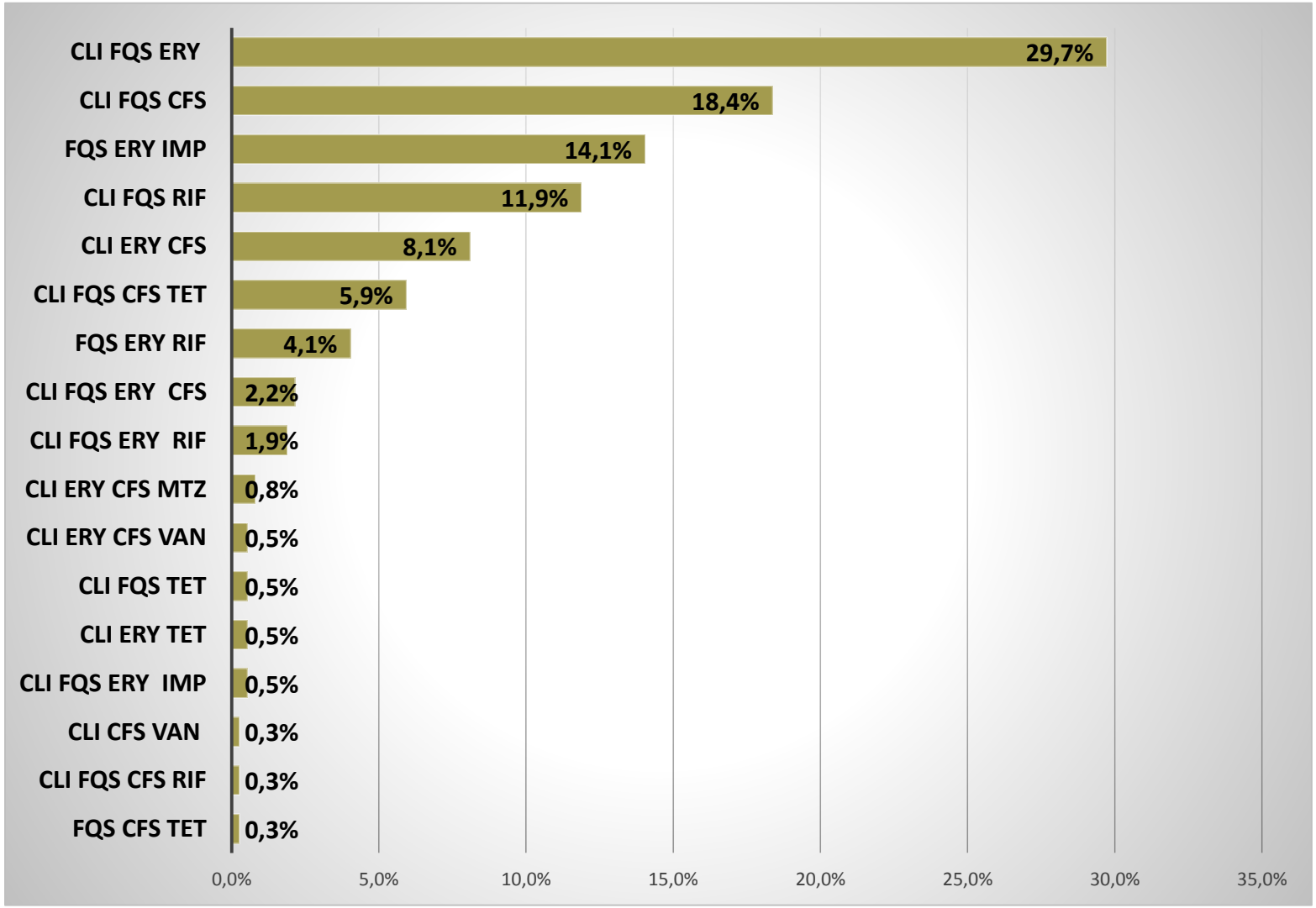
Multidrug resistance in European *Clostridium difficile* clinical isolates

Patrizia Spigaglia, Fabrizio Barbanti and Paola Mastrantonio* on behalf of the European Study Group on *Clostridium difficile* (ESGCD)†

- 55% of EU resistant strains were MDR
- 49% of MDR isolates were resistant to four different classes of antibiotics: ERY, CLI, MXF and RIF
- MDR strains belonged predominantly to: 001(39%), 017 (18%) and 012 (12%)

Review of 12 studies published between 2012 and 2014 (370 MDR *C. difficile* isolates)

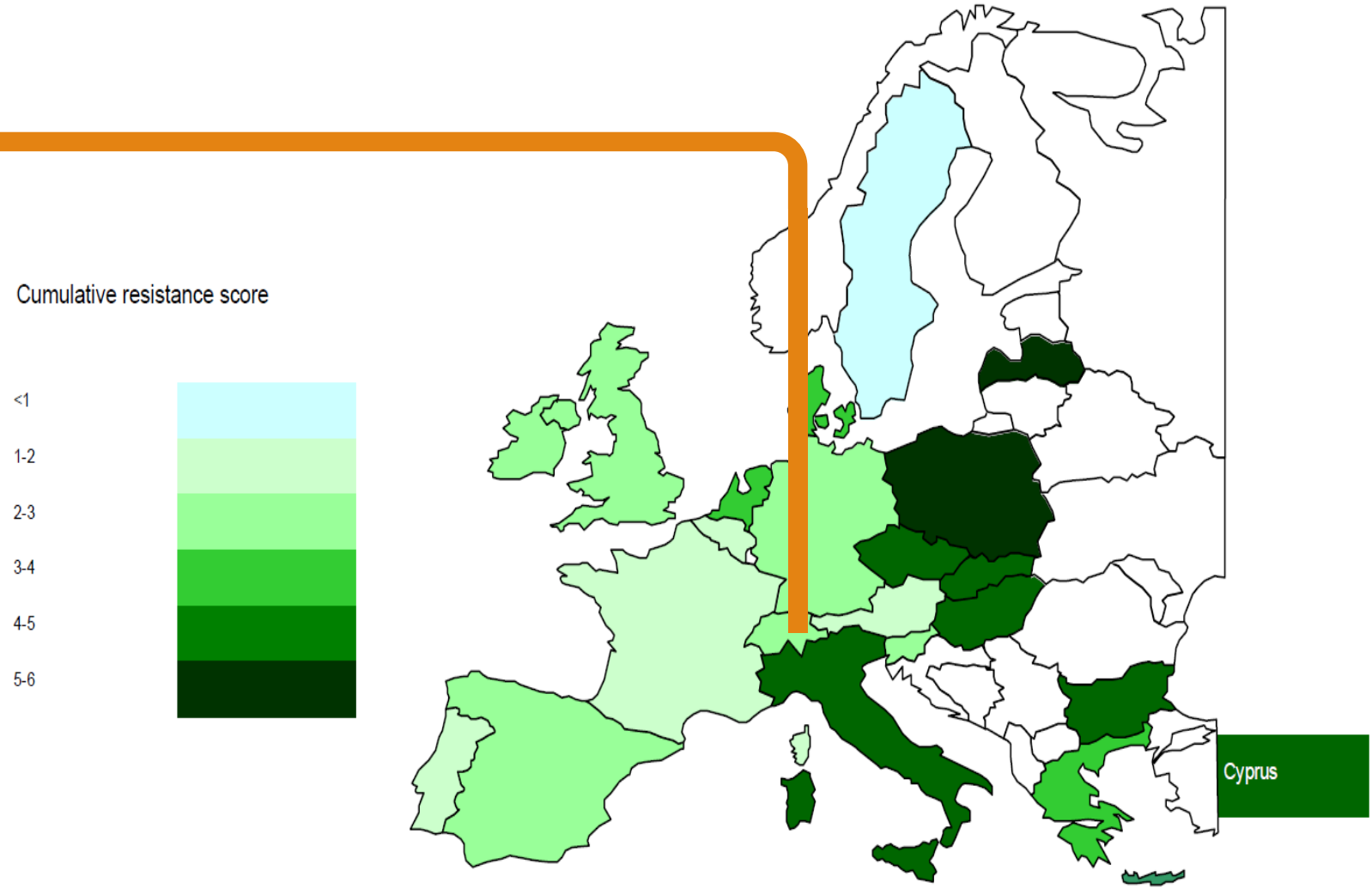
- The most common resistance patterns include resistance to: CLI, FQs, CFs, ERY and RIF
- RTs most commonly associated to MDR: 001/072, 012, 017, 027, 046, 176, 018
- The percentage of MDR strains ranges between 13 and 100% depending from the hospitals and the geographic regions



Lower antibiotic resistance levels among countries with a greater diversity of *C. difficile* RTs (Norther and Western Europe).

It is probably due to mandatory reporting programmes with consequent decrease of endemic RTs rates *Clin Microbiol Infect 2014. 21:248.e9-248.e16*

- In Italy: RT018 predominat from 2006 to 2013 when RT356 emerged
  J Clin Microbiol. 2010. 48: 2892–96
- RT018 and 356 show 87.5% of genetic similarity
  Both RTs are MDR (MXF, RIF, ERY)
  Microbiologia Medica 2014; 29:4722
- Selection for RIF resistance probably secondary to drug exposure (used in Italy for more than 20 years)



Reduced susceptibility to MTZ: an emerging phenotype

- Metronidazole (MTZ) is the drug of choice in the treatment of mild-to-moderate CDI

- Frequency of treatment failure or recurrence of CDI is high

  Treatment failure is higher with MTZ than VAN (22.4%vs 14.2%; P=0.002)

  Recurrence rate is similar (27.1%vs 24.0%, P=0.26)

  International Journal of Antimicrobial Agents . 2012. 40: 1-8

- Recently, reduced susceptibility to MTZ and heteroresistance have raised in *C. difficile* population

  JAC. 2008. 62: 1046–52
  AAC. 1999. 43: 2607–11
  JAC. 2001. 48:741–2.
  AAC. 200. 46: 1647–50.
  JAC. 2013. 68: 362–365

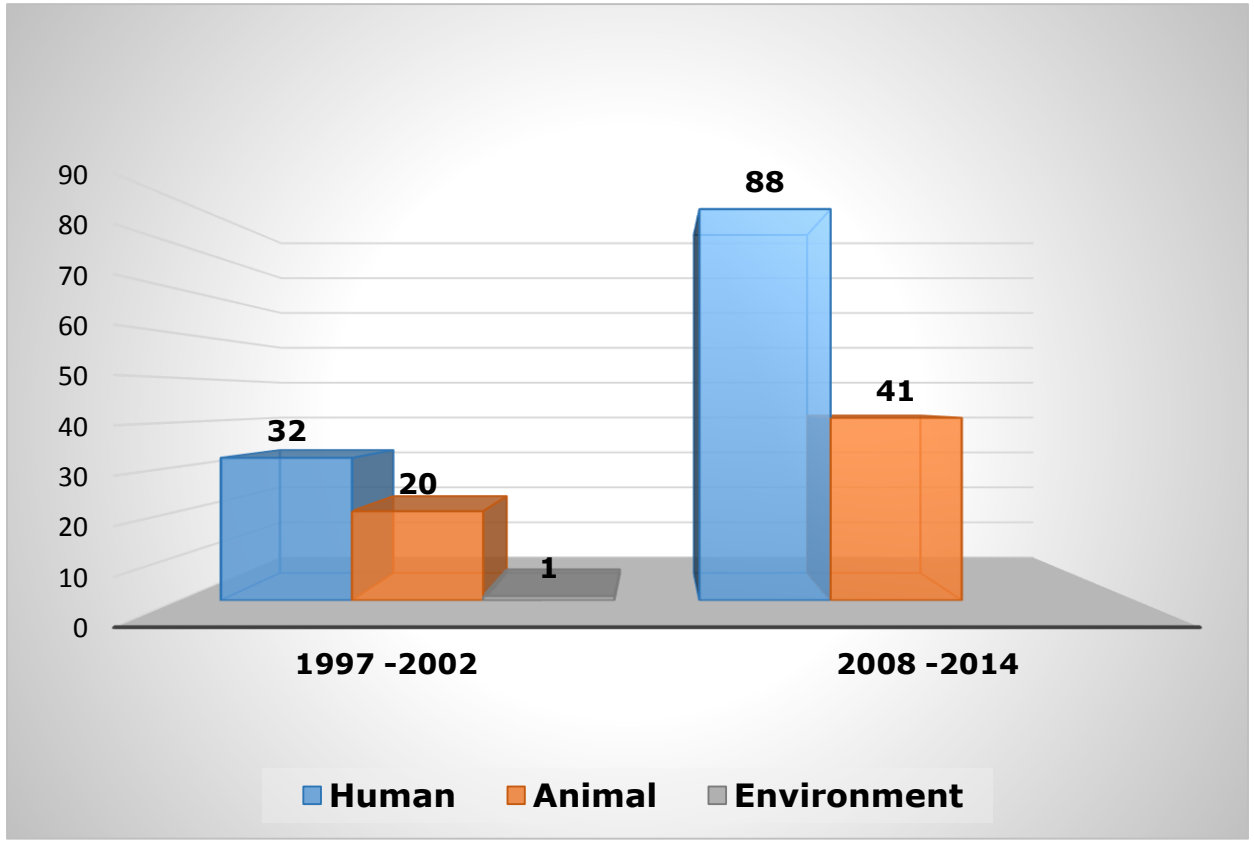
A higher geometric mean MICs to MTZ has been observed in some RTs

Epidemiological cut-off (ECOFF) for reduced susceptibility to MTZ is defined as MIC> 2mg/L (EUCAST)

Anaerobe. 2015. 33:105-108	• RT027 1.1 mg/L (Canada) • Associated with recurrent diseases
Clin Microbiol Infect 2014. 21:248.e9-248.e16	• RT027 1.42 mg/L (EU and UK) • RT106 0.65 mg/L • RT001/072 0.65 mg/L • RT356 0.61 mg/L
J Antimicrob Chemother 2013. 68: 362–365	• RT010 1.5 mg/L • Non toxigenic

MICs of others RTs range between 0.62 – 0.95 mg/L

C. difficile strains resistant to MTZ (MICs between 3 and ≥ 256 mg/L) have been reported (EUCAST-ECOFF > 2mg/L ; CLSI-MTZ breakpoint ≥ 32mg/L)



Review of 21 papers published between 1997 and 2014

Human
RT027; RT001; RT078; RT010

Animal
RT078 (swine, dog); RT010 (dog)

Horse
Swine
Dog
Zebra

Environment
RT010

Identification of strains with reduced susceptibility to MTZ is not easy…

Often, strains with reduced susceptibility to MTZ do not maintain the MIC values after passages in antibiotic free media or after freeze/thaw

- *J Clin Microbiol. 2008. 46:3028–3032); PLoS ONE. 2014. 9: e82622*

Agar Incorporation Method (AIM) has been demonstrated the method of choice to detect colonies with reduced susceptibility to MTZ

Etest<ADM< AIM

- *J Antimicrob Chemother 2008. 62: 1046–52; J Antimicrob Chemother 2013. 68: 362–365*

… so these strains can be underestimated

C. difficile strains characterized as susceptible to MTZ have been associated with cases of treatment failure

- *Anaerobe 1999. 5: 201–4; Clin Infect Dis 2008. 47:56–62*

Strains with reduced susceptibility to MTZ could have a role in treatment failure

Exposure to MTZ selects colonies of strains RT010 and 001 with increased MICs

Antibiotic subinhibitory concentrations could have a relevant role in selecting and maintaining colonies with higher MICs

- *J Antimicrob Chemother 2013. 68: 362–365*

Mean concentration of MTZ in the feces of patients during treatment ranges from < 0.25 to 9.5 mg/L

It can be hypothesized that these concentrations can be insufficient for the treatment of CDI due to strains with higher MICs

- *J Antimicrob Chemother 2001. 48:741–2*
- *J Antimicrob Chemother 2008. 62: 1046–52*
- *J Antimicrob Chemother 2013. 68: 362–365*

The majority of *C. difficile* strains are still susceptible to VAN and FDX

Epidemiological cut-off (ECOFF) for reduced susceptibility to VAN is defined as MIC> 2mg/L (EUCAST)

C. difficile isolates with MICs >1 mg/L for FDX have been defined intermediate

Clin Microbiol Infect. 2014. 21:248.e9-248.e16

- Vancomycin (VAN) is a first-line option in severe CDI
- Few strains with reduced susceptibility to VAN (MICs range 4-16 mg/L) as been found

Clin Microbiol Infect. 2014. 21:248.e9-248.e16
AAC. 2002. 46:1647-50
AAC. 2007. 51. 8: 2716-19
AAC. 2012. 56: 3943-49
Iranian Red Crescent Medical Journal. 2013. 15: 704-11
International Journal of Antimicrobial Agents 41 (2013) 80– 84

- Fidaxomicin (FDX) is currently used in case of severe CDI and recurrences
- *C. difficile* isolates are susceptible to FDX
  MIC_{50} ≤0.016 - 0.25 mg/L
  MIC_{90} 0.125 - 0.5 mg/L

Expert Rev Anti Infect Ther. 2010. 8: 555-64
Clin Microbiol Infect. 2014. 21:248.e9-248.e16
2010. 8: 555-64; Anaerobe. 2003. 47.2334-38

- Only strain with MIC=16 mg/L have been identified

CID.2012. 55 (Suppl 2): S143

J Antimicrob Chemother 2014; 69: 41–44
doi:10.1093/jac/dkt302 Advance Access publication 25 July 2013

Journal of Antimicrobial Chemotherapy

In vitro selection, via serial passage, of *Clostridium difficile* mutants with reduced susceptibility to fidaxomicin or vancomycin

Jennifer A. Leeds[1]*, Meena Sachdeva[1], Steve Mullin[2], S. Whitney Barnes[3] and Alexey Ruzin[1]

C. difficile mutants with reduced susceptibility to VAN (MICs 4-16 mg/L) or FDX (MICs 1-4 mg/L) has been isolated in vitro after passages on medium containing antibiotics

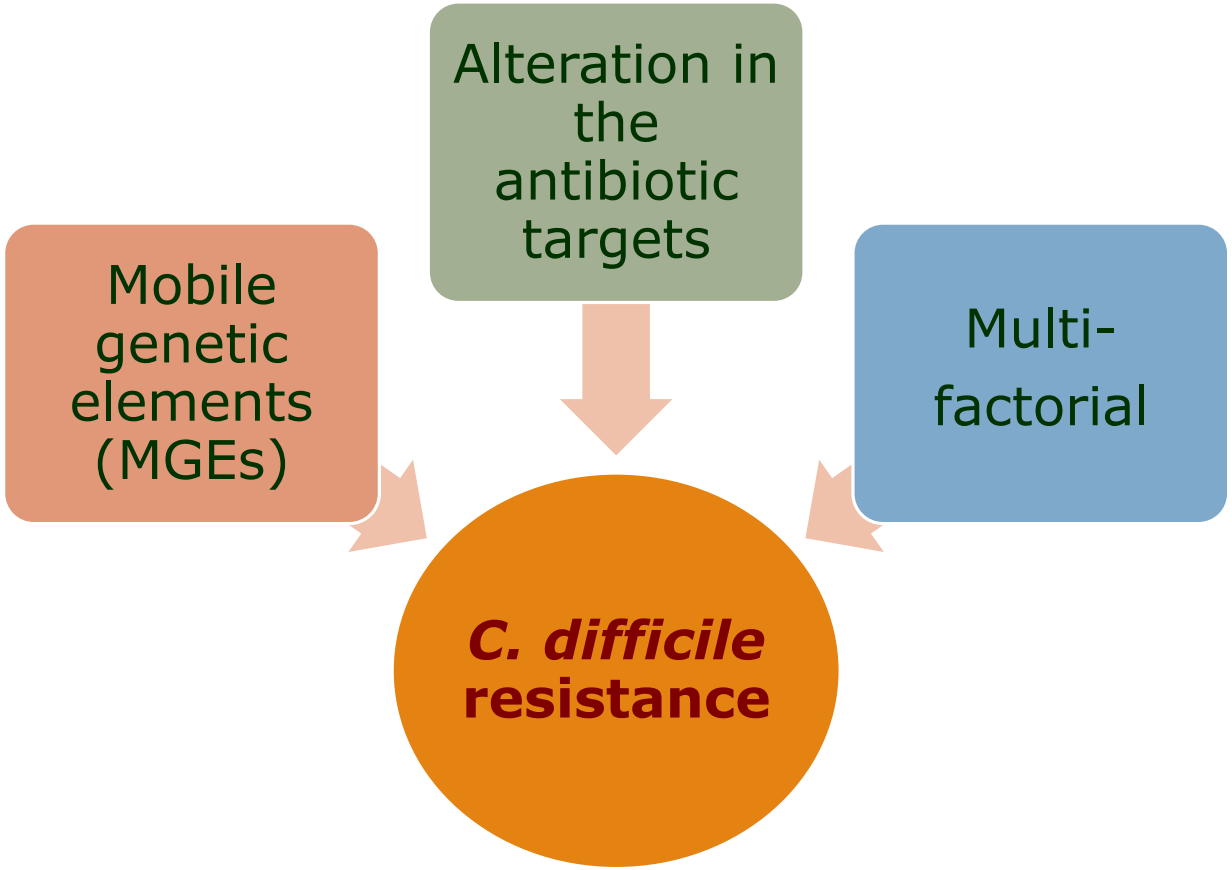
J Antimicrob Chemother. 2014 69: 41-44

Mutants show mutations mediating target modification (RNA polymerase, cell wall)

Strain	Selecting agent	Gene name	Nucleotide change	Amino acid change
NB95013-JAL0859	FDX	*marR/CD22120*	ΔT349	frameshift after amino acid 117
NB95013-JAL0863	VAN	*murG/CD2725*	C326T	P108L
		CD3659	G982T	E327stop
		sdaB/CD3222	ΔAGC (879–881)	ΔA295
NB95026-JAL0861	FDX	*rpoB/CD0066*	A3221G	Q1073R
NB95026-JAL0865	VAN	*rpoC/CD0067*	G733T	D244Y

Since the fecal concentration of these antibiotics is high (2000 µg/g for VAN and >1000 µg/g for FDX) the clinical significance of strains with reduced susceptibility is unclear

Emergence, spread and persistence of *C. difficile* antibiotic resistance: an increasingly complex topic



Mobile Genetic Elements (MGEs)

- Any region of nucleic acid that can move from one part of the genome to another or between genomes
- About 11% of the *C. difficile* genome is made up of MGEs
- MGEs provide *C. difficile* of remarkable genetic plasticity

OPEN ACCESS Freely available online

PLoS one

Genetic Organisation, Mobility and Predicted Functions of Genes on Integrated, Mobile Genetic Elements in Sequenced Strains of *Clostridium difficile*

Michael S. M. Brouwer, Philip J. Warburton, Adam P. Roberts, Peter Mullany, Elaine Allan*

C. difficile contains a plethora of conjugative transposons (CTns) and mobilisable transposons (MTns)

Usually these elements confer resistance to MLS_B antibiotics (ermB) or tetracycline (tetM)

Strain	Element	Genome excision	Properties	CDS start	CDS stop	Size (kb)	Percent G+C	Transfer frequency (σ)	Genbank accession number (if appropriate)
630	CTn*1*	Yes		CD0355	CD0386	28.9	38.6	$7.0*10^{-9}$ ($9.0*10^{-9}$), $2.0*10^{-8}$ ($1.7*10^{-8}$)	
	CTn*2*	Yes		CD0408	CD0436	42.2	35.1	$2.1*10^{-4}$ ($5.5*10^{-4}$)	
	Tn*5397*	Yes	Tc^R	CD0496	CD0511	20.7	38.3		
	CTn*4*	Yes		CD1091	CD1118	30.5	46.6	$2.3*10^{-6}$ ($4.2*10^{-6}$)	
	CTn*5*	Yes		CD1845	CD1878a	45.6	32.7	$2.8*10^{-5}$ ($2.6*10^{-5}$)	
	CTn*6*	No		CD3326	CD3348	21.3	42.8		
	CTn*7*	Yes		CD3370	CD3392	29.2	40.9	$9.6*10^{-9}$ ($4.4*10^{-9}$)	
	Tn*5398*	ND	Erm^R	CD2001	CD2010b	9.6	35.4		
R20291	CTn*1*-like	No		3453	3475	27.2	39.9		
	Tn*6103*	Yes		1740	1809	84.9	41.2	$<10^{-11}$	BK008007, JF422666
	Tn*6104*	Yes		1744	1764	15.6	48.2		
	Tn*6105*	No		1765	1775	15.8	49.8		
	Tn*6106*	No		1777	1788	11.3	49.9		
CD196	CTn*1*-like	ND		3407	3429	27.2	39.7		
QCD-23M63	Tn*6073*	Yes		3137	2962	29.1	39.3		BK008006, JF422665
	CTn*4*-like	ND		4815	4970	25.0	45.0		
	Tn*6107*	Yes		16791	16991	50.5	33.5		BK008008, JF422667
QCD-63Q42	CTn*1*-like	NA		7272	7692	71.7	42.0		
	CTn*1*-like	NA		9214	9039	30.9	39.7		
	CTn*5*-like	NA		17051	17266	52.2	32.4		
	Tn*6115*	NA		7732	7787	13.6	47.2		
	CTn*7*-like	NA		17276	17431	29.6	40.5		
ATCC-43255	CTn*1*-like	No		6310	6090	38.5	37.7		
	Tn*5398*-like	ND		10502	10527	5.4	36.9		
QCD-37X79	CTn*1*-like	NA		18585	18425	27.2	39.4		
	CTn*5*-like	NA		16948	17243	58.0	37.8		
QCD-66C26	CTn*1*-like	No		18275	18120	27.2	39.4		
	Tn*6110*	Yes		16663	16958	58.0	37.8		BK008009, JF422668
QCD-32G58	CTn*1*-like	No		4196	4166	27.2	39.4		
	Tn*6111*	Yes		2165	3914	53.4†	37.9†		JF422669
QCD-76W55	CTn*1*-like	NA		18479	18319	27.2	39.6		
QCD-97B34	CTn*1*-like	NA		18008	17853	27.2	39.5		

PLosOne. 2011. 8: e23014

CTns

capable of transferring themselves from a donor cell to a recipient using a conjugative-like mechanism

Tn916/Tn916-like elements:
Tn5397 **(tetM)**
Tn6194 **(ermB)**
CTn1/CTn1-like **(catD, ABC transporter)**
CTn6
Ctn7

Tn1549-like elements :
CTn2
CTn4
CTn5/CTn5-like **(ABC transporter)**

MTns

lack genes for conjugation and use genes of CTns or conjugative plasmids present in the same cell

- **Tn5398/ Tn5398-like elements (ermB)**
- **Tn6215 (ermB)**
- **Tn6115 (ABC transporter)**
- **Tn4453a/Tn4453b (catD)**

Tn5398 (MTn)	• *C. difficile* • *S. aureus* • *B. subtilis*	*AAC. 1987. 31: 1039-1045* *J Antimicrob Chemother. 1995. 35:305-315* *AAC. 1983. 23:784-786*
Tn5397 (CTn)	• *C. difficile* • *E. faecalis*	*AAC. 2010. 54: 4924-4926* *J General Microbiol. 1990.136: 1343-1349*
Other Tn916 –like (CTns)	• *C. difficile* • *B. subtilis*	*J Bacteriol. 2012. 194: 2125-2126* *Mobile Genetic Elements. 2012.2:8-12* *PLoS One. 2011. 6(8), e23014*

The mobilizable non-conjugative element Tn5398 has been mainly detect in *C. difficile* RT012

AAC. 2005. 49:2550-2553
Microbial Drug Resistance. 2014. 20: 555-560

Tn916-like conjugative elements are widespread among *C. difficile* strains of different RTs

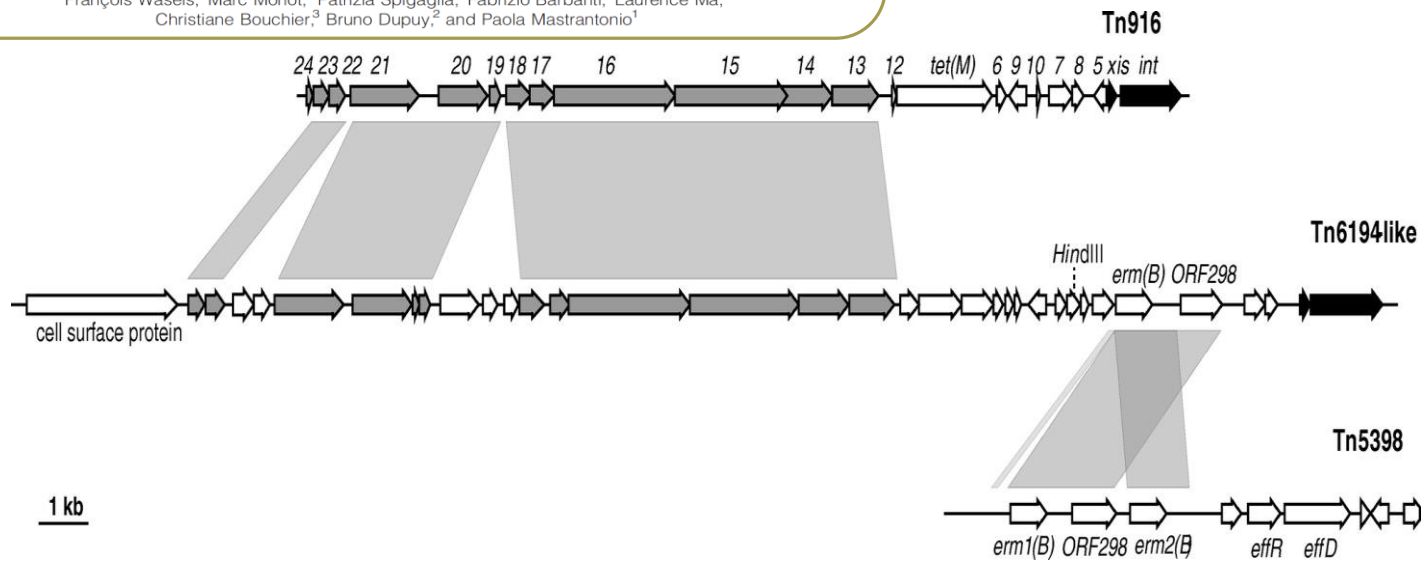
Research in Microbiology. 2015. 166:361-367
AAC. 2005. 49:2550-2553

MICROBIAL DRUG RESISTANCE
Volume 20, Number 6, 2014

DOI: 10.1089/mdr.2014.0015

Inter- and Intraspecies Transfer of a *Clostridium difficile* Conjugative Transposon Conferring Resistance to MLS_B

François Wasels,[1] Marc Monot,[2] Patrizia Spigaglia,[1] Fabrizio Barbanti,[1] Laurence Ma,[3] Christiane Bouchier,[3] Bruno Dupuy,[2] and Paola Mastrantonio[1]

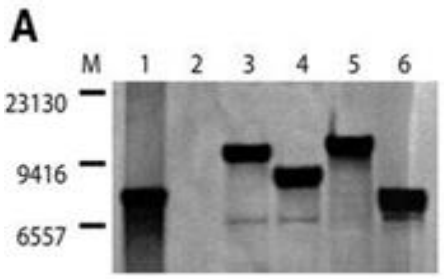


Tn6194 is the most frequently found CTn ermB-containing element in EU epidemic RTs

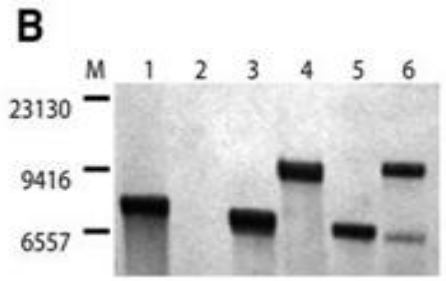
(including RT027, 001 and 017)

Nat Genet. 2012. 45:109-113
Proc. Natl Acad Sci USA 2010: 107: 7527-7332
J Med Microbiol. 2013. 62: 1461-1467
Microbial Drug Resistance. 2014. 20: 555-560

Recipient strain CD37 (RT009)



Recipient strain CD196R (RT027)



Tn6194-like element from CII7 (RT001) is able to in vitro transfer to *C. difficile* and *E. faecalis* JH2-2.

It integrates into the *C. difficile* recipients genome at different sites. A consensus 5'-C[TC]T[AG]GGAG[GA][GT]-3' was identified

Journal of Medical Microbiology (2013), 62, 1461–1467 DOI 10.1099/jmm.0.057117-0

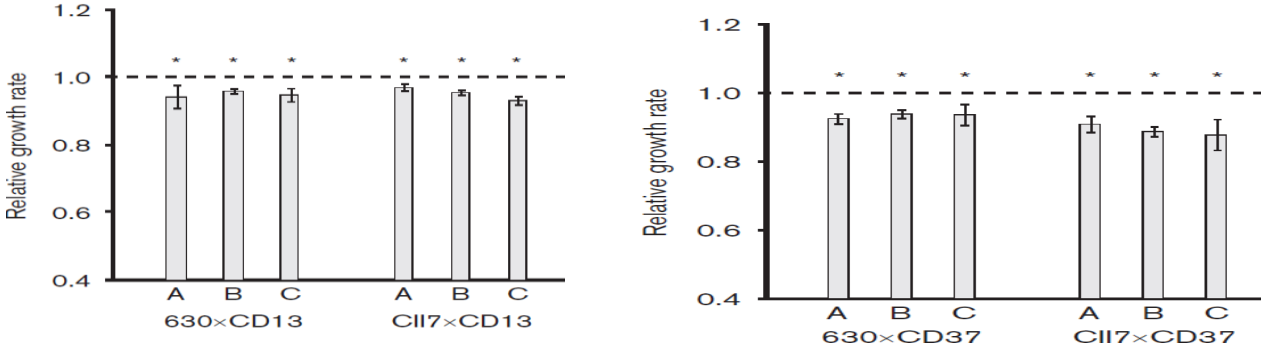
Clostridium difficile erm(B)-containing elements and the burden on the *in vitro* fitness

François Wasels, Patrizia Spigaglia, Fabrizio Barbanti and Paola Mastrantonio

Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy

Fitness cost of Tn6194-like element (strain CII7) and Tn5398 (strain 630) was investigated evaluating growth rates of transconjugants and recipient strain CD13 (RT039) or CD37 (RT009) and by competitive assays

Transconjugants tested vs CD13	*s**	Relative fitness per generation (SD)†	*p*‡
630xCD13A	−0.111	0.889 (0.014)	0.002
630xCD13B	−0.113	0.887 (0.008)	<0.001
630xCD13C	−0.093	0.907 (0.012)	<0.001
CII7xCD13A	−0.110	0.890 (0.012)	0.001
CII7xCD13B	−0.136	0.864 (0.012)	0.001
Transconjugants tested vs CD37			
630xCD37A	−0.035	0.965 (0.030)	0.299
630xCD37B	0.195	1.195 (0.056)	0.075
630xCD37C	−0.039	0.961 (0.029)	0.134
CII7xCD37A	−0.182	0.818 (0.013)	<0.001
CII7xCD37B	0.042	1.042 (0.032)	0.227
CII7xCD37C	−0.149	0.850 (0.078)	0.054



All transconjugants show growth rates significantly reduced

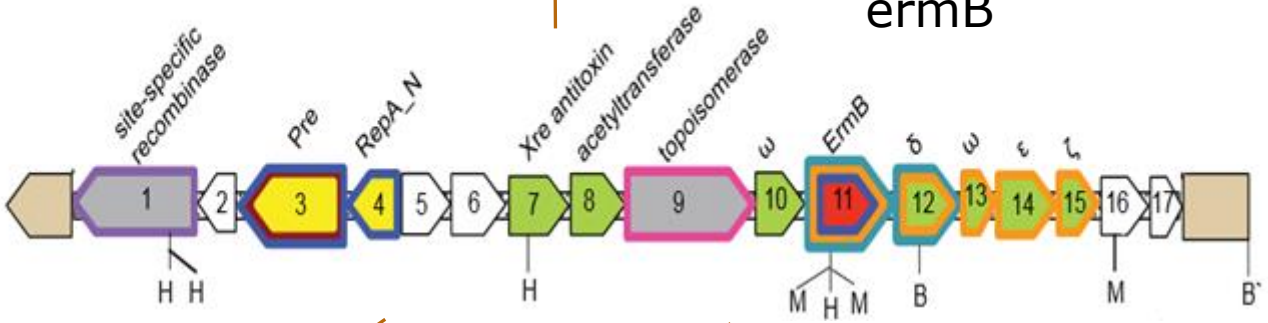
In competitive assays tranconjugants from recipient strain CD13 were disadvantaged, while those from CD37 were differently affected

Independently of the burden that the element imposes on fitness, other factors (the capacity of in vivo transfer, the different insertion sites and the intrinsic characteristics of strains) are involved in the successful spreading and persistance of an element among *C. difficile* population

Conjugation – like mechanism

MBio. 2013.4(6):e00840-13

Tn6215 (MTn)
ermB



Transduction

Phage ΦC2

MBio. 2013.4(6):e00840-13

Transformation – like mechanism

Mobile Genetic Elements. 2015. 5: 12-16

Tn6215 and Tn5398 can integrate the genome of *C. difficile* through exchange of large genomic fragments

Mobile Genetic Elements. 2015. 5: 12-16

Exchange of large genomic fragments has been also observed in *C. difficile* Pathogenicity locus (PaLoc) transfer

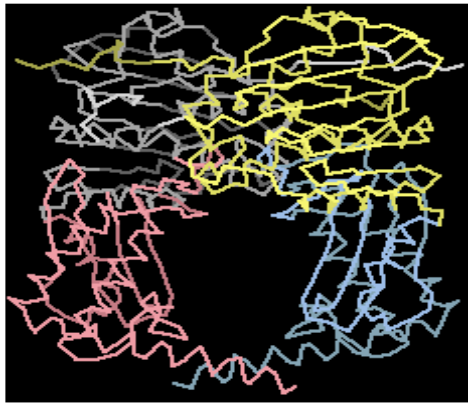
NATURE COMMUNICATIONS. 2013. 4:2601

Whole genome sequence comparison has demonstrated that exchange of large block of DNA between strains is an important driver of *C. difficile* genome evolution

PNAS.2010. 107: 7527-32

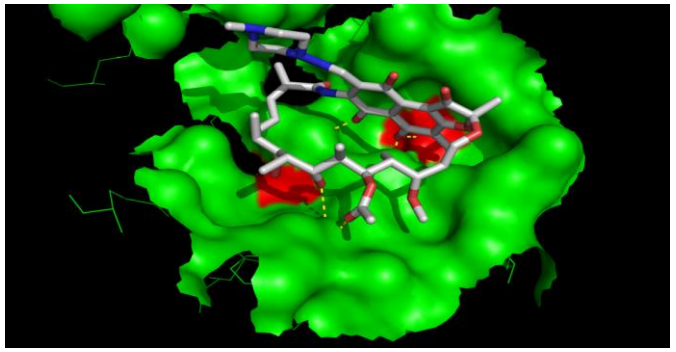
NATURE COMMUNICATIONS. 2013. 4:2601

Alteration in the target sites of antibiotics



FQs resistance:

alterations in the quinolone-resistance determining region (QRDR) of either GyrA or GyrB, the DNA gyrase subunits



RIF resistance:

alterations in the β-subunit of the RNA polymerase (RpoB)

Molecular and Cellular Probes 24 (2010) 61e67

Substitutions in GyrA or GyrB

Allele	Amino acid position	Substitution	Codon mutation	Reference sequence accession number (nucleotide positions)
gyrA				
1	**Thr82**	**Ile**	ACT → ATT	DQ821481–DQ821482
2	**Thr82**	**Ile**	**ACT → ATT**	AM890063
	Thr97	Thr	ACT → ACC	
3	Tyr62	Tyr	TAC → TAT	AM890064
	Thr82	**Ile**	**ACT → ATT**	
4	**Thr82**	**Ile**	**ACT → ATT**	AM890065
	Leu134	Leu	CTG → CTA	
5	Leu48	Leu	TTA → CTA	AM890062
	Lys75	Lys	AAG → AAA	
	Thr82	**Ile**	**ACT → ATT**	
	Gly113	Gly	GGG → GGA	
6	Thr97	Thr	ACT → ACC	DQ821483
	Ser151	Ser	TCG → TCA	
7	Leu48	Leu	TTA → CTA	AM890066
	Lys75	Lys	AAG → AAA	
	Gly113	Gly	GGG → GGA	
gyrB				
1	Ala 375	Ala	GCT → GCC	AM890067
	Arg389	Arg	AGA → AGG	
	Leu398	Leu	TTA → CTA	
	Ser416[b]	Ala	TCT → GCT	
	Phe421	Phe	TTC → TTT	
	Gly425	Gly	GGG → GGA	
	Ser427	Ser	TCA → TCG	
	Lys433	Lys	AAA → AAG	
	Asp437	Asp	GAT → GAC	
	Gly448	Gly	GGG → GGT	
	Lys455	Lys	AAA → AAG	
	Leu462	Leu	CTA → TTA	
2	Arg393	Arg	AGG → AGA	AM890068
	Leu398	Leu	TTA → CTA	
	Asp426	**Asn**	**GAT → AAT**	
	Gly448	Gly	GGG → GGT	
	Leu462	Leu	CTA → TTA	
3	Ser366[a]	Ala	TCA → GCA	DQ642011
	Ile370	Ile	ATC → ATA	
	Arg393	Arg	AGG → AGA	
	Leu398	Leu	TTA → CTA	
	Asp426	**Val**	**GAT → GTT**	
	Gly448	Gly	GGG → GGT	
	Leu462	Leu	CTA → TTA	
	Lys486	Lys	AAA → AAG	
4	**Asp426**	**Asn**	**GAT → AAT**	AM890069
5	Arg447	Lys	AGA → AAA	AM890070
6	Ser366[b]	Ala	TCA → GCA	DQ642012
	Ile370	Ile	ATC → ATA	
	Arg393	Arg	AGG → AGA	
	Leu398	Leu	TTA → TTG	
	Gly448	Gly	GGG → GGT	
	Leu462	Leu	CTA → TTA	
	Lys486	Lys	AAA → AAG	

J Med Microbiol. 2012. 61: 780-785

Substitutions in RpoB

RpoB amino acid substitution	Reduced rifaximin susceptibility	Ribotype (RT) (number of isolates)
Wild-type	No	Different RTs (246)
R505K	Yes	RT027 (46)
		RT072 (1)
T501T + L506L + G510G + G512G + F521F + E541E + K556K	No	RT027 (7)
		RT126 (4)
		RT078 (3)
		RT033 (2)
		RT078 (2)
		RT579 (2)
		RT045 (1)
		RT053 (1)
		RT063 (1)
H502N + R505K	Yes	RT012 (5)
		RT027 (4)
A555A	No	RT023 (3)
		RT027 (3)
		RT115 (1)
		RT063 (1)
		RT053 (1)
H502L	Yes	RT027 (1)
H502N	Yes	RT027 (2)
		RT047 (1)
H502Y	Yes	RT027 (2)
S475S + F481F + D492D + T501T + A508A + G510G + T539T + K556K + S575A	Yes	RT539 (1)
L487F + H502Y	Yes	RT027 (1)
D492N	Yes	RT027 (1)
D492V	Yes	RT053 (1)
H502N + A555A	Yes	RT058 (1)
R505K + I548M	Yes	RT027 (1)
S550F	Yes	RT027 (1)
S550Y	Yes	RT027 (1)

Thr82Ile in GyrA is the most widespread among toxigenic clinical isolates resistant to FQs (93%)

Antimicrob Agents Chemother 2002.46:3418-21
J Med Microbiol 2008.57:784-9

Arg505Lys is the most widespread among RT027 (65%) Resistant to RIF

AAC. 2008. 52:2813–7
J Med Microbiol 2012. 61: 780–5
J Clin Microbiol. 2011. 49: 4319–21
Clin Infect Dis. 2009. 48:425–9

AAC. 2015. 59: 1794-96

Fluoroquinolone Resistance Does Not Impose a Cost on the Fitness of *Clostridium difficile In Vitro*

François Wasels,[a] Sarah A. Kuehne,[b] Stephen T. Cartman,[b] Patrizia Spigaglia,[a] Fabrizio Barbanti,[a] Nigel P. Minton,[b] Paola Mastrantonio[a]

FQ-resistant mutants were generated by introduction of point mutations into either the gyrA or gyrB QRDR domains of the strain *C. difficile* 630 using a two-step allele exchange

Substitutions introduced:

Thr82Ile (common) Thr82Val (rare) GyrA

Asp426Asn (rare) Asp426Val (rare) GyrB

Thr82Ile had no detectable cost on the fitness

This substitution can be maintained in *C. difficile* population even in the absence of antibiotic selective pressure

TABLE 2 MICs of *C. difficile* 630 and the mutants obtained in this study

Strain(s)	MIC (mg/liter) of[a]: MXF	GAT	LVX	CIP
630 (wild type)	0.5	0.75	3	6
ISST82Ia, ISST82Ib, ISST82Ic	32	≥32	≥32	≥32
ISST82Va, ISST82Vb, ISST82Vc	4	8	≥32	≥32
ISSD426Na, ISSD426Nb, ISSD426Nc	4	8	≥32	≥32
ISSD426Va, ISSD426Vb, ISSD426Vc	4	6	≥32	≥32

[a] MXF, moxifloxacin; GAT, gatifloxacin; LVX, levofloxacin; CIP, ciprofloxacin.

TABLE 3 Competition assays between wild-type *C. difficile* strain 630 and fluoroquinolone-resistant mutants

Mutants tested vs 630	*s*[a]	Relative fitness/generation[b]	*P* value[c]
ISST82Ia, ISST82Ib, ISST82Ic	−0.0216	0.9784 ± 0.0757	0.4036
ISST82Va, ISST82Vb, ISST82Vc	−0.0952	0.9018 ± 0.0462	<0.0001
ISSD426Na, ISSD426Nb, ISSD426Nc	0.0877	1.0877 ± 0.0813	0.0052
ISSD426Va, ISSD426Vb, ISSD426Vc	0.0589	1.0590 ± 0.0749	0.0312

[a] Selection coefficient.
[b] Fitness relative to that of the susceptible strain.
[c] Statistical significance of difference in fitness.

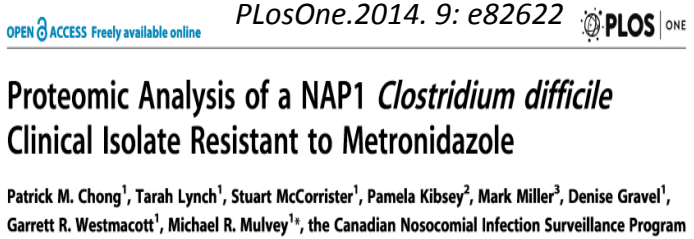
Resistance to antibiotics can be multi-factorial

Altered expression of redox-active ptoteins,iron metabolism and DNA repair

PLosOne.2013. 8: e53757

Characterization of a Stable, Metronidazole-Resistant *Clostridium difficile* Clinical Isolate

Tarah Lynch[1], Patrick Chong[1], Jason Zhang[1], Romeo Hizon[1], Tim Du[1], Morag R. Graham[1], Daniel R. Beniac[1], Timothy F. Booth[1], Pamela Kibsey[2], Mark Miller[3], Denise Gravel[4], Michael R. Mulvey[1]*, Canadian Nosocomial Infection Surveillance Program (CNISP)[¶]

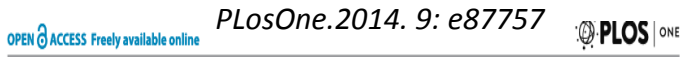


PLosOne.2014. 9: e82622

Proteomic Analysis of a NAP1 *Clostridium difficile* Clinical Isolate Resistant to Metronidazole

Patrick M. Chong[1], Tarah Lynch[1], Stuart McCorrister[1], Pamela Kibsey[2], Mark Miller[3], Denise Gravel[1], Garrett R. Westmacott[1], Michael R. Mulvey[1]*, the Canadian Nosocomial Infection Surveillance Program (CNISP)[¶]

Reduced susceptibility to MTZ



PLosOne.2014. 9: e87757

Spore Formation and Toxin Production in *Clostridium difficile* Biofilms

Ekaterina G. Semenyuk[1], Michelle L. Laning[1], Jennifer Foley[1], Pehga F. Johnston[1], Katherine L. Knight[1], Dale N. Gerding[2], Adam Driks[1]*

AAC. 2014. 58: 4957-60

Multidisciplinary Analysis of a Nontoxigenic *Clostridium difficile* Strain with Stable Resistance to Metronidazole

Ines Moura,[a] Marc Monot,[b] Chiara Tani,[c] Patrizia Spigaglia,[a] Fabrizio Barbanti,[a] Nathalie Norais,[c] Bruno Dupuy,[b] Emilio Bouza,[d] Paola Mastrantonio[a]

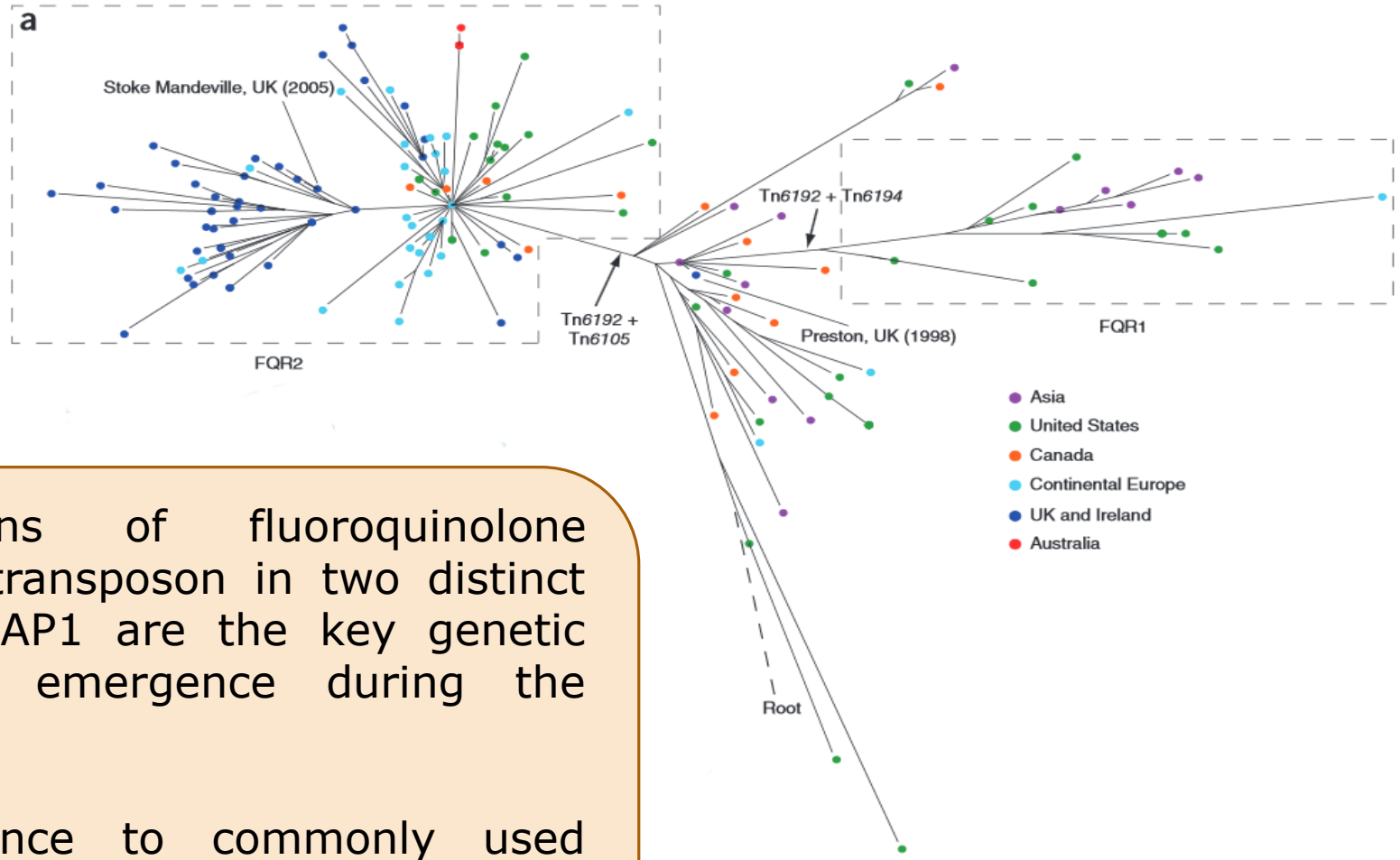
Sessile cells show increased resistance to MTZ

Alterated metabolic pathways involving pyruvate-ferrodedoxin oxidoreductase

nature genetics

Emergence and global spread of epidemic healthcare-associated *Clostridium difficile*

Miao He[1], Fabio Miyajima[2,3], Paul Roberts[2,3], Louise Ellison[1], Derek J Pickard[1], Melissa J Martin[4], Thomas R Connor[1], Simon R Harris[1], Derek Fairley[5], Kathleen B Bamford[6,7], Stephanie D'Arc[6,7], Jon Brazier[8], Derek Brown[9], John E Coia[9], Gill Douce[9], Dale Gerding[10], Hee Jung Kim[11], Tse Hsien Koh[12], Haru Kato[13], Mitsutoshi Senoh[13], Tom Louie[14], Stephen Michell[15], Emma Butt[15], Sharon J Peacock[1,16–18], Nick M Brown[17,18], Tom Riley[19], Glen Songer[20], Mark Wilcox[21], Munir Pirmohamed[2,3], Ed Kuijper[22], Peter Hawkey[23], Brendan W Wren[4], Gordon Dougan[1], Julian Parkhill[1] & Trevor D Lawley[1]



" The separate acquisitions of fluoroquinolone resistance and a conjugative transposon in two distinct lineages of *C. difficile* 027/BI/NAP1 are the key genetic changes linked to its rapid emergence during the early 2000s.

"….the acquisition of resistance to commonly used antibiotics is a major feature of the continued evolution and persistence of *C. difficile* 027/BI/NAP1 in healthcare settings"

- ✓ ***C. difficile*** **epidemiological changes are also associated to changes in antibiotic resistance**
- ✓ **National and international surveillances of antibiotic susceptibility patterns are needed**
- ✓ **Investigation on mechanisms responsible for antibiotic resistance**
- ✓ **New treatment strategies**

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