



## Prevalence of sulfite reducing *Clostridium* species in barbecued meat in Abidjan, Côte d'Ivoire

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### ABSTRACT

**Objective:** The study aimed to determine the prevalence of sulfite-reducing *Clostridium* species in beef meat cooked and sold in streets and in public restaurants in Abidjan.

**Methodology and results:** A total of 395 samples of beef meat were collected from eight municipalities of Abidjan. Sampling consisted of collecting of three types of ready to eat meat namely, cooked kebabs, cooked steaks and meats cooked in sauce. The isolated 222 (56.2%) strains of sulfite-reducing *Clostridium* species in all the samples analyzed. Four species were identified at different isolation frequencies, 13% (81) for *Clostridium perfringens*, 20.5% (27) for *Clostridium difficile*, 21.2% (91 strains) for *Clostridium sporogenes* and 23% for *Clostridium septicum*. With regard to the efficiency of various types of cooking, the results showed that 31.9% of 395 analyzed samples were not adequately cooked.

**Conclusion and applications of results:** It is concluded from this study that barbecued meat is a risk factor for *Clostridium* infection for the consumers in Abidjan. The cooked kebabs are the most contaminated products and should be avoided particularly by children and immuno-compromised persons. Measures to increase hygiene and awareness and improved handling and cooking of products should be promoted to reduce or eliminate the risks identified.

**Key words:** Sulfite reducing *Clostridium*, barbecued, meat, Abidjan

### INTRODUCTION

Pathogenic *Clostridium* species, *C. perfringens* and *C. difficile* are anaerobic sulfite reducing and endospore forming Gram-positive bacteria which cause histotoxic and gastrointestinal (GI) diseases in humans and animals (Labbe, 2002). *C. perfringens* and *C. difficile* cause an estimated 250,000 cases of food poisoning annually, leading to about 41 hospitalizations and seven deaths each year in the U.S. (Fallani et al., 2006). Many outbreaks associated with meat and poultry products, frequently occurred in North America

(Taormina et al., 2003). Lindstrom et al. (2006) estimated that in England *C. perfringens* was the second most common cause of foodborne illness (behind *Campylobacter*) and the second most frequent cause of death associated with foodborne disease after *Salmonella*.

Symptoms due to *C. perfringens* include gastroenteritis, including acute abdominal pain and diarrhea, usually appearing 8–16 h after ingestion of the vegetative form of the organism and can last 24–48 h (Li et al., 2006, Vijay et al., 2006).

*C. difficile* is a major cause of pseudomembranous colitis and antibiotic-associated diarrhea (Paredes-Sabja et al., 2009).

These organisms are ubiquitously distributed in the environment and can be readily isolated from dust, raw meat and poultry, and the intestinal tract of man and animals (Akhtar et al., 2008). The spores of this organism are more heat-resistant than most foodborne pathogens such as *Salmonella* and *Listeria* and can survive normal cooking or thermal processing conditions in manufacturing products such as ham, roast beef and corned beef (Labbe and Juneja, 2002; McCourt et al., 2005). Red meats and poultry products are frequently contaminated with *C. perfringens* and *C. difficile* (Li et al., 2006). After ingestion of *Clostridium*, once in

the small intestine, the cells sporulate, releasing an enterotoxin that is responsible for the pathological effects in humans as well as the typical symptoms of diarrhea and abdominal pain. The outbreaks usually result from improper handling and preparation of foods at home, retail, or food service operations, and rarely involve commercial meat processors (Taormina et al., 2003).

In Côte d'Ivoire, barbecues are routinely consumed by a large number of people during baptisms, marriages and some other major ceremonies. Unlike developed countries, there is no statistical data related to food-borne *Clostridium* following the consumption of beef. The purpose of this study was to enumerate and identify *Clostridium* strains isolated from the flora of cooked beef.

## MATERIALS AND METHODS

**Meat sampling:** In total, 395 samples of cooked beef were purchased in the streets of Abidjan, Côte d'Ivoire. The samples represented three cooking methods and included cooked kebabs, steaks and meat cooked in sauce.

**Isolation of bacteria:** The meat is crushed in the stomacher (Lab-Blender 400) and a suspension is obtained by adding ten grams of the broyat obtained in 90mL of Buffered Peptone Water which is an enrichment broth. It was left at ambient temperature of the laboratory for 30 minutes then a quantity of about 40 ml was extracted and introduced into two test tubes, each 20 ml. The test tubes were treated at 80°C for

ten minutes. Ten milliliters of the treated solution was transferred into test tubes containing the medium TSC (Bio-Rad, France), and incubated at 37 °C for 24 hours. The characteristic black colonies of sulfite-reducing *Clostridium* counted and some extracted and introduced into the Rosenow broth, and a Gram stain test done. A classic identification was done and then confirmed by the gallery API20A (Biomérieux, France).

**Statistical analysis:** The statistical analysis compared the bacterial loads of meat cooked by various methods. The analytical software ' Statistica ' was used and Duncan's multiple range tests was used to detect the significant differences between the cooking methods.

## RESULTS

Four species of sulfite-reducing *Clostridium* were isolated three species were present in all cooked meat regardless of their method of cooking. These were *C. difficile*, *C. sporogenes* and *C. septicum*. With the exception of *C. perfringens*, other species identified are of no particular interest in food microbiology (Table 1).

The burden of sulfite-reducing *Clostridium* species varied from one cooking method to another. Thus, 76.40% of cooked kebabs were positive for sulfite-reducing *Clostridium* with an average load of 20 cfu / g; 31.49% of cooked meats were positive with an average load of 3 cfu / g and 30.00% of cooked steaks were positive with an average load of 2 cfu / g (Table 2).

**Table 1:** Prevalence of sulfite-reducing *Clostridium* species isolated in beef meat in Abidjan, Cote d'Ivoire.

Cooked modalities	Samples	<i>Clostridium</i> species isolated			
		<i>C. perfringens</i>	<i>C. difficile</i>	<i>C. sporogenes</i>	<i>C. septicum</i>
Cooked kebabs	178	23 (12.92%)	58 (32.58%)	17 (09.55%)	69 (38.76%)
Cooked steaks	90	(<1%)	08 (08.88%)	05 (05.55%)	14 (15.55%)
Meats cooked in sauce	127	(<1%)	15 (11.81%)	5 (03.93%)	8 (06.29%)
Total	395	23 (05.82%)	81 (20.50%)	27 (21.25%)	91 (23.03%)

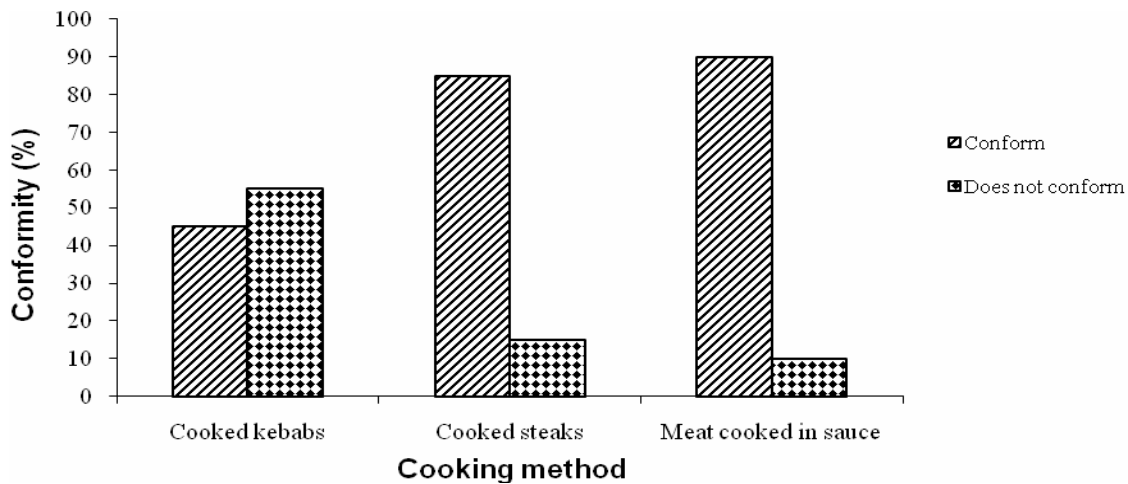
**Table 2:** Loads of sulfite-reducing *Clostridium* cooking modalities of beef.

Modalities	Préparation method	Number	Number positive for SRC	Average CFU/g
Kebabs	precooked	178	136 (76.40%)	21 ± 22
	cooked	178	136 (76.40%)	20 ± 21
Meats	precooked	127	40 (31.49%)	4 ± 7
	cooked	127	40 (31.49%)	3 ± 5
Steaks	precooked	90	27 (30.00%)	3 ± 5
	cooked	90	27 (30.00%)	2 ± 4

SRC: Sulfite-reducing *Clostridium*; CFU: Colony forming unity

More than half of the cooked kebab samples (55%) did not conform to expected quality standards, while

samples of cooked meat in sauce and cooked steaks conformed at 90 and 85%, respectively (Figure 1).



**Figure 1:** Quality of meat sold in Abidjan (Côte d'Ivoire) according to the cooking method.

## DISCUSSION

Four species of sulfite-reducing *Clostridium* were isolated at varying frequencies from the samples of beef meats analyzed. *C. difficile* was isolated from all cooking method but was highest in cooked kebabs (32.58%) and least in cooked steaks (08.88%).

The pathogenic species (*C. perfringens* and *C. difficile*) that are dreaded and suspected to be at the origin of several hospital-borne infections, causing mortal necrotizing diarrhea, pseudomembranous colitis and gaseous gangrenes (Granum, 1990) were isolated at considerable rates of 12.9 and 32.58 % in the cooked kebabs. This method of preparing meat involves chopping and the meat is subjected to intense manipulations before cooking. Furthermore the poor conditions of storage and prolonged preservation

facilitate the reproduction of sulfite-reducing *Clostridium* spores. Despite the presence of a heat source, *C. difficile* was isolated at a high rate of 11.81% and 08.88%, respectively, in the cooked meat in sauce and cooked steaks showing that this bacteria is highly resistant to heat. Ingested in large quantities *C. perfringens* is lethal to its host (Rodriguez-Palacios et al., 2007; Sabji-Paredes et al., 2008). The cooked kebabs are the most contaminated and thus most dangerous human and animal health (Broda et al., 1996).

Cooked kebabs have a low cost and thus are affordable to most consumers. The levels of contamination of kebab samples appears very high (55.62 %) with an average load of 12 cfu / g of food. These results are in

agreement with the works of Miwa and et al. (1996) on the flesh and the excrements of beef. The meat cooked in sauce and steaks cooked with less handling and are cut into larger chunks had significantly reduced growth and proliferation of bacteria (Li et al, 2006, Akhtar et al., 2008). The high unhealthiness of the various places of sale as well as the abundance of dust are some of the causes of the high rate of contamination meat by sulfite-reducing *Clostridium* (Andersson et al., 1995; Rodriguez-Palacios et al., 2007). The stock of unsold meat is also badly preserved and sold the following

## CONCLUSION

Sulfite-reducing *Clostridium* species are mostly pathogenic bacteria constituting a serious public health problem since its spores are resistant to heat and live long in the soil. The intense manipulations without precaution and the poor packaging are major factors in the contamination of the cooked meat. In view of the results of this study, the risk of contracting an infection

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day, which enables CRS to reach the stage of spore formation (Sunagawa et al., 1987).

Once ingested in large quantities spores hatch causing necrotizing diarrhea, pseudomembranous colitis, which is often fatal for humans and animals (Norinaga et al. 1998; Harrym et Vijay, 2002, Lindstrom et al 2006). Cooked steaks and meats cooked in sauce had higher rates of conformity to expected quality standards at 90 and 85.83%, respectively. These products have relatively low rates of contamination with an average load of 3 and 4 cfu / g of food, which reflect. The better handling and cooking applied to these meats.

following consumption of cooked beef is real because of the presence of *Clostridium perfringens* in the samples analyzed. Therefore to reduce the risk, the meat must be cooked enough and protected from dust that can carry spores of *Clostridium*. In addition, meat handlers must be trained in good hygiene practices

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