Sudan Journal of Science and Technology
Journal homepage:
http://jst.sustech.edu/

Determination of the Campylobacter Contamination of Cattle Meat in Khartoum State

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ARTICLE INFO

ARTICLE HISTORY
Received: 3/7/2018
Accepted10/9/2018
Available online: December 2018

Keywords:
Campylobacteriosis, *Cattle* meat, Sensitivity test, Khartoum

ABSTRACT

This study was conducted to determine the Campylobacter contamination of cattle meat in Khartoum State, from April 2016 to August 2017. A total of 40 swab samples were collected from Al-Kadaro Slaughterhouse in Khartoum North (Bahry) and Karrari Slaughterhouse in Omdurman for total viable counts (TVCs), and to determine the susceptibility of the *campylobacter* to different drugs. Those samples categorized as follow: 20 swab samples From 6 cattle carcasses were randomly selected from each slaughterhouse (Swab from the knife and another swab from worker hands), 6 swabs from the carcasses after skinning, 6 after evisceration and other 6 swabs sample from the carcasses after two hours of chilling using sterile swabs. TVCs were 2.48 ± 0.037 log10 cfu/ml after evisceration and 2.32 ± .028 log10 cfu/ml after 2 hours of chilling. While the highest level of TVC occurred after skinning which is 4.44 ± 0.83 log10 cfu/ml, both hands workers and knives were *Campylobacter* free. The percentage of *Campylobacter* contamination was 55% of total samples. Sensitivity test against certain antibiotics ranged from 4.6 to 59.1% for the total samples, the highest percentage of sensitivity occurred in clostin 10 µg, while the percentage of resistance ranged from 40.9 to 95.4% in azithromycin and kanamicin 30 µg.

INTRODUCTION:

The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughter houses and retail establishments (Gill, 1998; Abdalla *et al.,* 2009). Most microbial contaminants of carcasses represent commensal bacteria, some microorganisms such as *Salmonella spp*., *Campylobacter spp., Escherichia coli* O157:H7, and *Listeria monocytogenes* pose a threat to consumer health (Gustavsson and Borch, 1993; Samelis *et al.*, 2001).

There were significant increases in total bacterial count at skinning points than that at washing operations; also, dirty workers hands, clothes and equipments of the slaughterhouse acted as intermediate sources of contamination of meat (Gill, 1998; Gilmour *et al.,* 2004; AbdelSadig, 2006; Abdalla et *al.,* 2009).

Campylobacter jejuni and Campylobacter coli are the most frequent causes of acute bacterial gastroenteritis in humans, representing an unrelenting worldwide public health problem. *C. jejuni* accounts for over 90% of cases, with the majority of the remainder caused by *C. coli* (Cody *et al., 2012).* Campylobacteriosis manifested by diarrhea that is often bloody, abdominal cramping, fever and vomiting (Allos, 2001). Although most *Campylobacter* associated diarrhea is self-limited, complications can occur. One complication is Guillain-Barre´ Syndrome (GBS), an acute, symmetric, ascending paralysis that is estimated to occur 30 times for every 100,000 *Campylobacter* cases (Wierzba *et al.,* 2008) and the case fatality ratio approaches 10% (Nachamkin *et al., 1998)*. Many cases of campylobacteriosis are self-limiting and require only supportive therapy. Antibiotics may be useful for some cases of enteritis, especially those that are severe. Macrolides and fluoroquinolones are commonly prescribed for Campylobacteriosis; however, resistance to these and other antibiotics also occurs (The centre for food security and public health, 2013). Of the reviewed articles only one study on *Campylobacter* colonization in ducks by Nonga and Muhairwa (2009) addressed the issue of antimicrobial susceptibility of the *Campylobacter* isolates. Resistance profiles of the isolates to different antimicrobials were determined by the disk diffusion method (Bauer *et al.,* 1966) on Mueller-Hinton agar. The isolates tested against the following antimicrobials: streptomycin 10µg, amoxicillin 10µg, ampicillin 10µg, ciprofloxacin 5µg, cefuroxime sodium 30µg, gentamicin 10µg, cloxacillin 5µg, tetracycline 30µg, nitrofurantoin 30µg, amikacin 30µg, erythromycin 15µg and norfloxacin 10µg. The bacterial growth inhibition zone was measured to assess resistance of the isolates using guidelines stipulated by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The objectives of this study were to identify the *Campylobacter spp*., and to determine the susceptibility of *Campylobacter spp* to different drugs (sensitivity test), and also to determine the main points of contamination of carcasses during slaughtering operations.

MATERIALS AND METHODS:

Sampling: Four swab samples were collected from knives and workers hands, also another 36 swab samples were collected randomly from different sites of carcasses at the moment of skinning, evisceration and refrigeration by using sterile swabs. The study was conducted to determine *Campylobacter* contamination of cattle carcasses in Alkadero and Karri Slaughterhouses between April 2016 to August 2017. Twenty swab samples From 6 cattle carcasses were randomly selected from each slaughterhouse (Swab from the knife and another swab from worker hands), 6 swabs from the carcasses after skinning, 6 after evisceration and another 6 swabs sampled from the carcasses after two hours of chilling using sterile swabs. A total of 40 swab samples were collected for total viable counts (TVCs). To detect contamination and to determine the susceptibility of the *campylobacter* to different drugs from

cattle carcasses were randomly selected and sampled from different sites. The samples were stored in a cooling box and transported to the laboratory in Veterinary Research Centre (Soba), where the microbiological analysis was performed 2 days after collection to allow the bacterial growth anaerobically.

Isolation and Identification of Campylobacter: Whole of the 40 swab samples were inoculated on the modified charcoal-cefoperazone - deoxycholate agar (CCDA) using streaking method in sterile petri dish and incubated at 42°C for 48 hours under microaerobic condition (5% O2, 10% CO2 and 85% N2). Suspected colonies of *Campylobacter* were identified under phase contrast microscope for detection of characteristic motility using the hanging drop technique and morphological character according to Smibert (1984) *Campylobacter* isolates were identified using biochemical tests (Gossens *et al*., 1990; Frost *et al*., 1998).

Antimicrobial agents: The antibiotics tested in this study were: gentamycin 10 µg, cephalexin 30 µg, cefepime 30 µg, streptomycin 10 µg, clostin 10 µg, kanamycin 10 µg, kanamycin 30 µg, neomycin 30 µg, azithromycin 10 µg, azithromycin 30 µg and amoxicillin 10 µg.

Antibiotic susceptibility test: Disc Diffusion Method according to National Committee for Clinical Laboratories Standard (NCCLS, 2002). The top of single and well isolated colony was touched with sterile lope, then transferred into 2ml normal saline. The turbidity of actively growing broth culture adjusted to 0.5 McFarland Standard. Sterile cotton swab was dipped into the adjusted suspension and firmly pressed against the inside of the tube above the fluid level, soaked swab spread evenly over the entire surface of the plate of Nutrient Agar Medium. Plates were then allowed to dry for 5 minutes. Antibiotic impregnated disc applied to the surface of the inoculated plates with sterile forceps. Six-Five disc were placed in petri dish, Then plates were incubated at 42°C after 24 hours of incubation plates were examined and the diameter of the zone of complete inhibition to the nearest whole mm were measured.

Statistical analysis: The data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 16.0, SSPS Inc. Chicago, IL, USA). All bacterial counts were analyzed using One Way ANOVA and the statistical significance was set at *P-value* of ≤ 0.05.

RESULTS:

In this study samples were obtained from the cattle carcasses at different critical points between knives, workers hands, skinning process, evisceration and refrigeration using sterile swabs in Khartoum state. Samples were tested for C*ampylobacter spp* using traditional phenotypic characterization and biochemical tests. The prevalence of positive *Campylobacter spp* was 55% among the total samples investigated. The study revealed a statistically significant difference at *P-value* ($p \leq 0.05$) of the contamination by *Campylobacter spp* at the different critical points specially after skinning process and there is no significant difference after evisceration and after two hours of chilling (Table 1). TVCs was 2.48 ± .037 log10 cfu/ml after evisceration and 2.32 ± .028 log10 cfu/ml after two hours of chilling. While the highest level of TVC occurred after skinning which is 4.44±.83 log10 cfu/ml, both hands

workers and knives were *Campylobacter* free (Table 1).

Table 1: Comparison of mean total viable count of Campylobacter *spp.* $\log_{10}$ CFU/ml ± SD in cattle meat in Khartoum State

Items	Post Skinning	Post Evisceration	Post Chilling
Workers Hands	Negative	Negative	Negative
Knives	Negative	Negative	Negative
Carcasses	4.44 ± .83	2.48 ± .037	2.32 ± .028

Significant difference at *P-value* ($p \leq 0.05$)

Sensitivity test against different antibiotics ranged from 4.6 to 59.1% for the total samples, the highest percentage of sensitivity occurred in clostin 10 µg while the percentage of resistance ranged from 40.9 to 95.4% in azithromycin and kanamicin 30 µg (Table 2).

Table 2: Percentages of Antibiotic Susceptibility Test for *Campylobacter spp.* in cattle meat in Khartoum State

Antibiotics	% of Sensitivity to different antibiotics	% of Resistance to different antibiotics
Amino glycosides		
Gentamicin (CN10 µg)	31.8	68.2
Streptomycin (S10 µg)	27.3	72.7
Neomycin (N30 µg)	18.2	81.8
Kanamycin (K10 µg)	40.9	59.1
Kanamycin (K30 µg)	4.6	95.4
Cephalosporins		
1st Generation		
Cephalexin (CN30 µg)	22.8	77.2
4th Generation		
Cefepime (CPm30 µg)	27.3	72.7
Non-ribosomal peptide		
Colistin (CL10 µg)	59.1	40.9
Macrolides		
Azithromycin (Az10 µg)	9.1	90.9
Azithromycin (Az30 µg)	4.6	95.4
Penicillin		
Amoxicillin 10 µg	50	50

DISCUSSION:

Campylobacter species are a major cause of bacterial gastroenteritis worldwide. In addition to *C. jejuni* and *C. coli*, responsible for 90% and 10% of all cases of human enteric infection, respectively, other *Campylobacters* (*C. upsaliensis*) have also been implicated as gastrointestinal pathogens (Cody *et al., 2012;* Lawson *et al.,* 1999 and Lastovica, 2006). The level of the TVC is set and agreed to be a criterion for assessing the microbial contamination of carcasses and a useful mean to know the hygienic status of meat (Hänninen *et al.,* 2003). This study revealed a statistically significant difference ($P \leq 0.05$) between those critical points mentioned above specially after skinning process and there isn't significant difference both after evisceration and two hours of chilling (Table 1). This finding is similar to what has been found by Gill, (1998) who reported bacterial contamination of meat during the different slaughtering operations. The highest level of TVC was found after skinning process 4.44 ±.83 log10 cfu/ml. This could probably be due to the exposure of meats to the hides and hoofs which is containing multiple types of pathogens, and this result agreed with Gill (1998) who mentioned that; there were significant increases in total bacterial count at skinning points than

that at washing operations. Also it may be due to the cross contamination occurred between infected and non infected carcasses and absolutely the main cause is attributed to the bad hygiene and condition for skinning process.

The lowest rates of contamination occurred in critical control points were found to be in the abattoir refrigerators after chilling of the carcasses and this result agreed with Maike *et al.* (2010) who mentioned that chilling and freezing suppress the *campylobacter* growth.Many cases of Campylobacteriosis are self-limiting and require only supportive therapy. Antibiotics may be useful for some cases of enteritis, especially those that are severe. Macrolides and fluoroquinolones are commonly prescribed for Campylobacteriosis; however, resistance to these and other antibiotics also occurs (The centre for food security and public health, 2013). In this study the susceptibility of *Campylobacter spp* to antimicrobial was found to colistin CL10 µg with 59.1% (Table 2), and this result agreed with Suzete *et al.* (2008) who mentioned that most of the strains of *Campylobacter* were sensitive to colistin and whole of them susceptible to gentamicin. While the resistance occurred in kanamycin K30 µg and azithromycin Az30 µg with 95.4% of samples (Table 2).

According to Schutz (1991) the occurrence of hygienic faults and of a high level of microbiological contamination of carcasses in slaughter houses are not due to an absence of hygiene equipment or to failure to use what equipment there is, but rather to faulty slaughter techniques. The spread of pathogen can also be reduced by developing slaughter technique (Christensen and Luthje, 1994). Such as enclosing the rectum has reduced the pathogenic contamination (Andersen *et al.,* 1991). According to Gerats (1990) there is an association between slaughter technique and the hygienic practice of workers. Those workers who commit many slaughter mistakes neglect hygienic practices. Involving good sanitary measures during slaughtering processes will lead to the reduction of the amount and/or removal of the microorganisms and other hazards.

HACCP should be applied properly during slaughtering operations by using sufficient clean water and safe disinfectants. To make all these, extensive education and training programs for workers should immediately be started.

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