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Clostridium perfringens and *Clostridium difficile* in cooked beef sold in Côte d'Ivoire and their antimicrobial susceptibility

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ABSTRACT

The aim of this study was to evaluate the prevalence of *Clostridium difficile* and *Clostridium perfringens* in cooked beef sold in the streets in Côte d'Ivoire and their antimicrobial susceptibility. A total of 395 kidney and flesh samples of cooked beef were collected from vendors at Abidjan and subjected to *C. difficile* and *C. perfringens* isolation and identification by using biochemical tests, API 20A system and PCR detection. Subsequently, the antimicrobial susceptibility test was performed for confirmed isolates. Our results showed the prevalence of 12.4% for *C. difficile* (11.04% in kidney and 13.45% in flesh) and 5.06% for *C. perfringens* (2.32% in kidney and 7.17% in flesh). Metronidazole and vancomycin remained the most potent antimicrobial agents against *C. difficile* while metronidazole and penicillin G were the most potent agents against *C. perfringens*. The resistance rates to tetracycline, doxycycline, chloramphenicol and erythromycin against *C. difficile* and *C. perfringens* isolates ranged from 2.05% to 8.16% and from 20% to 50%, respectively. Among all antimicrobial agents tested against *C. difficile*, percentages of resistance to quinolones ciprofloxacin, norfloxacin and nalidixic acid as well as to gentamicin and cefotaxime were the highest. Eight resistant phenotypes were defined for *C. difficile* isolates and eleven resistant phenotypes for *C. perfringens* isolates. Clindamycin/gentamicin/cefotaxime/ciprofloxacin/norfloxacin/nalidixic acid resistance was the most common phenotype for *C. difficile* (55.10% of isolates) while norfloxacin/nalidixic acid resistance was the most common phenotype for *C. perfringens* (20% of isolates).

1. Introduction

Street foods are defined as ready-to-eat foods and beverages prepared and/or sold by vendors on the street from push-carts or buckets or balance poles or stalls or from shops having fewer than four permanent walls [1]. Street foods provide a source of affordable nutrients to the majority of the people especially the low-income group in the developing countries. But, the safety of these foods is affected by several factors starting from the quality of the raw materials, to food handling and storage practices. So they are contaminated by several pathogens such as *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus* and *Salmonella* spp [2–4].

Clostridium difficile has been traditionally regarded as a nosocomial human pathogen [5]. In fact, several authors reported that this bacterium is an important cause of infectious diarrhea that usually develops in patients after hospitalization and antibiotic treatment. The symptoms of *C. difficile* infection range from asymptomatic colonization to mild diarrhea and severe life threatening pseudomembranous colitis [6]. *C. difficile* also appears to be an important cause of enteric disease in a variety of animal species, suggesting that animals and humans may share a common source [5]. In accordance herewith, recent reports show a remarkable overlap between isolates from animals and humans [7]. In recent studies, *C. difficile* has been isolated from food animals such as poultry and sheep [8], pigs [9,10], chickens, goats and cattle [11] and calves [12]. This finding has logically led studies on *C. difficile* in meat and meat products. Canadian studies reported that 12% of samples of beef and pork ground meat were culture positive and the prevalence in chicken meat was 12.8% [13].

Like *C. difficile*, *C. perfringens* is one of the most widespread pathogenic bacteria, and has been associated with a wide range of

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histotoxic and gastrointestinal diseases in both humans and animals [14,15]. *C. perfringens* is also widely distributed in a variety of foods, especially meat and poultry products, and is recognized as an important cause of food poisoning throughout the world [16]. Cooked meat products, such as ham, roast beef and corned beef, are frequently associated with foodborne outbreaks of *C. perfringens* gastroenteritis [17–19].

In spite of the importance of *C. difficile* and *C. perfringens* as pathogenic bacteria widely distributed in meat and meat products, there is little data about the prevalence of these microorganisms contamination in cooked beef sold in the streets. And also very little data have been reported on antimicrobial resistance characterization of these two bacteria isolated from cooked beef sold in the streets. This study evaluated firstly the presence of *C. difficile* and *C. perfringens* isolates in cooked beef sold in the streets at Abidjan, Côte d'Ivoire and secondly assessed the frequency of antimicrobial resistance in these *C. difficile* and *C. perfringens* isolates at the phenotype level.

2. Materials and methods

2.1. Sampling

A total of 395 samples of cooked beef (172 samples of kidney and 223 samples of flesh) were purchased at street food vendors in Abidjan, Côte d'Ivoire between 2009 and 2010. Only one sample of kidney or flesh was purchased from each vendor. The sampling places were randomly selected throughout eight municipalities (Abobo, Adjame, Cocody, Koumassi, Marcory, Port-Bouet, Treichville, Yopougon) within Abidjan. Samples were collected aseptically in sterilized plastic bags by using sterilized utensil and immediately transported to the laboratory in ice-cooled containers within 2 h. The analysis for bacteria isolation started as soon as samples arrived to the laboratory.

2.2. Isolation and identification of C. difficile and C. perfringens

Samples were crushed in the stomacher and ten gram (10 g) of each sample was placed into a sterile plastic bag containing 90 mL of sterilized Buffered Peptone Water and homogenized for 1 min using a stomacher (Seward, West Sussex, UK). In order to quantify spores, 40 mL of the homogenate solutions were heated in screw cap tubes in a water bath at 80 °C for 10 min. Anaerobic total plate and spore counts were performed in duplicates on TSC medium (Bio-Rad, France). After incubation at 37 °C for 24 h, black colonies were purified in TSC agar and identified by Gram staining and the following standards biochemical tests: gas production, lecithinase and lipase productions, motility, starch hydrolysis, indole production, coagulation and retraction of cysteinated milk. Suspected isolates were preliminarily identified as *C. difficile* and *C. perfringens* by using a commercially available identification system, API 20A (BioMerieux, Paris, France) and confirmed by PCR detection. PCR was performed for detection of genes encoding production of triose phosphate isomerase (*C. difficile* isolates) and toxin A (*C. perfringens* isolates) as previously described [20,21]. The reference strains *C. difficile* ATCC 9689 and *C. perfringens* ATCC 3624 were used as positive controls. Isolates were stored in 25% glycerol at −80 °C for further analyses.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing to 16 antimicrobials was carried out for 69 isolates (49 isolates for *C. difficile* and 20 isolates for *C. perfringens*) by the disk diffusion method on Mueller–Hinton agar + 5% sheep blood (Biorad, Paris, France), according to Clinical and Laboratory Standards Institute (CLSI) guidelines [22]. *C. difficile* ATCC 9689 and *C. perfringens* ATCC 3624 were used as quality control strains. The following agents were chosen: metronidazole (Met), vancomycin (Van), clindamycin (Cl), tetracycline (Te), doxycycline (Do), erythromycin (Ery), gentamicin (Gm), chloramphenicol (Chl), trimethoprim–sulfamethoxazole (Sxt), ampicillin (Amp), ciprofloxacin (Cip), nalidixic acid (Na), norfloxacin (Nor), cefotaxime (Cfx), penicillin (Pen) and lincomycin (Lin). Diameters of the zone of inhibition around the disc were measured to the nearest millimeter using a metal caliper, and according to Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) criteria [23], resistance was defined as follows: Met < 21 mm; Van < 17 mm; Cl < 15 mm; Te < 17 mm; Do < 17 mm; Ery < 17 mm; Gm < 16 mm; Chl < 23 mm; Sxt < 10 mm; Amp < 16 mm; Cip < 22 mm; Na < 15 mm; Nor < 22 mm; Cfx < 15 mm; Pen < 18 mm; Lin < 17 mm.

3. Results

3.1. Prevalence of C. difficile and C. perfringens

C. difficile was isolated in 11.04% of 172 kidney samples and in 13.45% of 223 flesh samples (Table 1) which were collected from street food vendors throughout eight municipalities. *C. difficile* was isolated from all the municipalities except Cocody and the rates of contamination ranged from 4.54% to 28.00% for kidney samples and from 8.69% to 25.92% for flesh samples. The overall prevalence of *C. difficile*-positive cooked beef sold in the streets was the highest at Abobo (22.00%) and the lowest at Marcory (6.66%).

C. perfringens was only isolated from kidney samples collected throughout three of the eight municipalities (Abobo, Adjame and Cocody) and it was isolated from flesh collected throughout seven of the eight municipalities. The prevalence of *C. perfringens* in cooked beef was lower than *C. difficile* one's (5.06% versus 12.40%). The highest prevalence of *C. perfringens* was obtained at Abobo

Table 1
Prevalence and spore counts of *Clostridium difficile* in cooked beef (kidney and flesh) sold in the streets.

Municipalities	Kidney			Flesh			Prevalence (%)
	No. of samples	No. (%) of samples positive for *C. difficile*	Means of spore counts (ufc/g)	No. of samples	No. (%) of samples positive for *C. difficile*	Means of spore counts (ufc/g)	
Abobo	23	04 (17.39)	5.69	27	07 (25.92)	2.25	11 (22.00)
Adjame	25	07 (28.00)	1.61	32	03 (09.37)	0.64	10 (17.54)
Cocody	20	00 (00.00)	<1	23	00 (00.00)	<1	00 (00.00)
Yopougon	20	03 (15.00)	2.45	34	07 (20.58)	0.97	10 (18.51)
Port-Bouet	20	02 (10.00)	2.80	27	03 (11.11)	1.11	05 (10.63)
Koumassi	21	01 (04.76)	1.22	28	04 (14.28)	0.48	05 (10.20)
Marcory	22	01 (04.54)	1.41	23	02 (08.69)	0.56	03 (06.66)
Treichville	21	01 (04.76)	0.33	29	04 (13.29)	0.13	05 (10.00)
Total	172	19 (11.04)	2.22	223	30 (13.45)	0.88	49 (12.40)