SUPPLEMENT

Whole-genome sequencing reveals nosocomial *Clostridioides difficile* transmission and a previously unsuspected epidemic scenario

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LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary Figure S1. Phylogenetic relationships among isolates from ribotype 001 contructed with PhyML. Symbols indicate the acquisition of *C. difficile* based on surveillance definitions. Bootstrap values >90% are indicated in the tree.

Supplementary Figure S2. Phylogenetic relationships among isolates from ribotype 027 and AI-33 (isolate CD-17-00124). Symbols and bootstrap values as in Suppl. Figure S1.

Supplementary Figure S3. Phylogenetic relationships among isolates from ribotype 078. Symbols and bootstrap values as in Suppl. Figure S1.

Supplementary Figure S4. Phylogenetic relationships among isolates from ribotype 106. Symbols and bootstrap values as in Suppl. Figure S1.

Supplementary Figure S5. Phylogenetic relationships among isolates from ribotype 014/020. Symbols and bootstrap values as in Suppl. Figure S1.

Supplementary Figure S6. Phylogenetic relationships among isolates from ribotype 446. Symbols and bootstrap values as in Suppl. Figure S1.

Supplementary Figure S7. Putative transmission events connecting ribotype 001 isolates. The figure shows three clusters involving four (red), three (yellow) and two (blue) patients, respectively. Data from all patients that shared closely related *C. difficile* isolates (i. e., ≤2 core-genome SNPs) with other patients are illustrated. Green bars indicate the patients' times of hospital stay and black diamonds indicate dates of *C. difficile* isolation. Connecting lines indicate closely related isolates (i. e., ≤2 core-genome SNPs), with solid lines indicating time intervals ≤90 days and dashed lines indicating >90 days. Asterisks indicate patients with closely related isolates (i. e., ≤2 core-genome SNPs) but not linked to a previous case. Patients *001_B* and *001_F* had occupied the same bed (97 days apart). Acronyms for ward names are as follows: Anaesthesia and Resuscitation Department (ANRH); Angiology and Vascular Surgery (VASH); Cardiology (CAAH); Gastroenterology (GASH); General and digestive surgery (CGDH); Gynaecology (GINH); Haematology (HEMH); Infectious diseases (EINH); Intensive Care Department (MIVH); Internal medicine (MINH); Nephrology (NFRH); Neurology (NRLH); Oncology (ONCH); Otolaryngology (ORAH); Pneumology (NMLH); Plastic Surgery (CPLH); Psychiatry (PQAH); Traumatology (TRAH/TOGH); Urology (UROH). In case the patient stayed in different wards, the underlined ward indicates where the *C. difficile* was isolated.

Supplementary Figure S8. Putative transmission events connecting ribotype 027 isolates. Symbols and lines as in Suppl. Figure S7. The figure shows one cluster involving 17 patients. Patients *027_G* and *027_H* had occupied the same bed (0 days apart) and patients *027_G* and *027_J* had occupied the same bed (100 days apart).

Supplementary Figure S9. Putative-transmission events connecting ribotype 078 isolates. Symbols and lines as in Suppl. Figure S7. The figure represent four clusters, involving 13 (blue), three (red), three (purple) and two (yellow) patients, respectively. Patients *078/126_I* and *078/126_K* had occupied the same bed (50 days apart).

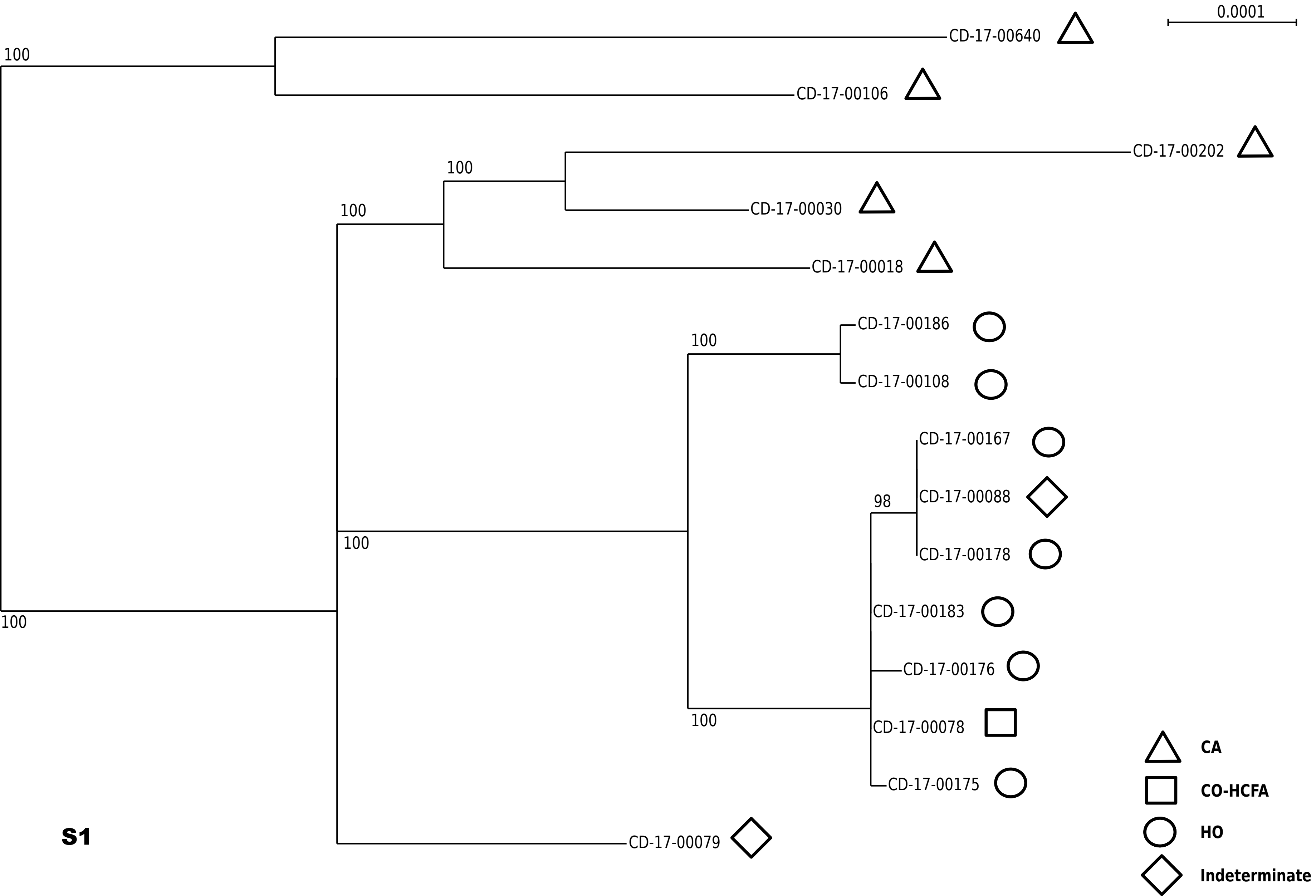
Supplementary Figure S10. Putative-transmission events connecting ribotype 106 isolates. Symbols and lines as in Suppl. Figure S7. The figure shows seven clusters, involving 18 (red), three (olive), two (blue), two (sky blue), two (brown), two (pink) and two (yellow) patients, respectively.

Supplementary Figure S11. Putative-transmission events connecting ribotype 014/020 isolates. Symbols and lines as in Suppl. Figure S7. The figure shows one cluster involving two patients.

Supplementary Figure S12. Putative-transmission events connecting ribotype 446 isolates. Symbols and lines as in Suppl. Figure S7. The figure shows one cluster involving three patients.

Supplementary Figure S13. Flow diagram indicating numbers of analyses.

Ribotype 001

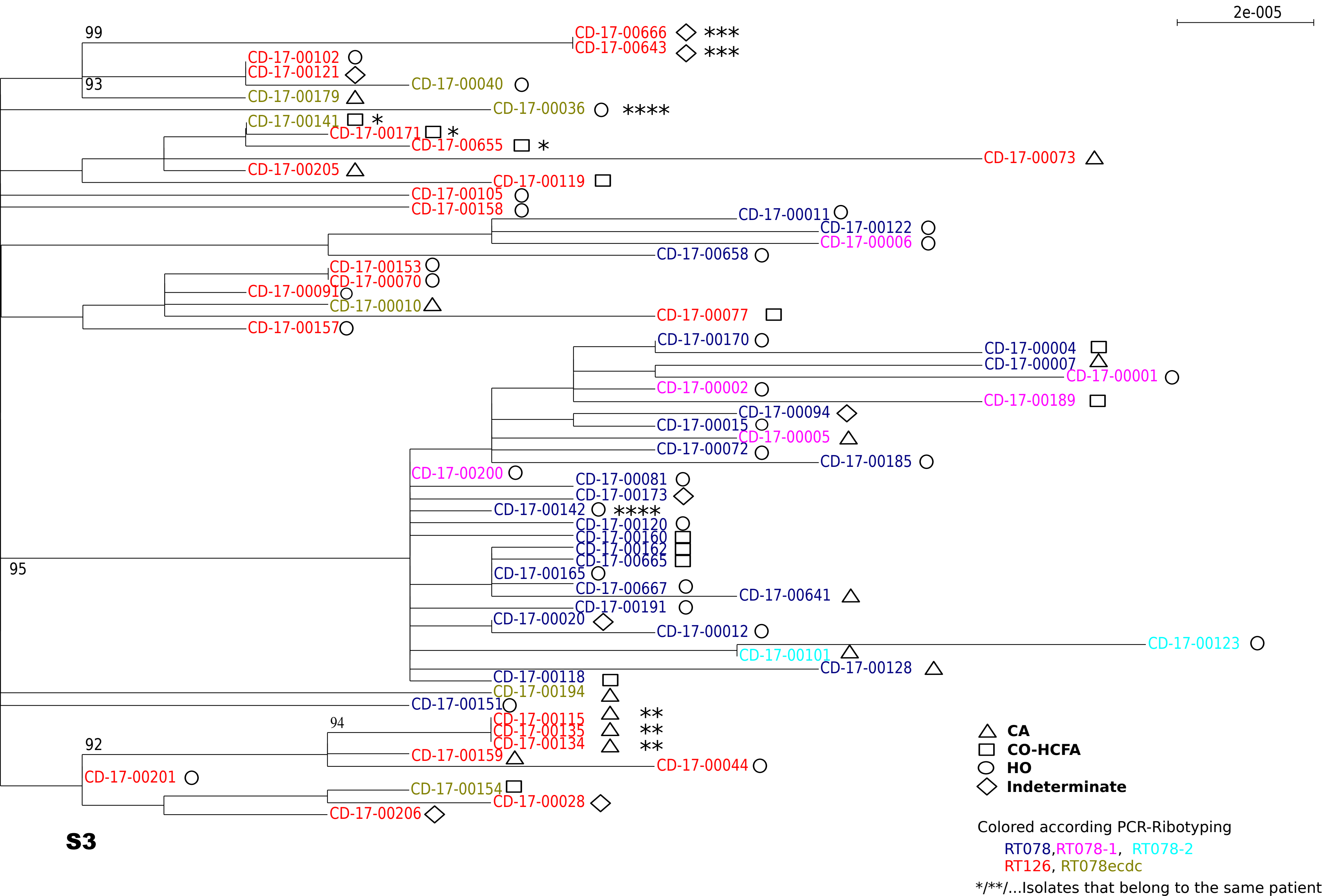


S1

Ribotype 027

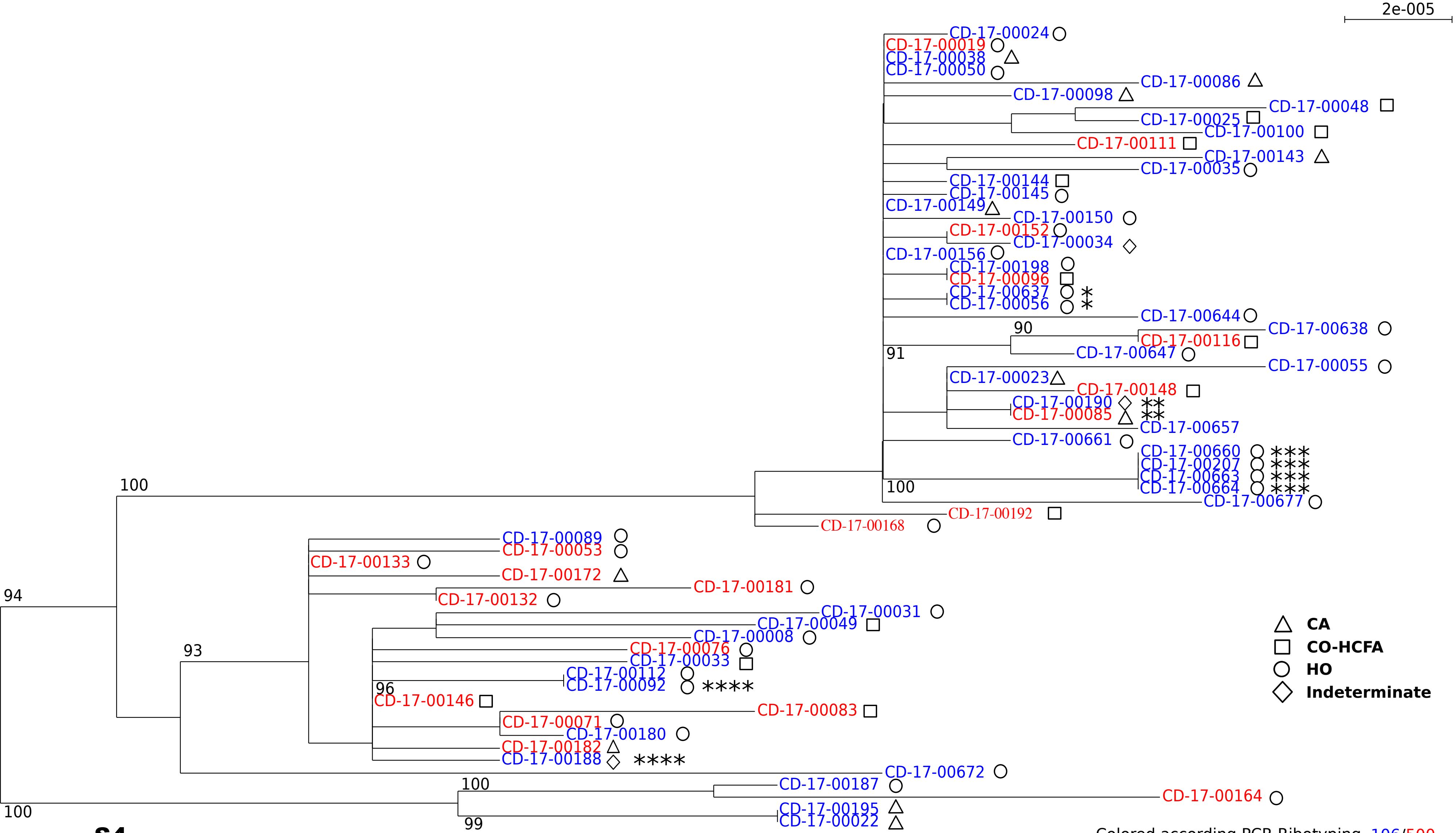
*/**/...Isolates that belong to the same patient

Ribotype 078



S3

Ribotype 106

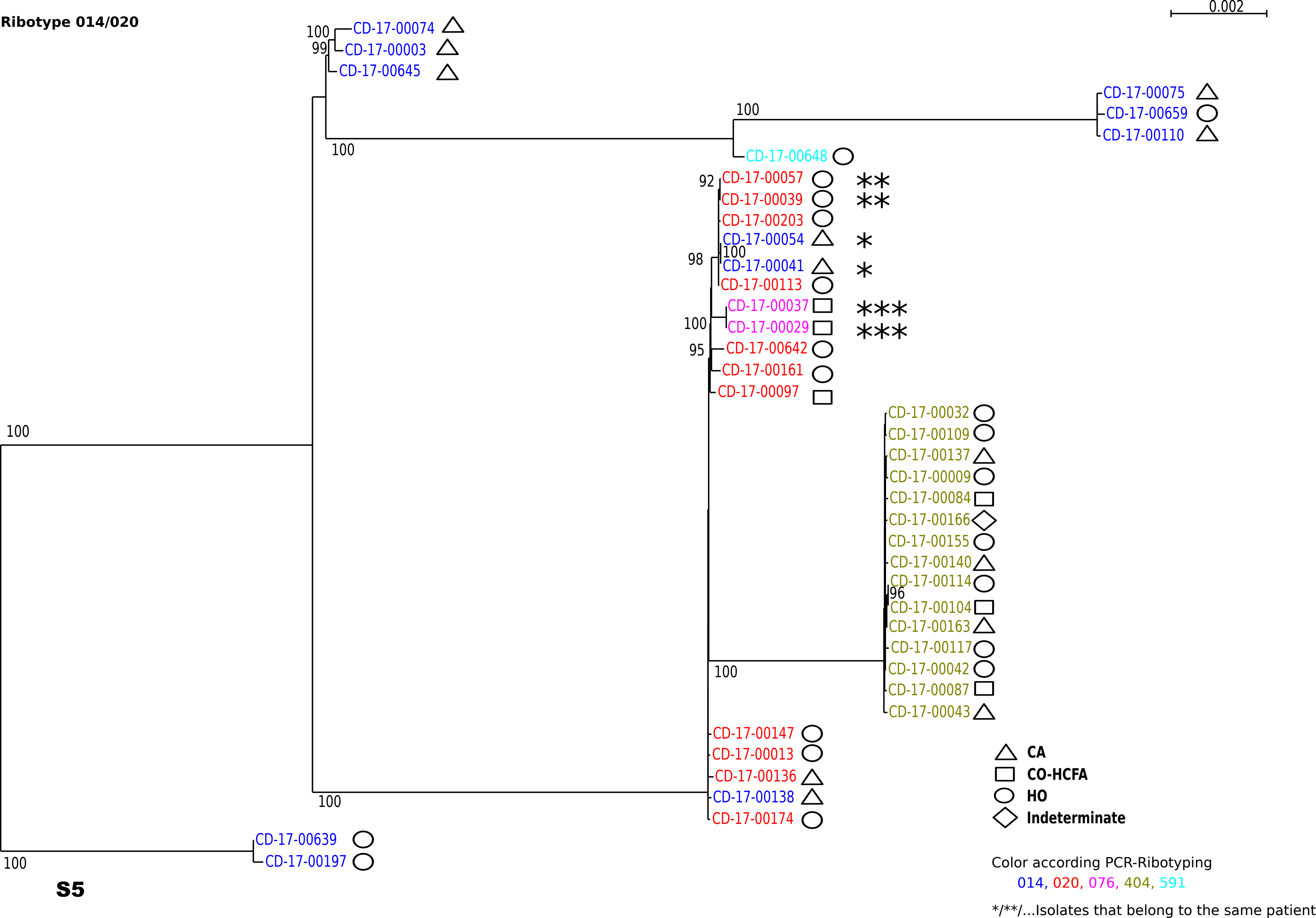


S4

Colored according PCR-Ribotyping 106/500

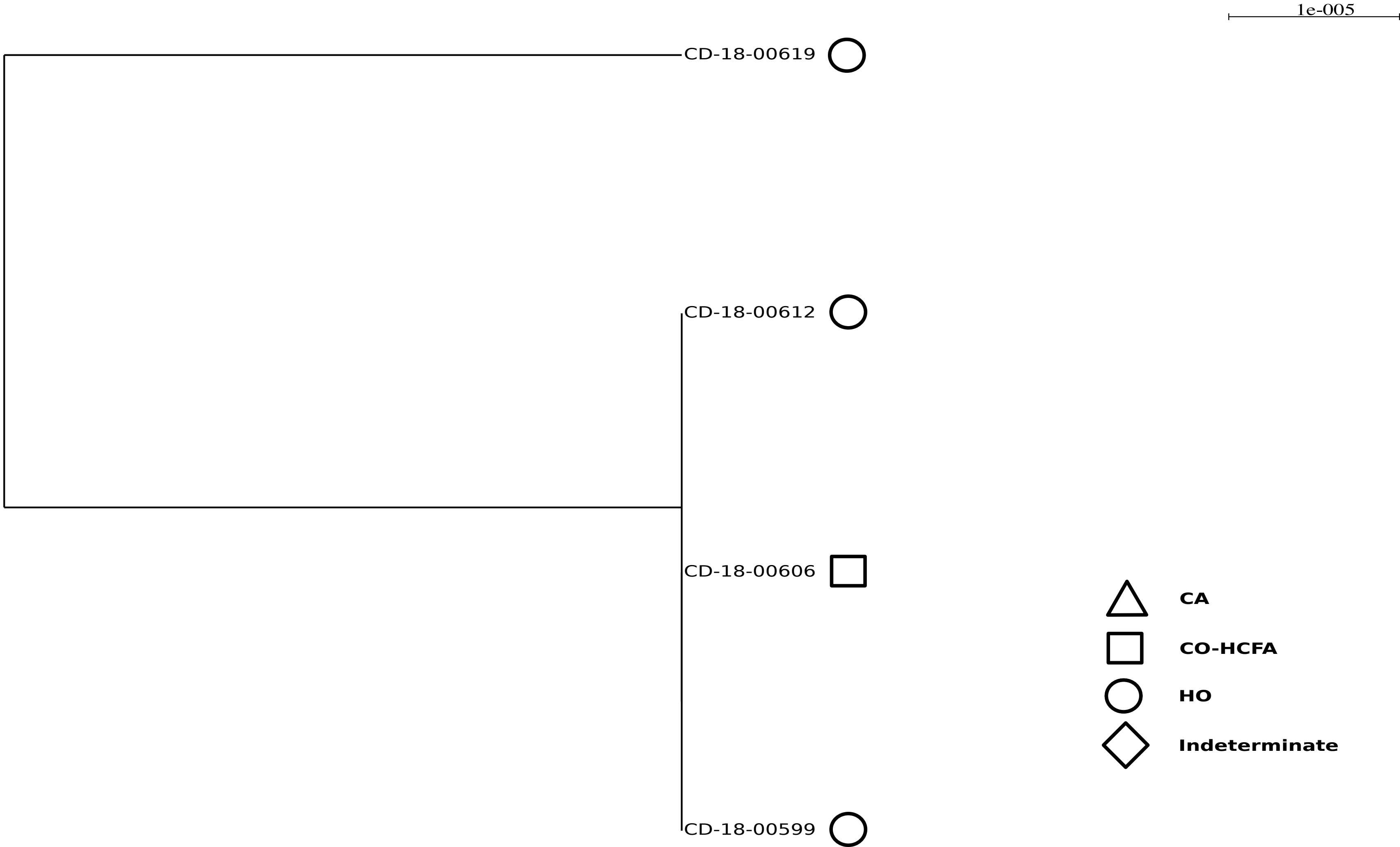
*/**/...Isolates that belong to the same patient

Ribotype 014/020

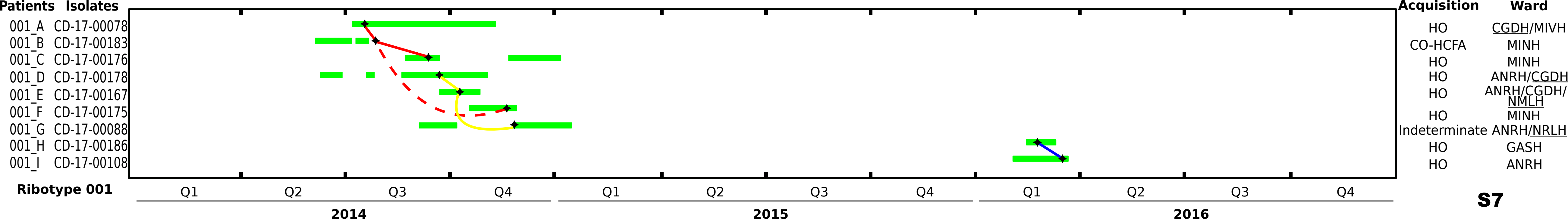


S5

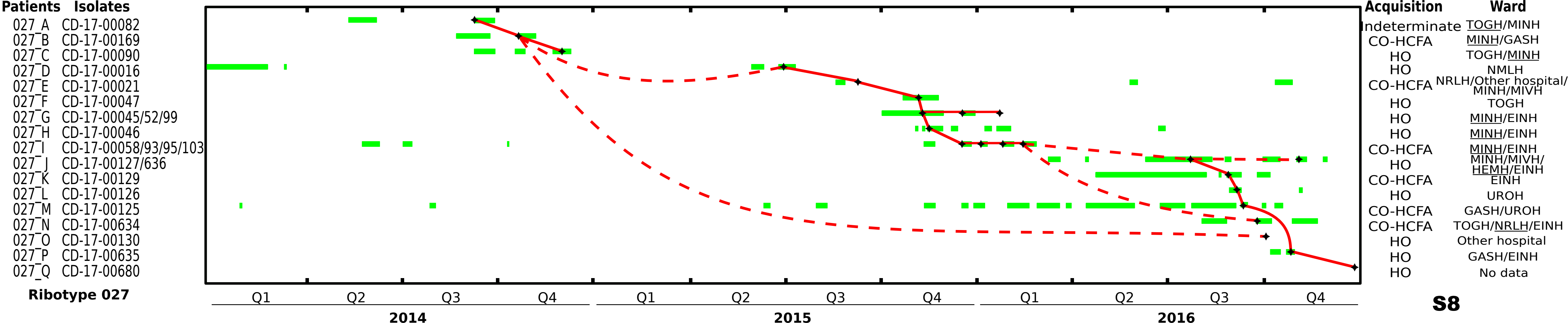
Ribotype 446



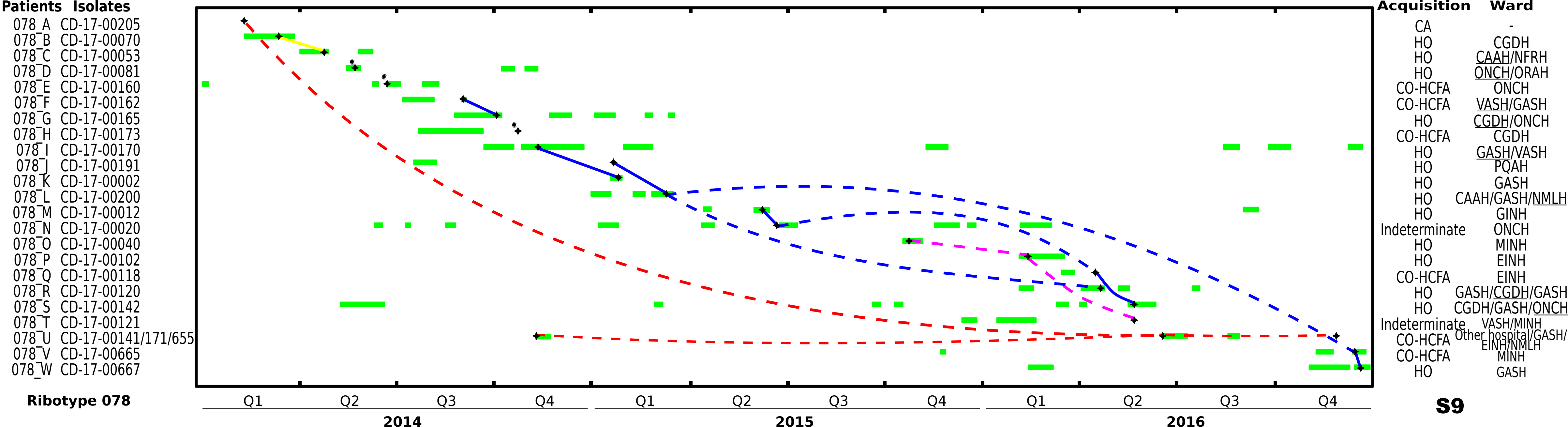
S6


Patients Isolates
001_A CD-17-00078
001_B CD-17-00183
001_C CD-17-00176
001_D CD-17-00178
001_E CD-17-00167
001_F CD-17-00175
001_G CD-17-00088
001_H CD-17-00186
001_I CD-17-00108
Ribotype 001
Q1 Q2 Q3 Q4
2014
Q1 Q2 Q3 Q4
2015
Q1 Q2 Q3 Q4
2016
Acquisition Ward
HO CGDH/MIVH
CO-HCFA MINH
HO MINH
HO ANRH/CGDH
HO ANRH/CGDH/NMLH
HO MINH
Indeterminate ANRH/NRLH
HO GASH
HO ANRH

S7


Patients
Isolates
Acquisition
Ward
027_A CD-17-00082 Indeterminate TOGH/MINH
027_B CD-17-00169 CO-HCFA MINH/GASH
027_C CD-17-00090 HO TOGH/MINH
027_D CD-17-00016 HO NMLH
027_E CD-17-00021 CO-HCFA NRLH/Other hospital/ MINH/MIVH
027_F CD-17-00047 HO TOGH
027_G CD-17-00045/52/99 HO MINH/EINH
027_H CD-17-00046 HO MINH/EINH
027_I CD-17-00058/93/95/103 CO-HCFA MINH/EINH
027_J CD-17-00127/636 HO MINH/MIVH/ HEMH/EINH
027_K CD-17-00129 CO-HCFA EINH
027_L CD-17-00126 HO UROH
027_M CD-17-00125 CO-HCFA GASH/UROH
027_N CD-17-00634 CO-HCFA TOGH/NRLH/EINH
027_O CD-17-00130 HO Other hospital
027_P CD-17-00635 HO GASH/EINH
027_Q CD-17-00680 HO No data
Ribotype 027
Q1
Q2
Q3
Q4
2014
Q1
Q2
Q3
Q4
2015
Q1
Q2
Q3
Q4
2016

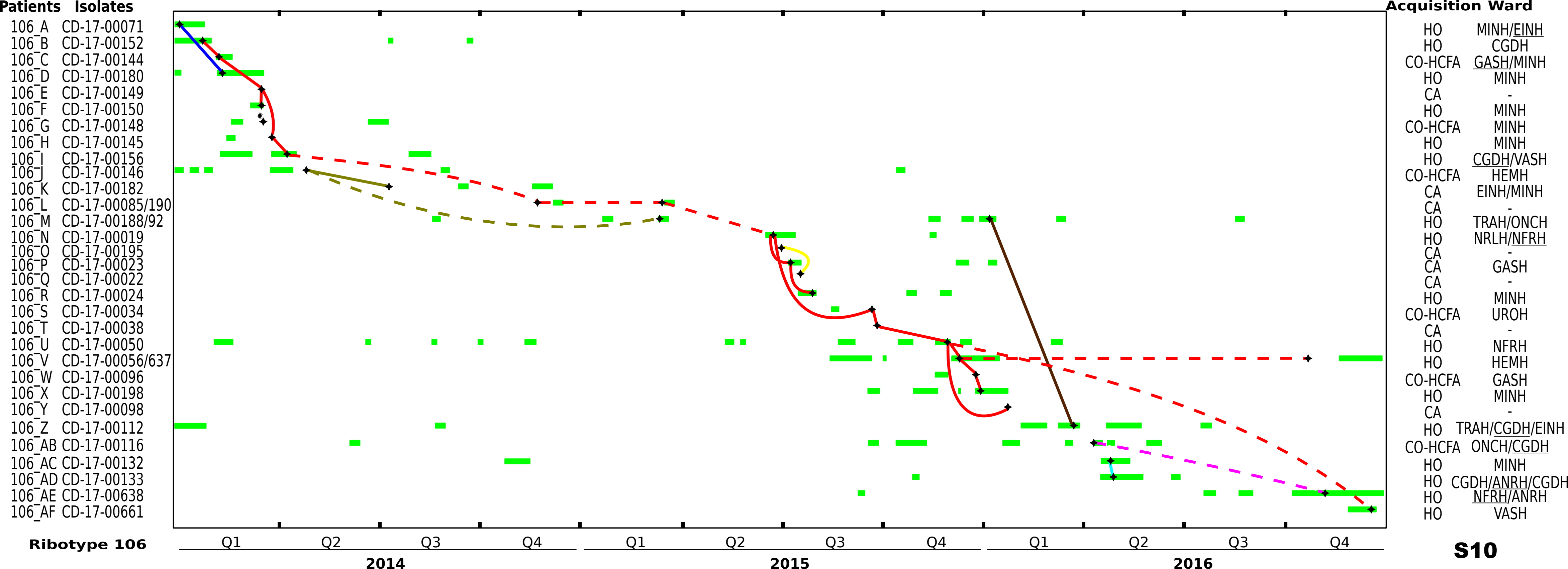
S8



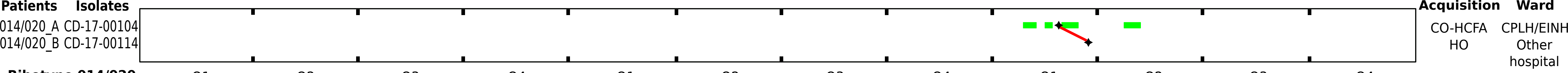
Patients	Isolates	Acquisition	Ward
078_A	CD-17-00205	CA	-
078_B	CD-17-00070	HO	CGDH
078_C	CD-17-00053	HO	CAAH/NFRH
078_D	CD-17-00081	HO	ONCH/ORAH
078_E	CD-17-00160	CO-HCFA	ONCH
078_F	CD-17-00162	CO-HCFA	VASH/GASH
078_G	CD-17-00165	HO	CGDH/ONCH
078_H	CD-17-00173	CO-HCFA	CGDH
078_I	CD-17-00170	HO	GASH/VASH
078_J	CD-17-00191	HO	PQAH
078_K	CD-17-00002	HO	GASH
078_L	CD-17-00200	HO	CAAH/GASH/NMLH
078_M	CD-17-00012	HO	GINH
078_N	CD-17-00020	Indeterminate	ONCH
078_O	CD-17-00040	HO	MINH
078_P	CD-17-00102	HO	EINH
078_Q	CD-17-00118	CO-HCFA	EINH
078_R	CD-17-00120	HO	GASH/CGDH/GASH
078_S	CD-17-00142	HO	CGDH/GASH/ONCH
078_T	CD-17-00121	Indeterminate	VASH/MINH
078_U	CD-17-00141/171/655	CO-HCFA	Other hospital/GASH/ EINH/NMLH
078_V	CD-17-00665	CO-HCFA	MINH
078_W	CD-17-00667	HO	GASH

Ribotype 078

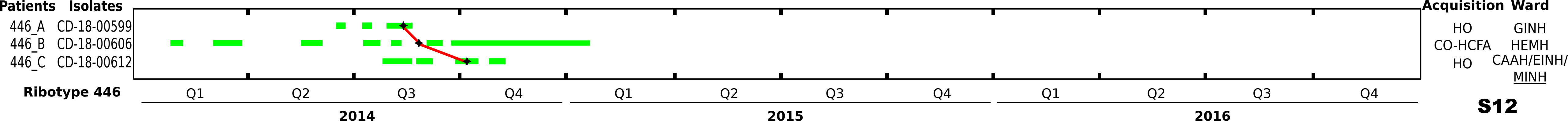
S9

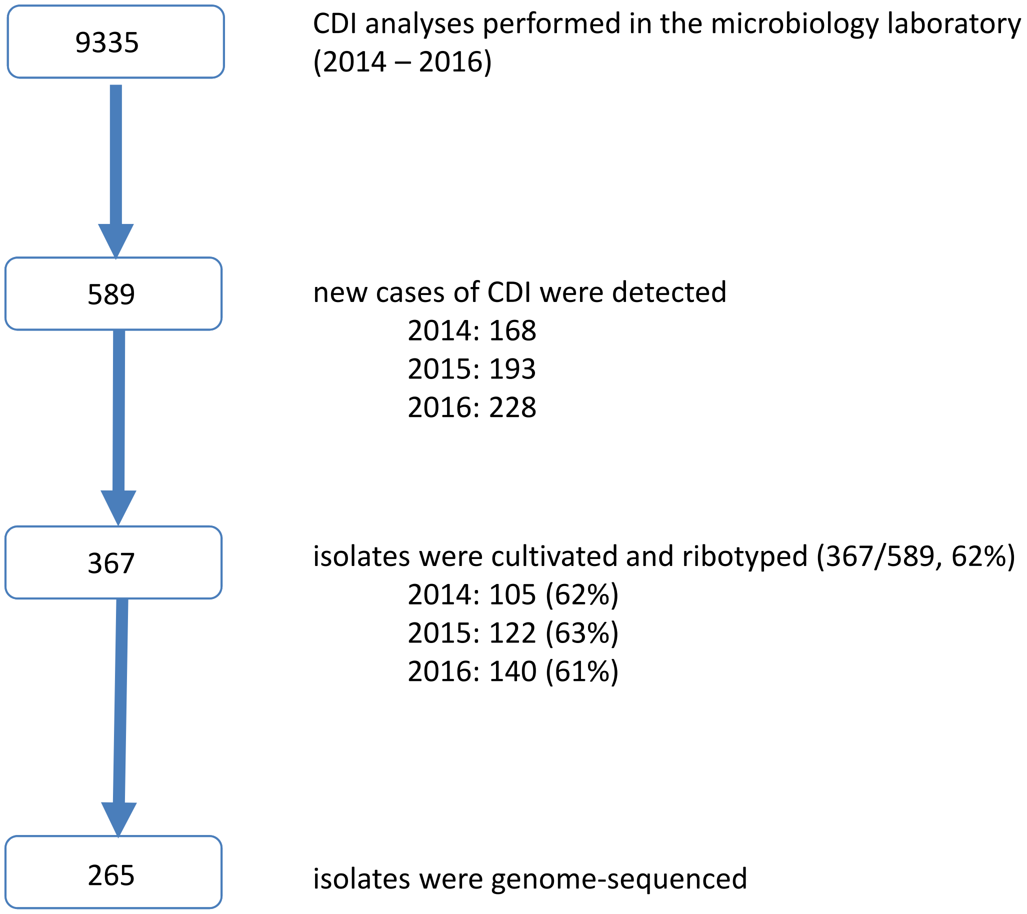

Patients Isolates
106_A CD-17-00071
106_B CD-17-00152
106_C CD-17-00144
106_D CD-17-00180
106_E CD-17-00149
106_F CD-17-00150
106_G CD-17-00148
106_H CD-17-00145
106_I CD-17-00156
106_J CD-17-00146
106_K CD-17-00182
106_L CD-17-00085/190
106_M CD-17-00188/92
106_N CD-17-00019
106_O CD-17-00195
106_P CD-17-00023
106_Q CD-17-00022
106_R CD-17-00024
106_S CD-17-00034
106_T CD-17-00038
106_U CD-17-00050
106_V CD-17-00056/637
106_W CD-17-00096
106_X CD-17-00198
106_Y CD-17-00098
106_Z CD-17-00112
106_AB CD-17-00116
106_AC CD-17-00132
106_AD CD-17-00133
106_AE CD-17-00638
106_AF CD-17-00661
Acquisition Ward
HO MINH/EINH
HO CGDH
CO-HCFA GASH/MINH
HO MINH
CA -
HO MINH
CO-HCFA MINH
HO MINH
HO CGDH/VASH
CO-HCFA HEMH
CA EINH/MINH
CA -
HO TRAH/ONCH
HO NRLH/NFRH
CA -
CA GASH
CA -
HO MINH
CO-HCFA UROH
CA -
HO NFRH
HO HEMH
CO-HCFA GASH
HO MINH
CA -
HO TRAH/CGDH/EINH
CO-HCFA ONCH/CGDH
HO MINH
HO CGDH/ANRH/CGDH
HO NFRH/ANRH
HO VASH
Ribotype 106
Q1 Q2 Q3 Q4
2014
Q1 Q2 Q3 Q4
2015
Q1 Q2 Q3 Q4
2016

S10


Patients
Isolates
014/020_A
CD-17-00104
014/020_B
CD-17-00114
Ribotype 014/020
Q1
Q2
Q3
Q4
Q1
Q2
Q3
Q4
Q1
Q2
Q3
Q4
2014
2015
2016
Acquisition
CO-HCFA
HO
Ward
CPLH/EINH
Other hospital

S11


Patients
Isolates
446_A
CD-18-00599
446_B
CD-18-00606
446_C
CD-18-00612
Ribotype 446
Q1
Q2
Q3
Q4
Q1
Q2
Q3
Q4
Q1
Q2
Q3
Q4
2014
2015
2016
Acquisition
Ward
HO
GINH
CO-HCFA
HEMH
HO
CAAH/EINH/
MINH
S12



9335

CDI analyses performed in the microbiology laboratory (2014 – 2016)

589

new cases of CDI were detected

2014: 168
2015: 193
2016: 228

367

isolates were cultivated and ribotyped (367/589, 62%)

2014: 105 (62%)
2015: 122 (63%)
2016: 140 (61%)

265

isolates were genome-sequenced

S13