

Antibiotic Resistance of *Clostridium Perfringens* Isolated from Raw Camel Milk in Isiolo County, Kenya

Benard O Aliwa* and Kaindi Dasel Wambua Mulwa

Department of Food Science, Nutrition, and Technology, University of Nairobi, P. O. Box 29053-00625, Nairobi, Kenya

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*Corresponding author

Benard O Aliwa, Department of Food Science, Nutrition, and Technology, University of Nairobi, P. O. Box 29053-00625, Nairobi, Kenya; Mob: +254720345846; Email: bennkaliwa@gmail.com (or) bochieng@uonbi.ac.ke

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Abstract

Clostridium perfringens is a gram-positive, anaerobic, motile rod spore forming bacteria. It causes food poisoning and spoilages in milk and milk products. The camel husbandry and health management practices in the Arid and Semi-Arid Lands (ASALs) of Kenya increase the possibility of contamination, transmission, and development of antibiotic resistance zoonotic organisms. Therefore, the objective of this study was to estimate the prevalence of antibiotic resistant *Cl. perfringens* strains in camel milk in Isiolo County, Kenya. A total of 308 raw camel milk were aseptically harvested from randomly selected camels from 15 herds along selected camel milk clusters in Isiolo County. 48.05% (148/308) of all the sampled camel milk were positive for *Clostridium* species and out of the 148 samples that were positive for *Clostridium* species, 19.1% (59/148) were positive for *Cl. perfringens*. Out of the 59 isolates that were confirmed as *Cl. perfringens*, the isolates that were resistant to tested antibiotics, in decreasing order, were ampicillin (61.02%), Sulphamethazole (47.46%), Cotrimazole (45.76%), Streptomycin (44.07%), Chloramphenicol (42.37%), Kanamycin (40.68%), Tetracycline (37.29%), and Gentamycin (35.59%). Therefore, these findings established contamination of raw camel milk with *Cl. perfringens* characterized with antibiotics resistance capabilities posing a health risk to the local communities that consume the unpasteurized camel milk.

Introduction

Clostridium perfringens, a gram-positive anaerobic rod spore forming bacteria, spores are ubiquitous in unhygienic farm environment including soil, dust, water, sewage, sediments and vegetation [1-2]. Animals are readily exposed to *Cl. perfringens* in farm and grazing environment; from where they enter the gastrointestinal tract (GIT) and other body tissues and rapidly proliferate and cause zoonotic diseases. The camel husbandry and health management practices in the Arid and Semi-Arid Lands (ASALs) of Kenya increase the possibility of contamination, transmission, and development of antibiotic resistance zoonotic organisms.

The development of antimicrobial resistant microorganisms results from misuse, overuse, under/inadequate use of antimicrobials, animal health practices common in ASALs of Kenya. This is due to inadequate provision of veterinary services leading to self-medication of camels [3-4]. The ASALs' climate and unhygienic environment like erratic rainfall, leading to inadequate grazing pastures and shrubs, inadequate and unhygienic animal watering resources, and dusty grazing, watering and housing environment makes camel husbandry management practices conducive for development of antimicrobial resistance and spread of zoonotic microorganisms. Therefore, the WHO Global Strategy and intervention framework of containment of antimicrobial resistance through slowing down emergence and reducing the spread of antimicrobial resistant microorganisms is difficult to attain in ASALs.

It has been reported that zoonotic pathogens, like spore-former *Cl. perfringens*, are more sensitive to climate change than human- or animal-only pathogens [5], suggesting that zoonotic pathogens are likely to be impacted more seriously by climate change. Some climate change drivers like erratic moisture and rainfall, and high temperatures are frequently found in the ASALs, hence likely to facilitate high mutation rate, horizontal gene transfer, development of antimicrobial resistant and transmission of food, water- and soil-borne resistant pathogens [6].

Previously, camels were considered resistant to most of the diseases commonly affecting livestock, but current research indicates that camels are susceptible to a large number of pathogenic agents [7]. Camel calf diarrhea and high camel calf and herd morbidity and mortality rate has been reported by veterinary researchers and pastoralist in Kenya as being the common disease affecting suckling dromedary calves, with serious effects on herd growth. This can be attributed to late veterinary intervention, a common case in ASALs due to inaccessible of veterinary services to where camels are kept [4]. Camel calf diarrhea is caused by mixed microbial infection, notably *Salmonella* spp., *Escherichia coli* and *Clostridium perfringens* [8]. There is high chance of this

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causative organisms being secreted in camel milk and causing milk-borne infection to human. Under harsh ASALs climatic condition *Cl. perfringens* being a spore-former can undergo mutation leading to development antibiotic resistant strains that can under the unhygienic milk production and harvesting condition contaminate camel milk and subsequently consumers, hence public health concern. However, there is no information and data on prevalence of spore-forming and antibiotic resistant *Cl. perfringens* in camel milk in Kenya. Therefore the objective of this study was to estimate the prevalence of antibiotic resistant *Cl. perfringens* strains in camel milk in Isiolo County, Kenya.

Materials and Methods

Study Site

The study was carried out along the Mlango-Ngarendare-Burat, KambiGarba-Ngaremarā-Chumvi-Gambela, and Boji-Kulamawe-Baranbate camel milk clusters in Isiolo County (Figure 1). Isiolo County is a typical arid and semi-arid lands (ASALs) area located in Northern eastern region of Kenya. The County has camel population of approximately 43,300 camels, kept under peri-urban and pastoral camel production systems. The Isiolo County has a thriving camel milk production and marketing business. It supplies 90% of camel milk to the Nairobi terminal camel milk market.

Sample Collection and Preparation

Raw camel milk was aseptically harvested from 308 camels

randomly selected from 15 camel herds along selected camel milk clusters in Isiolo County, Kenya. Immediately after harvesting, the milk samples were kept in ice box containing ice packs and transported to Isiolo County Veterinary Office laboratory. At the County Veterinary Office laboratory the raw camel milk samples were kept in deep freezer maintained at -20°C until transported to Molecular Laboratory, at the Department of Food Science, Nutrition and Technology, University of Nairobi and stored in deep freezer maintained at -50°C.

Isolation and Identification of *Clostridium perfringens* species

Frozen raw camel milk samples were thawed overnight at room temperature. Isolation of *Clostridium* species was performed by streaking camel milk samples on Reinforced *Clostridium* agar (Blalab, Merck Ltd, South Africa) with added egg yolk emulsion and incubated anaerobically at 37°C for 24-48 hrs. The typical *Cl. perfringens* isolates were identified on the basis of colony morphology, motility, and β -hemolysis reaction [9-11]. Characteristic *Cl. perfringens* isolates were black in colour, surrounded with clear zone, large and flat with wide spreading, irregular, coarse rhizoid margin and raised centre. The clear opaque zone surrounding colonies indicate lecithinase activity. *Cl. perfringens* is non-motile and normally grows at 44°C, whereas some other clostridia are inhibited at this temperature. *Cl. perfringens* is non-motile and normally grows at 44°C, whereas some other clostridia are inhibited at this temperature.

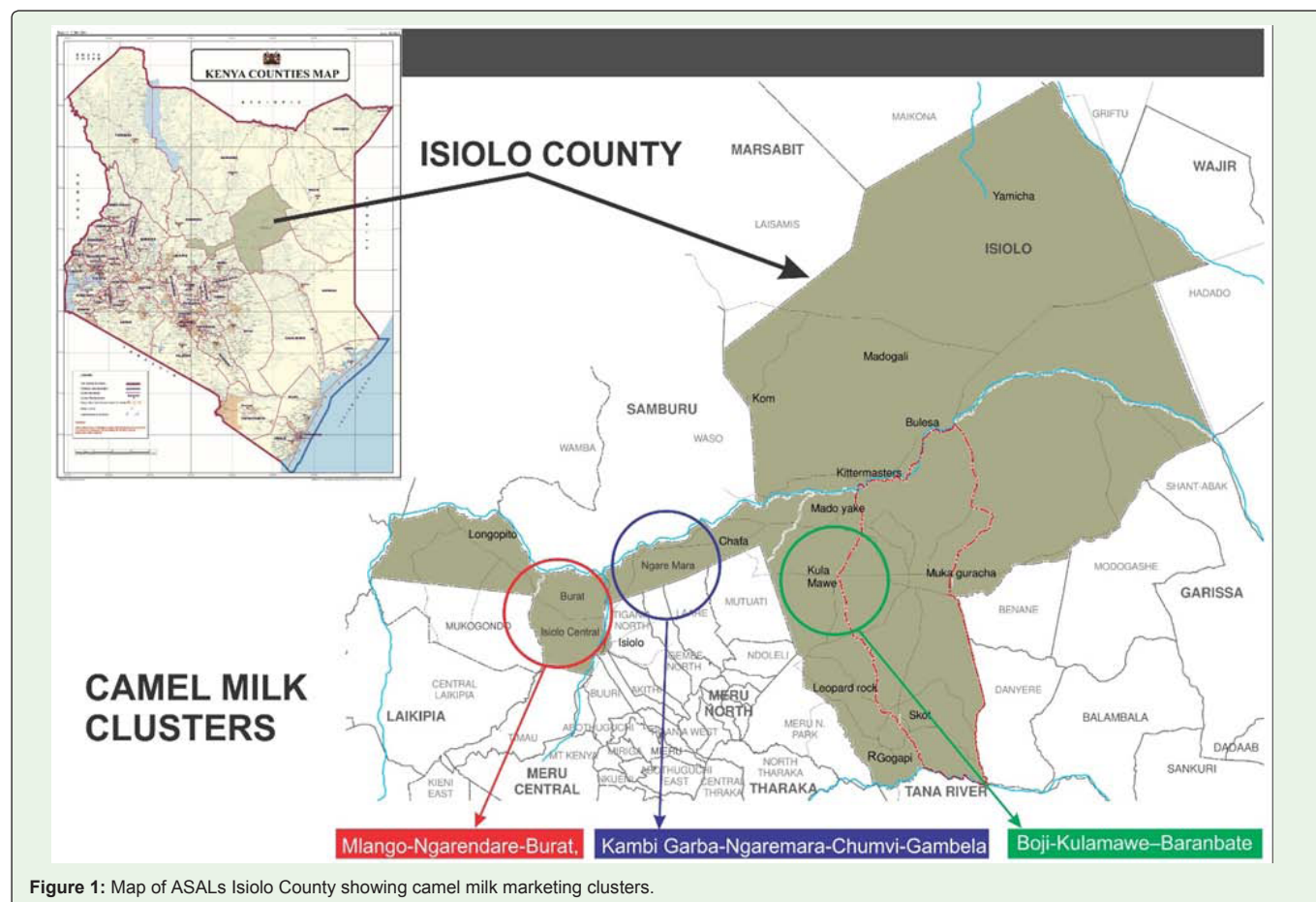


Figure 1: Map of ASALs Isiolo County showing camel milk marketing clusters.

Motility test

Suspected *Clostridium* isolates were inoculated by stabbing into the center of the Sulfide-Indole-Motility (SIM) Medium, a semi-solid that allows for the detection of bacterial motility, and incubated at $35 \pm 2^\circ\text{C}$ for 24 - 48 hours. Motility was demonstrated by growth away from the stab spot and turbidity or cloudiness throughout the medium. Non-motile organisms grow only along the stab spot and leave the surrounding medium clear. *Cl. perfringens* is non-motile.

Blood agar for hemolytic activity

Suspected *Clostridium* isolates were spread on horse blood agar media and incubated anaerobically for 24 hours at 37°C . The Blood agar plates were examined for round, smooth, circular, gray white color colonies surrounded by a typical zone of hemolysis (β -hemolysis) which is characteristic of *Cl. perfringens* produces large β -hemolytic colonies on Blood Agar, but some *Cl. perfringens* strains produce a double zone of hemolysis.

Determination of antibiotic resistance among *Clostridium perfringens* isolates

Antibiotic resistance testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton (MH) agar. The typical *Cl. perfringens* isolates were cultured overnight in Trypticase-soy broth (TSB) supplemented with 0.6% yeast extract. Approximately

0.1ml of broth culture was spread plated onto MH agar, diffusion KGL 2/4 octodiscs containing antibiotics namely: - Ampicillin (25 μg), Tetracycline (25 μg), Cotrimazole (25 μg), Streptomycin (10 μg), Kanamycin (30 μg), Gentamicin (10 μg), Sulphamethazole (200 μg) and Chloramphenicol (30 μg) (Himedia) introduced onto plates and plates incubated at 37°C in 5% CO_2 for 18 hours. Octodiscs are flat circular rings having 8 discs coated with antibiotics that aid in determination of multi-antibiotic resistance organisms (Figure 2). The plates were examined for growth, clear zone surrounding the antibiotic disc and no clearing surrounding the antibiotic disc. No clearing surrounding the antibiotic disc indicated antibiotic resistance.

Results

Out of 308 samples of raw camel milk examined 48.05% (148/308) were positive for *Clostridium* species. Out of 148 samples of raw camel milk that showed presence of *Clostridium* species, 19.1% (59/308) samples showed presence of β -hemolysis on blood agar and non-motility on SIM agar, indicating presence of *Cl. Perfringens*. Out of the 59 isolates that were positive for *Cl. perfringens*, the isolates that were resistant to tested antibiotics, in decreasing order, were ampicillin (61.02%), Sulphamethazole (47.46%), Cotrimazole (45.76%), Streptomycin (44.07%), Chloramphenicol (42.37%), Kanamycin (40.68%), Tetracycline (37.29%), and Gentamycin (35.59%) (Table 1).

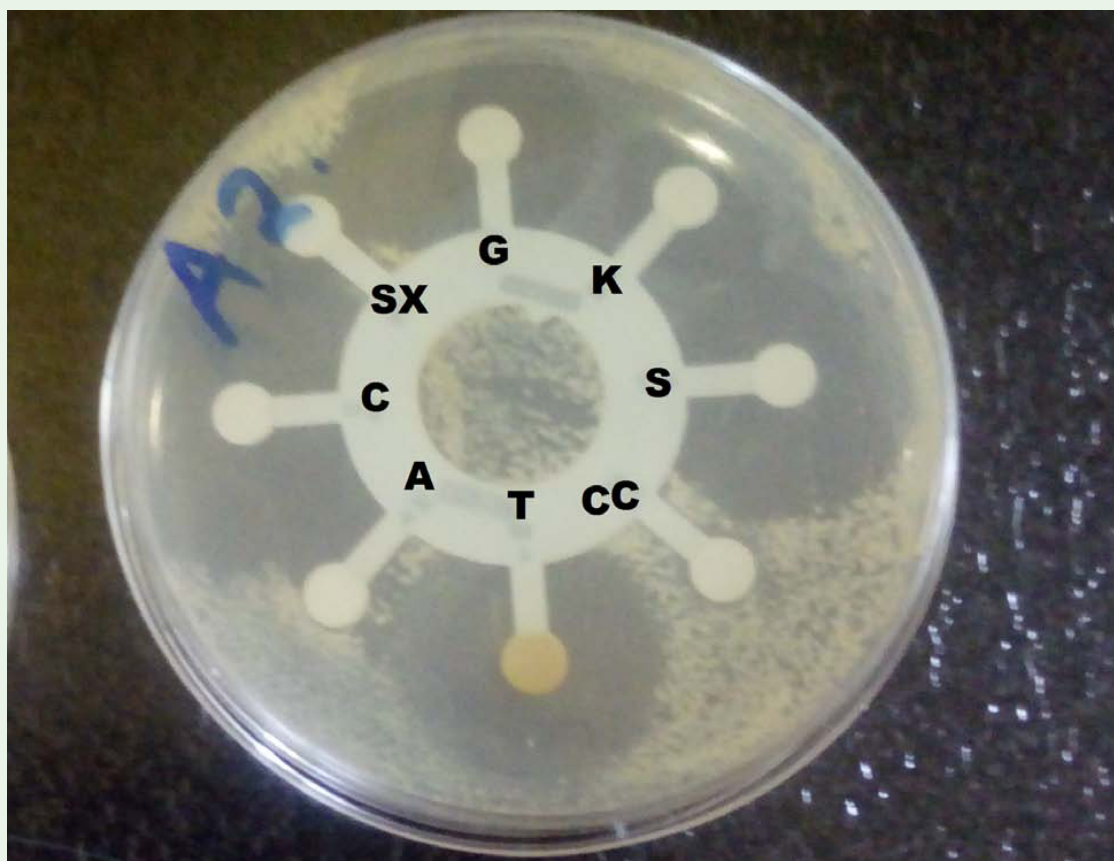


Figure 2: Showing multiple antibiotic resistance of *Clostridium perfringens* isolate to various antibiotics. Ampicillin (A), Tetracycline (T), Cotrimazole (CC), Streptomycin (S), Kanamycin (K), Gentamicin (G), Sulphamethazole (Sx), Chloramphenicol (C).

Table 1: Antibiotic resistance (expressed as %) of *Clostridium perfringens* isolated from camel milk in Isiolo County, Kenya.

	Antibiotic	Number Resistant Isolate (n=59)	Percentage of Resistant Isolates
1	Ampicillin	36	61.02
2	Tetracycline	22	37.29
3	Cotrimazole	27	45.76
4	Streptomycin	26	44.07
5	Kanamycin	24	40.68
6	Gentamicin	21	35.59
7	Sulphamethazole	28	47.46
8	Chloramphenicol	25	42.37

Discussion

This study demonstrates presence of multi-drug resistant of *Cl. perfringens* isolates in camel milk in the study area. Treatment of animal requires antimicrobial agents like antibiotics, however, improper use of this antimicrobial agents can results in development of antimicrobial resistance. The high resistance to antibiotic could be attributed to easy availability of antibiotics over the counter and misuse, overuse, under/inadequate use of antibiotic during treatment of camels by pastoralists' [4]. The antibiotic resistant *Cl. perfringens* can contaminate raw camel milk either through animal faecal contamination, unhygienic harvesting, and handling or from dusty environmental sources [12-13]. From this study it was established that 48.05% (148/308) of the analyzed milk samples were positive for *Clostridium* species. In a study conducted by [9], the findings revealed that 52% of the raw milk obtained from dairy animals was contaminated with *Clostridium* species. The small difference between the two findings can be related to difference in sample size used in the two studies and also type of the samples used. The occurrence of *Clostridium* species in milk products is hazardous posing a serious health risk to milk consumers, especially in the absence of pasteurization or sufficient boiling. Therefore, these findings underscore the need to enhance milk handling hygiene and improved policy on antibiotic use in treatment of camels.

The easy availability of antibiotics over the counter, despite regulations to the contrary, contributes to increased inappropriate usage of antimicrobial drugs in Kenya. Resistance to antimicrobial agents (AMR) result in treatment failures and increased health care costs, hence emergent antibiotic resistance is a serious public health risk and global problem. (49.2%) of the *Cl. Perfringens* isolates in this study showed multi drug resistance to the antibiotics. Majority, 39 out of 59 (61%) of the isolates were resistant to Ampicillin while minority (35%) being resistant to gentamycin. The rates of antibiotic resistance differ significantly depending on geographical locations and the existing national policy on the use of antibiotics (14). For example, various studies have established highest level of *Cl. Perfringens* resistance to clindamycin and cephalosporins (55% and 51%, respectively). Most of the isolates were susceptible to aminoglycosides including gentamycin, kanamycin, and streptomycin (65%, 60%, and 56%, respectively). 37% of the *Cl. Perfringens* isolates were resistant to Tetracycline based on the findings from this study this was in agreement with findings by (15), whereby it was established 20 % to 50% of the *Cl. Perfringens* isolates were resistant against tetracycline. However, the isolates were obtained from different

samples. From this study the findings revealed that 42% of the *Cl. Perfringens* isolate were resistant to chloramphenicol this is contrary to the findings obtained by [16], whereby 46% of the *Cl. Perfringens* were resistant to chloramphenicol. This difference in the findings can be attributed to the fact that the two researches were conducted in different geographical setting one in Kenya and the other in Egypt. According to [16], the rates of antibiotic resistance differ significantly depending on geographical locations and the existing national policy on the use of antibiotics.

Conclusion

An extensive resistance to multiple antibiotics was detected in *C. perfringens* isolates from camel milk in this study. Therefore, episodic monitoring of trends in Antibiotic resistance patterns is logical, since there is possibility of *C. perfringens* being a source of resistance genes transferring to other species of bacteria that are found in the camel milk. There is significant potential for therapeutic challenges in the future unless care is taken to avoid the selection of multi-resistant organisms.

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