



FIRST ISOLATION OF CLOSTRIDIODES DIFFICILE FROM SMOKED AND DRIED FRESHWATER FISH IN CAMBODIA

C. RODRIGUEZ^{1,2}, H. MITH³, B. TAMINIAU⁴, L. BOUCHAFA⁴, J. VAN BROECK⁵, K. SOUMILLION⁵, E. NGYUVULA⁵, E. GARCIA-FUENTES^{1,2}, N. KORSACK⁴, M. DELMEE⁵, G. DAUBE⁴



¹ UGC Aparato Digestivo, Hospital Universitario Virgen de la Victoria, Málaga, Spain

² Instituto de Investigación Biomédica de Málaga, Málaga, Spain

³ Faculty of Chemical and Food Engineering, Institute of Technology of Cambodia, Phnom Penh, Cambodia

⁴ University of Liege, Faculty of Veterinary Medicine, Department of Food Science & FARAH, Liège, Belgium

⁵ National Reference Center Clostridium difficile, Microbiology Unit, Catholic University of Louvain, Brussels, Belgium

*presenting author: cris.rdrz@gmail.com



INTRODUCTION

Proper hygiene practices in food processing are essential for prevention of foodborne disease outbreaks. In Cambodia, freshwater aquaculture is the most important source of food production. Fresh fish meat is considered a highly perishable food, which need the use of different manipulations and preservation techniques not only to reduce the water activity, but also to inhibit the development of several undesirable bacteria. These bacteria are naturally present in raw product or could be acquired during manipulation by cross-contamination.

PURPOSE

The objective of this study was to investigate the presence of *C. difficile* in one of the main food supplies of this country, smoked and dried freshwater fish, and to determine if healthy individuals are exposed to *C. difficile* by food ingestion. *C. difficile* isolates obtained for the first time in Cambodia were characterized by PCR-ribotyping, toxin gene profile and antibiotic resistance.

METHODS

- ✓ Samples of smoked and dried freshwater fish were collected from local markets originating from five provinces of Battambang, Kampong Chhnang, Kampong Cham, Kampong Thom and Siem Reap in Cambodia.
- ✓ Samples sold as ready to eat products corresponded to nine species of smoked and dried freshwater fish.
- ✓ *C. difficile* was isolated by direct and enrichment culture, using CCFAT selective medium. Confirmation was performed by detection of the *tpi* gene and the toxin genes by classical PCR.
- ✓ PCR-ribotyping based on capillary gel electrophoresis was also performed.
- ✓ Resistances to erythromycin, vancomycin, clindamycin, tetracycline, metronidazole and moxifloxacin were tested.

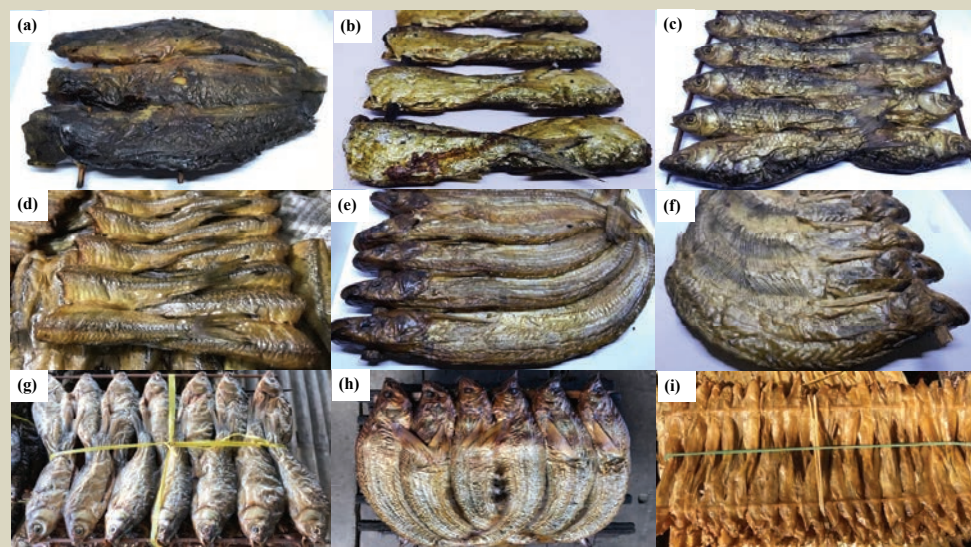


Figure 1. Smoked and dried fish samples coming from nine freshwater fish species in Cambodia (a) *Clarias macrocephallus* (b) *Paralauabuca typus* (c) *Cirrhinus siamensis* (d) *Micronema bleekeri* (e) *Rasbora tornieri* (f) *Ompok bimaculatus* (g) *Thynnichthys thynnoides* (h) *Belodontichthys truncatus* (i) *Clupeoides borneensis*

RESULTS

Type and origin of the samples

Table 1. Tested samples of smoked and dried freshwater fish species originated from different provinces in Cambodia

Code	Local Name	Scientific name	Origin (province)
SR1	Trey Kaes	<i>Micronema bleekeri</i>	Siem Reap
SR2	Trey Andoeng	<i>Clarias macrocephallus</i>	Siem Reap
BB3	Trey Ta Oan	<i>Ompok bimaculatus</i>	Battambang
BB4	Trey Andoeng	<i>Clarias macrocephallus</i>	Battambang
BB5	Trey Riel	<i>Cirrhinus siamensis</i>	Battambang
BB6	Trey Sleku Ruessy	<i>Paralauabuca typus</i>	Battambang
KPCN7	Trey Kaes	<i>Micronema bleekeri</i>	Kampong Chhnang
KPCN8	Trey Andoeng	<i>Clarias macrocephallus</i>	Kampong Chhnang
KPCN9	Trey Riel	<i>Cirrhinus siamensis</i>	Kampong Chhnang
KPCN10	Trey Sleku Ruessy	<i>Paralauabuca typus</i>	Kampong Chhnang
KPCN11	Trey Jongva Moul	<i>Rasbora tornieri</i>	Kampong Chhnang
KPCH12	Trey Andoeng	<i>Clarias macrocephallus</i>	Kampong Cham
KPT13	Trey Ta Oan	<i>Ompok bimaculatus</i>	Kampong Thom
KPT14	Trey Riel	<i>Cirrhinus siamensis</i>	Kampong Thom
KPCH15	Trey Kaes	<i>Micronema bleekeri</i>	Kampong Chhnang
KPCH16	Trey Sleku Ruessy	<i>Paralauabuca typus</i>	Kampong Chhnang
KPCH17	Trey Riel	<i>Cirrhinus siamensis</i>	Kampong Chhnang
KPCH18	Trey Ta Oan	<i>Ompok bimaculatus</i>	Kampong Chhnang
KPCH19	Trey Lenh	<i>Thynnichthys thynnoides</i>	Kampong Chhnang
KPCH20	Trey Bondol Ampov	<i>Clupeoides borneensis</i>	Kampong Chhnang
KPCH21	Trey Klang Hay	<i>Belodontichthys truncatus</i>	Kampong Chhnang
KPCH22	Trey Kaes	<i>Micronema bleekeri</i>	Kampong Chhnang
KD23	Trey Lenh	<i>Thynnichthys thynnoides</i>	Kandal
KD24	Trey Sleku Ruessy	<i>Paralauabuca typus</i>	Kandal
KD25	Trey Riel	<i>Cirrhinus siamensis</i>	Kandal

Figure 2. Traditional food-processing and preparation methods of the smoked and dried freshwater fish, which determines the final quality of the product

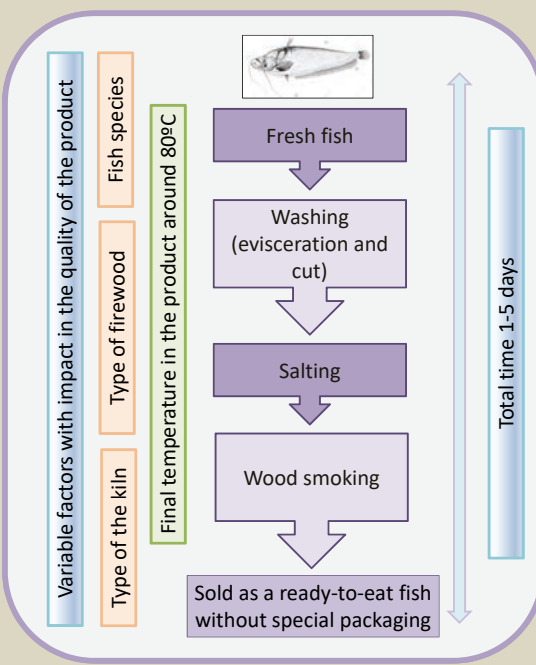
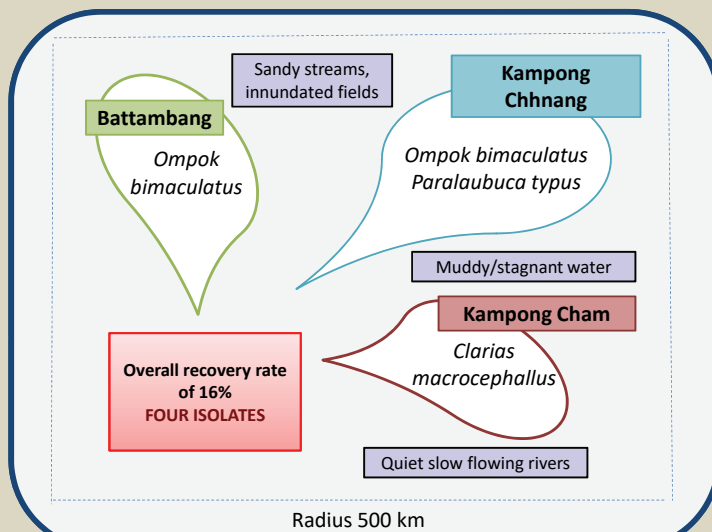


Table 2. Contamination levels and characterisation of *C. difficile* isolates

C. difficile positive samples

Figure 3. Positive *C. difficile* smoked and dried freshwater fish samples collected from 3 different Cambodian provinces



PCR-Ribotype	Toxin Profile	Detection level	Antibiotic resistances
UCL 36	A- B- CDT-	Enrichment culture	Clindamycin
Rare profile	A+ B+ CDT+	Direct culture 100 ufc/g	Clindamycin
Rare profile	A+ B+ CDT+	Enrichment culture	Clindamycin Moxifloxacin Tetracycline
Rare profile	A+ B+ CDT+	Enrichment culture	Clindamycin

CONCLUSIONS

We describe for the first time the presence of the pathogen *C. difficile* in ready-to-eat smoked and dried freshwater fish and we first isolate this bacterium in Cambodia. *C. difficile* was detected before and after enrichment, which indicates a contamination during handling and/or a contamination of the raw fish, followed by an insufficient heat treatment to kill the spore. The presence of *C. difficile* in smoked and dried fish implies a potential risk of human exposure, contamination and human infection.