Molecular Epidemiology of *Clostridium difficile* in Hong Kong

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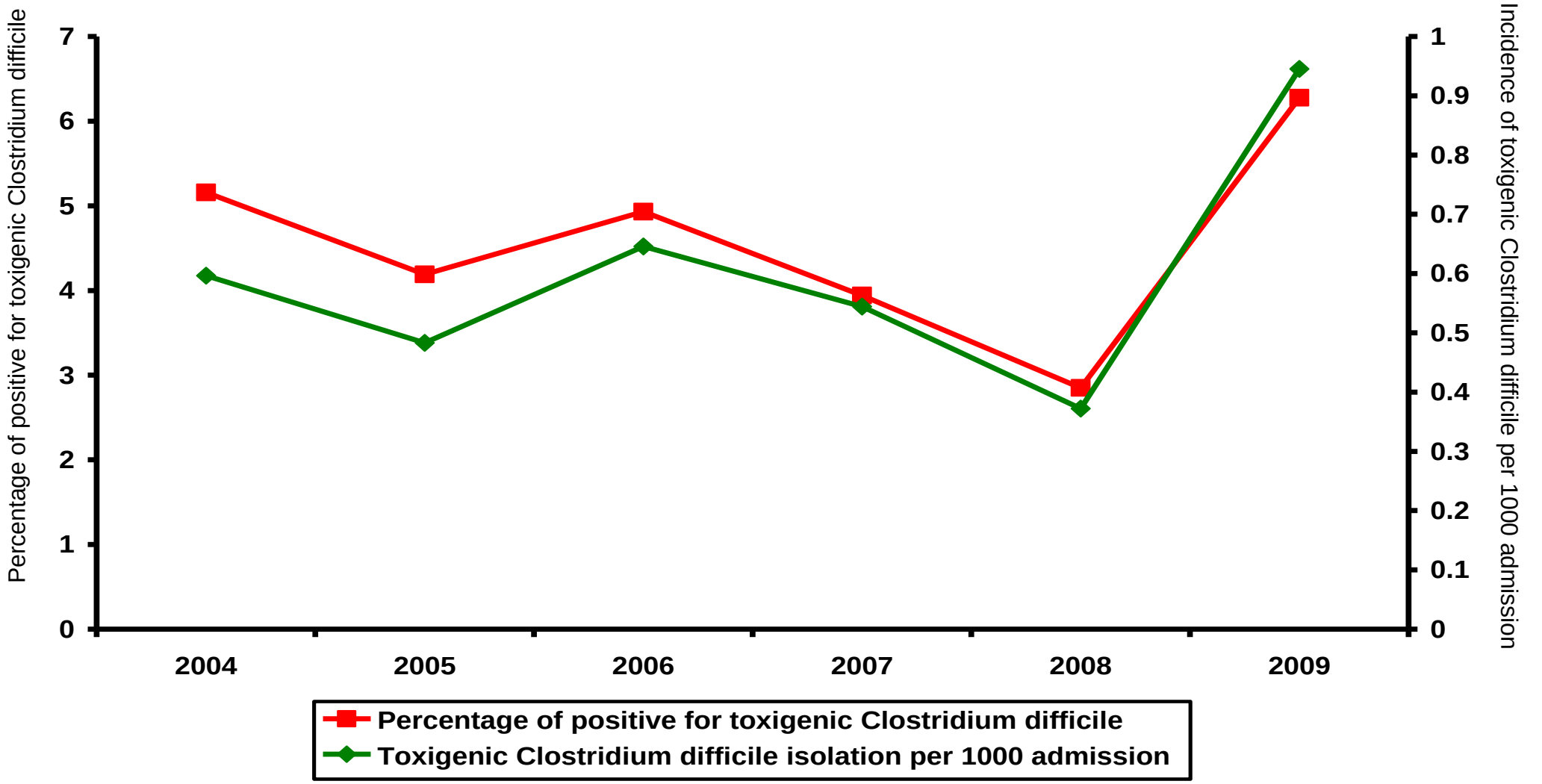
Clostridium difficile

- Anaerobic, Gram-positive, spore-forming bacillus
- Antibiotic associated diarrhea (AAC) and psuedomembranous colitis
- Global dissemination of a hypertoxigenic strain of PCR ribotype 027, toxinotype III, and **N**orth **A**merican **P**ulse-field type **1 (R-027 NAP1)**
  - more severe colitis
  - higher mortality rate
- A sporadic case of R-027 was reported in Hong Kong in 2008
  - tcdA+, tcdB+, binary toxins+, tcdC(deletion)

Trend of isolation of toxigenic *Clostridium difficile* in a healthcare region in Hong Kong



Study design

- One year study in 2009
- A healthcare region of 5 hospitals:
  - 1 acute care university teaching hospital (1,400 beds)
  - 4 chronic care hospitals (110-524 beds)
- Hospitalized patients suffering from GI tract infection:
  - stool specimens collected for culture and cytotoxin assay for *C. difficile*

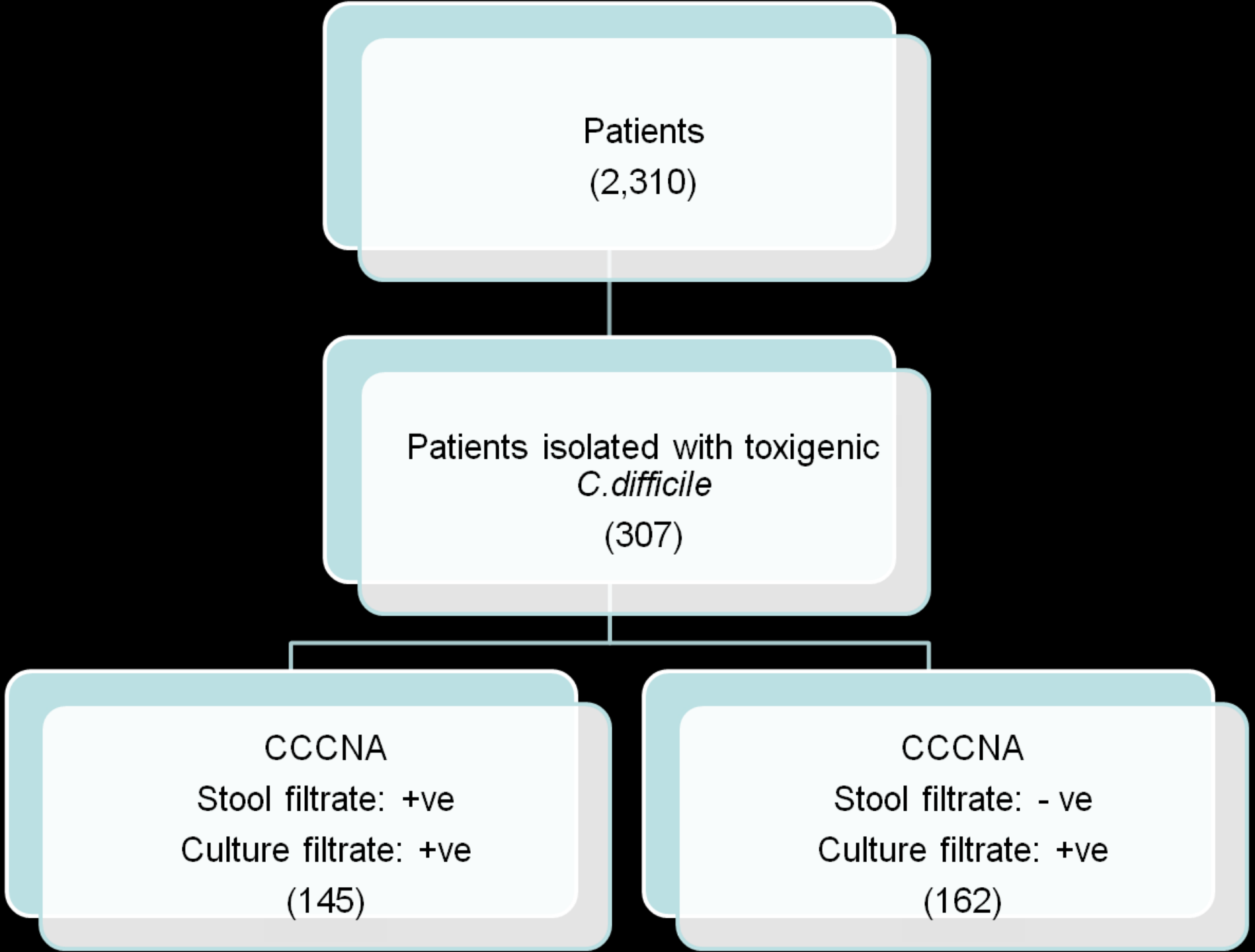
Methods

Isolation of *C. difficile:*

- Cycloserine -Cefoxitin-Fructose Agar (CCFA)
- ID by VITEK ANI card
- E-test (metronidazole, vancomycin, ciprofloxacin)

Cytotoxin detection:

- Hela Cell (Qualitative) + Antitoxin neutralization
  - Stool filtrate
  - *C.difficile* culture filtrate
- ELISA for *C. difficile* Toxin A and B (Quantitative)
  - TOX A/B IITM kit (TechLab, Netherlands)
  - PCR ribotype 002

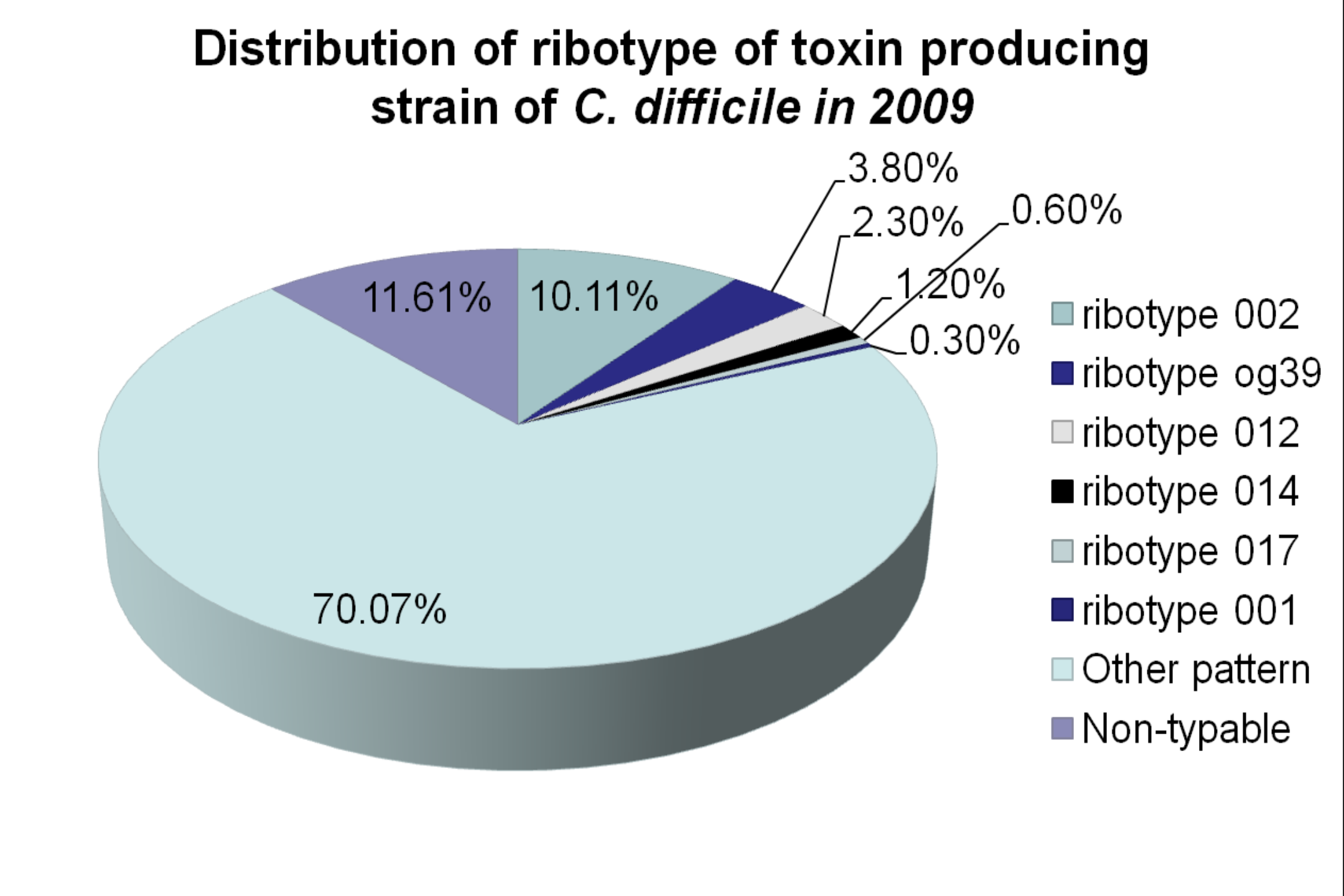

Patients
(2,310)
Patients isolated with toxigenic
C.difficile
(307)
CCCNA
Stool filtrate: +ve
Culture filtrate: +ve
(145)
CCCNA
Stool filtrate: - ve
Culture filtrate: +ve
(162)

Molecular typing

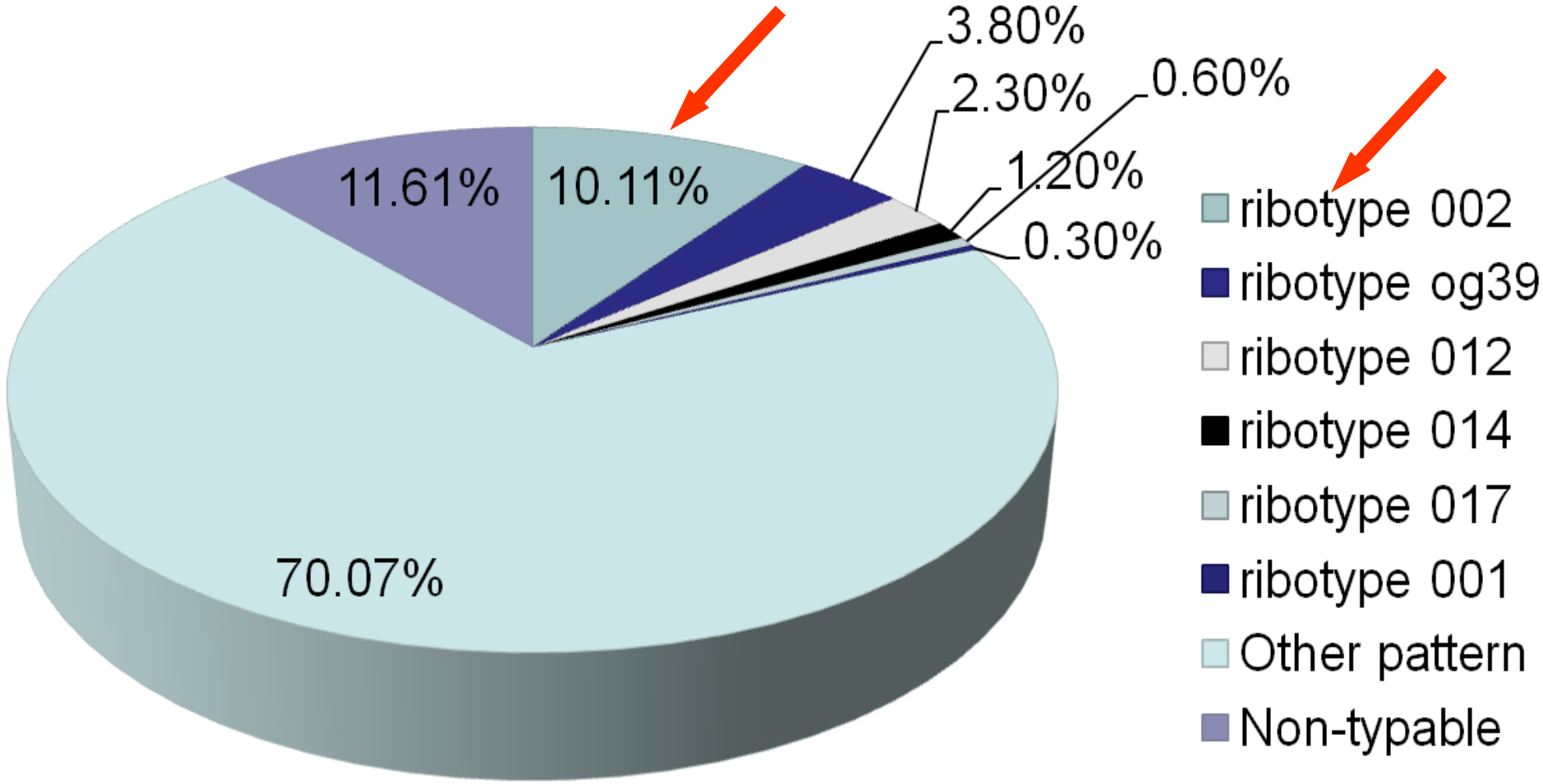
PCR ribotyping (Philip Bidet *et al* 2000)

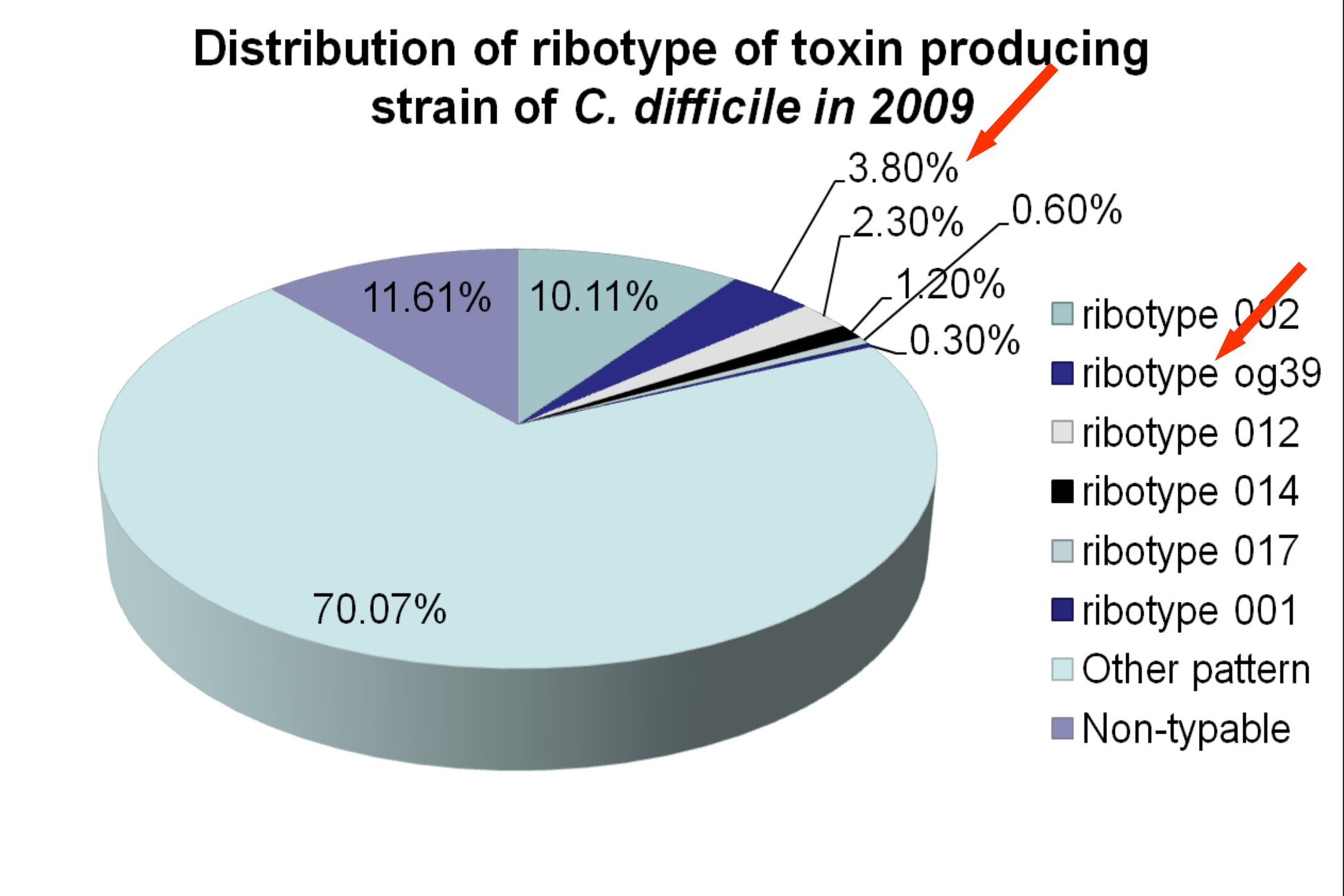
- 23 control strains with known ribotypes (gift from Dr E. J. Kuijper)
- phylogenetic analysis by Bionumeric ver 6.0

Surface layer protiein A (SlpA)- typing (Joost *et al* 2009)


Distribution of ribotype of toxin producing strain of C. difficile in 2009
3.80%
2.30%
0.60%
1.20%
0.30%
11.61%
10.11%
70.07%
ribotype 002
ribotype og39
ribotype 012
ribotype 014
ribotype 017
ribotype 001
Other pattern
Non-typable

Distribution of ribotype of toxin producing strain of *C. difficile in 2009*

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Sporulation ability of R-002

Miles and Misra Method (Hedges *et al*, 1978):

Sub-cullture bacterial suspension to 2 sets of plates -

- (A) incubated for 3 days anaerobic 37°C
- (B) incubated for **2 days** aerobic 37°C, followed by incubation of 3 days anaerobic 37°C

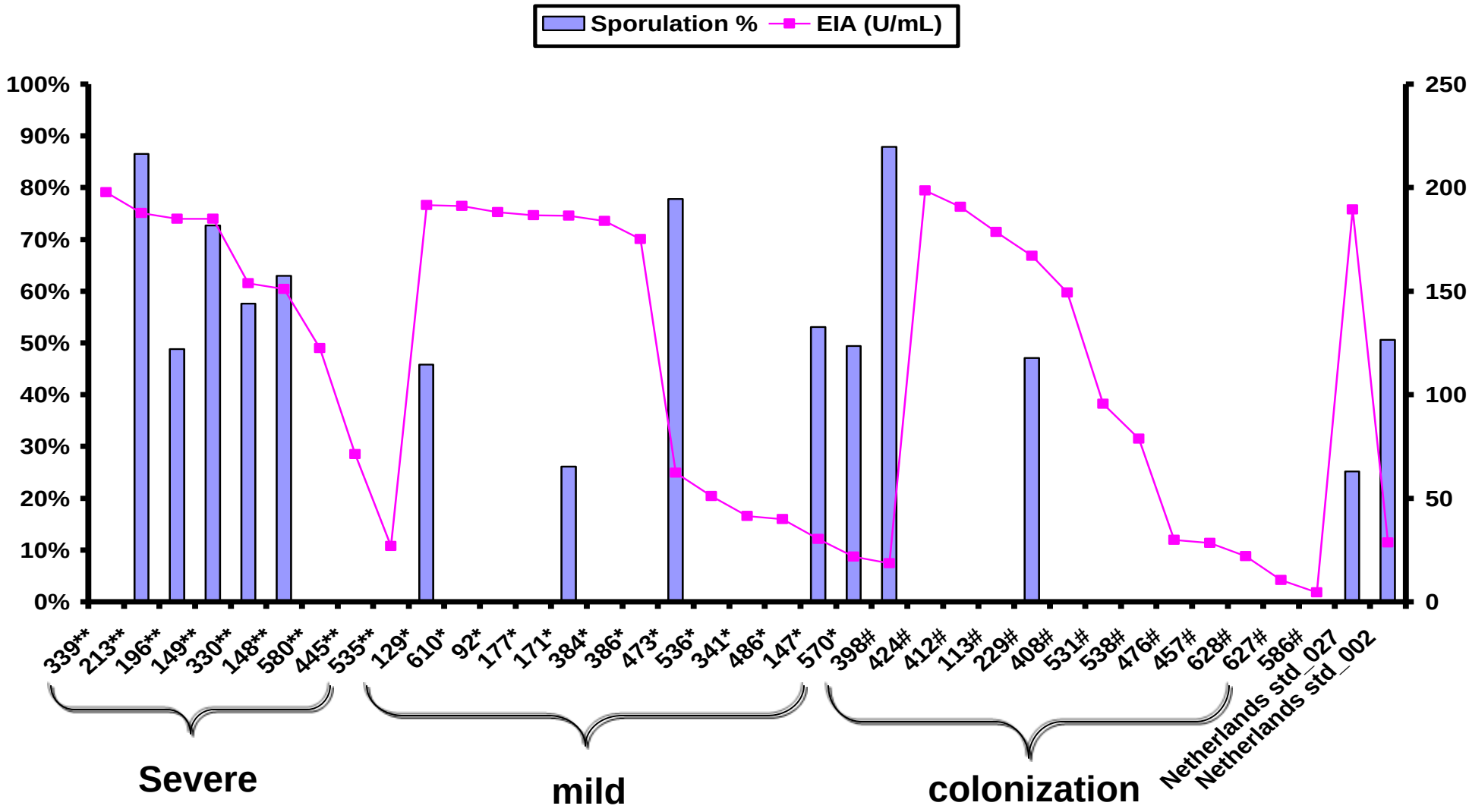
Sporulation frequency (%) : B / A

PCR 002 (Clinical Background)

- Symptomatic infection / asymptomatic colonization
  - Symptomatic infection defined as patients suffering from diarrhea at the time of specimen collection.

- Scoring system for the severity of symptomatic infection:
  - One point: Age > 60; temperature > 38.3°C; Albumin level < 2.5mg/dL; peripheral WBC count >15,000 cell/mm3 within 48h of symptoms onset.
  - Two points (severe *C.difficile* associated diarrhea [CDAD]):
    - Endoscopic evidence of pseudomembranous colitis
    - Treatment in ICU

Sporulation and toxin production of 35 strains of *Clostridium difficile* ribotype 002 isolated in Hong Kong (2009)



	Symptomatic infection	Asymptomatic colonization	P-value
No. of patients	17# • CDAD: 10 • Mild infection: 7	12#	--
Toxin level of C. difficile R-002	123.85 U/ml	96.13U/ml	0.29
Sporulation frequencies of C. difficile R-002	28.9%	3.5%	**0.02***

All 17 patients with symptomatic infection were defined as healthcare-associated infection

PCR 002 in Hong Kong

- Resistant to ciprofloxain (MIC >32µg/ml)
- tcdA and tcdB toxin genes +
- Intact tcdC genes (no deletion)
- No binary toxin gene
- Mean level of toxin production = 112.38 U/ml
- Mean sporulation frequency of 28.9%
  - non-002 ribotypes (3.5% ; $p<0.001$).

Summary

- R-002 is the predominant *C.difficile* ribotype in Hong Kong
- Mean sporulation frequency of R-002 strains was significant higher than other ribotypes
- Symptomatic infection correlates significantly with higher rate of sporulation, but not with toxin production level in R002
  - Epidemic strains reported with an increased inherent sporulation (Akerlund *et al*, 2008)
  - Transmission of C*. difficile* in healthcare was primarily mediated by spores (Alfa *et al*, 2008)
- R-002 in Hong Kong becomes an epidemic strain and contributes to the major C*. difficile* nosocomial transmission in Hong Kong?

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