

Presence of *Clostridium difficile* in fresh mushrooms at retail stores

C. Rodríguez^{*1,2}, B. Taminiau³, E. García-Fuentes^{1,2}, N. Korsak³, G. Daube³

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

² Instituto de Investigación Biomédica de Málaga, Málaga, Spain

³ University of Liege, Faculty of Veterinary Medicine, Department of Food Science & FARA, Liège, Belgium

*cris.rdrz@gmail.com

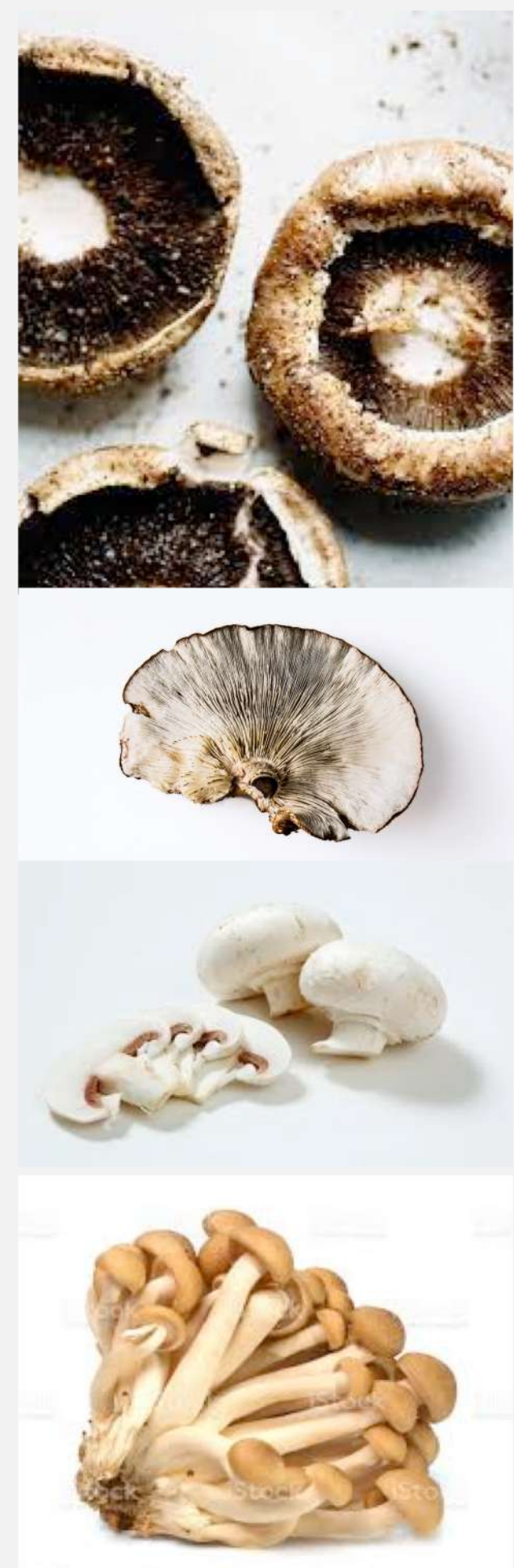
INTRODUCTION

Clostridioides difficile (*C. difficile*) is an opportunistic spore-forming intestinal pathogen, mainly associated with nosocomial infections. The **ecological niche** of the bacterium are **soils**, and therefore *C. difficile* spores are often found in **animals and foods**, suggesting a potential for **foodborne transmission**. The use of **contaminated composted products** with *C. difficile* spores during farming practices along with the ability of the **spores to survive** the composting process are probably the main factors implicated in frequent contamination of fresh vegetables and crops.

PURPOSE

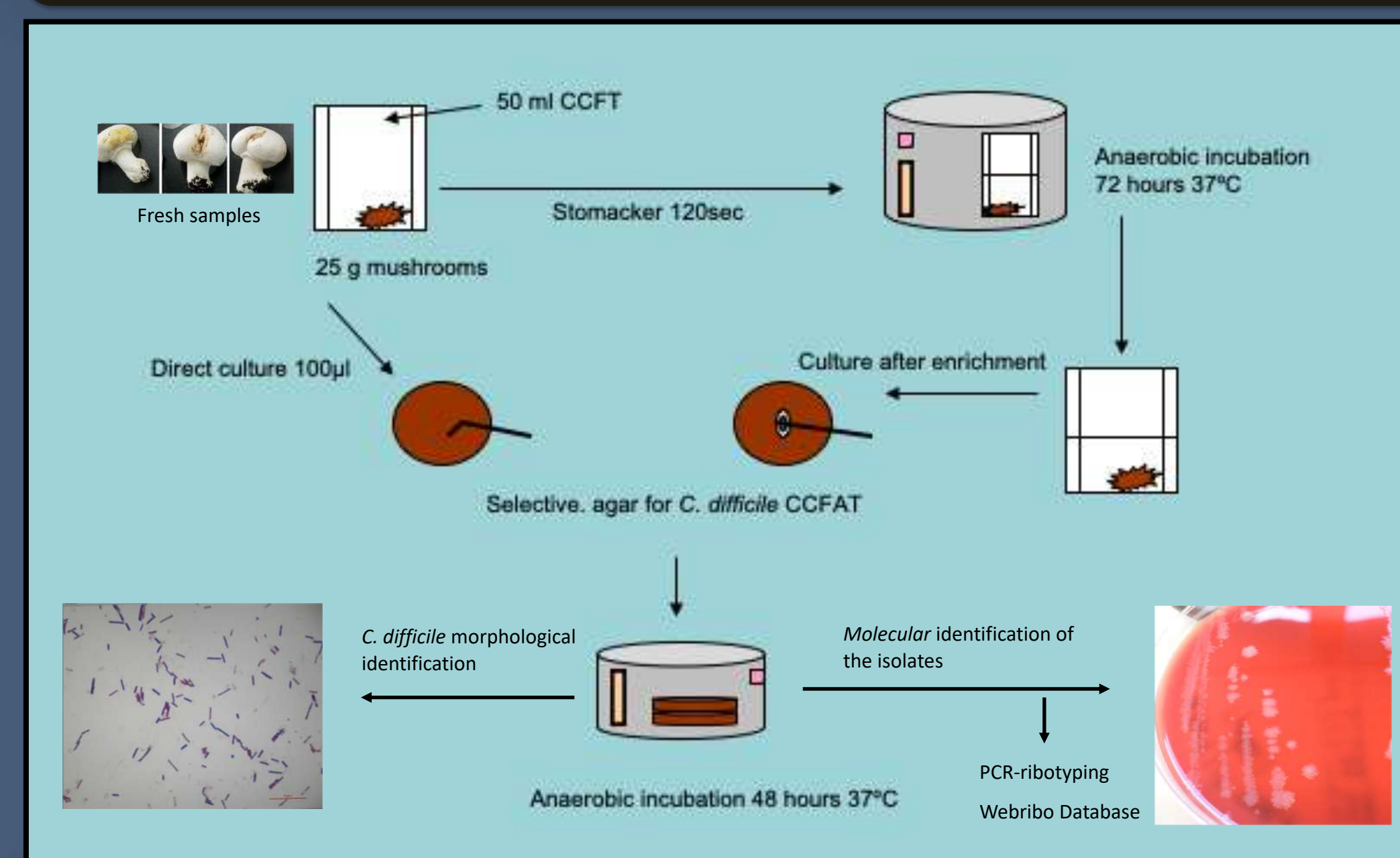
Microbiological studies in cultivated mushrooms are scarce and none of them have been focused on the presence of toxigenic *C. difficile*. The aim of this study was to investigate for the first time the prevalence of *C. difficile* in **fresh mushrooms in Spain**, and to characterize phenotypically and genotypically the *C. difficile* isolates in terms of toxin production, PCR-ribotyping and antimicrobial resistant rates.

METHODS



- ✓ **Samples (n=50)** were collected mainly from 5 different local markets who in turn worked with suppliers from **different regions of Spain**.
- ✓ Sampling was performed over 5 months, including winter and spring seasons.
- ✓ *C. difficile* was isolated by **direct and enrichment culture**, using the selective medium cycloserine cefoxitin fructose taurocholate medium.
- ✓ **Suspected colonies** were identified using a latex agglutination test. **Confirmation** was performed by detection of *tpi* gene and toxin genes *tcdA*, *tcdB* and binary toxin gene *cdtA* by classical PCR.
- ✓ PCR-ribotyping based on capillary gel electrophoresis was also performed.
- ✓ The antibacterial activities were determined by **disk diffusion method** and evaluated by measuring the diameter of the inhibitory zones. **Results were expressed as means of triplicates**
- ✓ Six standard antibiotics: **moxifloxacin** (5 µg/disk), **clindamycin** (2 µg/disk), **tetracycline** (30 µg/disk), **metronidazole** (5 µg/disk) and **vancomycin** (5 µg/disk), were tested for each isolate

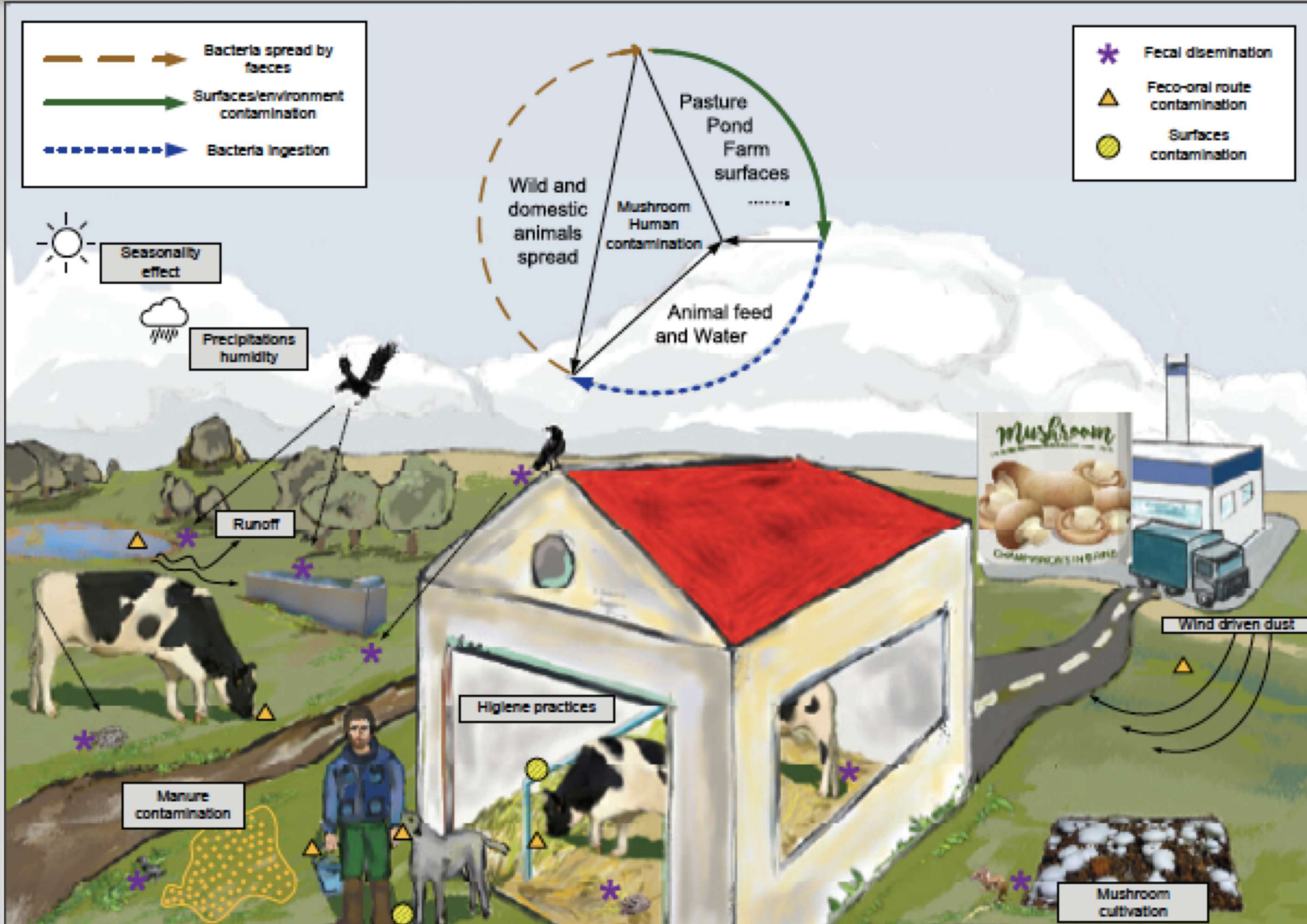
Figure 1. Details of the protocol used for the detection of *C. difficile* in fresh mushrooms collected from retail stores in Spain



The culture medium used CCFT for the enrichment culture and agar plates was selective for *C. difficile* and it was freshly prepared in the laboratory.

RESULTS

Dissemination of *C. difficile* spores in the natural environment: farming contamination



Detection of *C. difficile* in fresh mushrooms

Figure 2 (on the left). Illustration of *C. difficile* spore dissemination in a farming environment. Potential Routes of surfaces, vegetables, animals and human contamination.

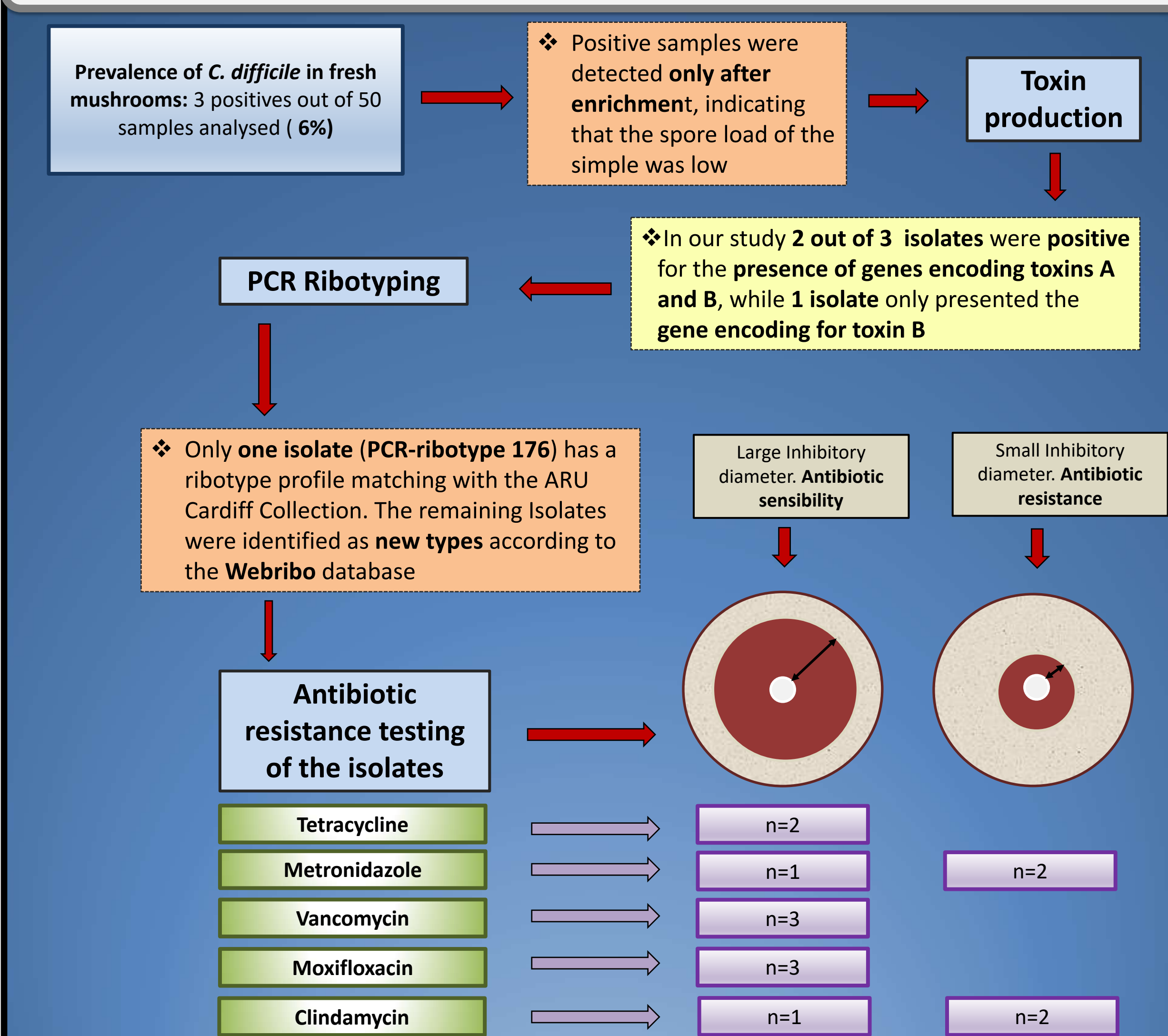


Figure 3 (above). Schematic representation of the main results obtained in this study.

CONCLUSIONS

Mushrooms are an important ingredient in the Mediterranean diet, which are often eaten **fresh without cooking** in salads and side dishes. The findings of this study reveal a **low contamination of fresh mushrooms** sold in retail markets in Spain. However, the **isolates obtained were toxigenic** and exhibited **resistances to antibiotics**, underlining the need of a general recommendation **"to wash properly before consumption"** in the product label.