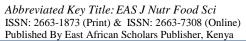
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Original Research Article

Risk assessment of *E.coli* contamination in broiler food chain in Khartoum North, Sudan

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Abstract: Poultry meat, as animal-source food, is considered one of the important causes of food-borne illness due to the high possibility of bacterial contamination. The objective of this study was to assess the overall risk of E.coli contamination in broiler food chain in Khartoum North - Sudan. The overall risk of contamination with E.coli was estimated after a qualitative assessment of risk being estimated in poultry farms, slaughterhouse, retailers and consumers respectively. Thirty checklists were taken from poultry farms followed by 30 swab samples collected from one slaughterhouse. The swabs were processed for microbiology and the number of colony forming units (CFU) was then calculated. Additional thirty checklists were collected from retailers, and 50 questionnaires were collected from consumers. High risk at the primary production was detected as a result of high frequencies and impacts of the examined risk factors. The risk of the contamination with E.coli in the slaughterhouse was medium, denoting that the risky event is likely to occur more than once in the next three years. The retailer's risk was high suggesting a great potential of the risky event taking place either during the year in which the study was carried out, or in frequent intervals. The risk added by consumer was high, meaning that the risky event most likely would appear either during the year in which the study was conducted, or in frequent intervals. It is concluded that the overall risk assessment of E. coli contamination in broiler food chain in Khartoum North was high.

Keywords: broiler, E. coli, meat, risk assessment, Sudan.

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1. INTRODUCTION

Food-Food-borne diseases (FBD), which is caused by agents entering the body through the intake of contaminated food materials, are one of the primary public health concerns (Babiker, M.et al.,, 2013). The FBD affects the people's well-being as well as imposing economic impacts (Akbar and Anal, 2013). In many developing countries, FBD outbreak caused by bacteria result in a substantial burden on health care systems, and remarkable reduction in economic productivity as well(Tan., et al. 2013; Minami et al., the main food-borne pathogens, Escherichia Coli is the most common and frequent pathogen responsible for food poisoning and food related infections. E.coli are responsible for 25% of the infant diarrhea in developing countries (WHO, 2000) Enteropathogenic, enteroinvasive and enterotoxigenic types of E. coli can be a leading cause of food-borne diarrhea has (Akbar and Anal, 2011). It become evident that poultry meat can transfer the pathogens, in particular E. coli (Manges, A. 2016). Furthermore, unhygienic practices along with the use of contaminated materials in food processing have been significantly associated with FBD caused by E. coli (Akbar and Anal, 2013). A study conducted in different poultry slaughter houses in Khartoum State, Sudan.(Mohamed-Noor, S *et al.*, 2012) revealed that *E. coli was* encountered in about 15% to18% in poultry meat (Bodering, A *et al.*, 2018).

Risk assessment is known as a scientific evaluation of known or potential adverse health effects resulting from human exposure to food-borne hazard (WHO, 2015). According to the Codex Alimentarius Commission, risk analysis is defined as a set of processes consisting of three components: risk assessment; risk management and risk communication (FAO/WHO 2010).Qualitative risk assessments are commonly used for screening risks to determine whether they merit further investigation. Such kind of risk assessment also can be useful in the 'preliminary risk management activities (FAO/WHO, 2002).In general, the risk assessment is a scientifically based process which consists of the following steps: (1) hazard identification, (2) hazard characterization, (3) exposure assessment and (4) risk characterization.

Hazard is something potentially harmful to animals, human, plant and environment (FAO, 2011). It

is important step to identify what that might go wrong in whatever activity is being considered (MacDiarmid and Pharo, 2003) Exposure assessment is made by estimation of frequency and concentration of *E.coli* throughout the production line from the farm to consumer. The sequence of steps which lead to unwanted outcome is called risk pathway, the analysis of which is the main tool used in risk assessment (FAO, 2011). Therefore, risk pathway scenario tree is appropriate and effective way in depicting biological pathways (MacDiarmid and Pharo, 2003).

In the present study the hazard was set as *E. coli*, and the therefore the objective was to assess the qualitative risk of *E.coli* contamination in broiler food chain in Khartoum North, Sudan.

2. MATERIALS AND METHODS

2.1 Study area and samples collection

Cross sectional study was carried out in October 2019 in Khartoum North - Sudan. Thirty checklists were taken from poultry farms followed by 30 swab samples collected from one slaughterhouse during two processing steps, defeathering and prepackaging (fifteen samples from each). Birds were

brought to the slaughter house from different farms in Khartoum North. The swab samples were then transported to the laboratory in thermal boxes.

2.2 E coli Identification

Serial dilution(1:10) of All samples were processed by buffered peptone water. One ml of the appropriate dilution(10^{-1} - 10^{-4}) was rapidly poured into Eosin Methylene Blue (EMB) agar. The samples were then incubated at 37° C for 24 hours. The number of colonies was counted in each plate and the mean count was determined. Each colony represented a bacterium or colony forming unit (CFU).

2.3 Questionnaires and checklists design

Thirty and Fifty questionnaires were collected from retailers and consumers respectively.

2.4 Risk assessment

The risk assessment was based mainly on hazard identification and exposure assessment. This is due to the lack of data on human's effective bacterial limit. The qualitative assessment of the risk is shown in table 1.

Table 1: Qualitative risk assessment of *E. coli* contamination in broiler food chain

		Description
VL	Very low	Rare (the risky event may occur in exceptional
		circumstances).
L	Low	Possible (the risky event may occur in the next three years).
M	Medium	Likely (the risky event is likely to occur more than once in
		the next three years).
Н	High	Almost certain (the risky event is likely to occur this year or
	High	in frequent intervals).

2.5 Hazard identification

The characteristic of the bacterial contamination E.coli - set as the hazard- was detected by bacteriological examination.

2.6 Exposure assessment

The exposure assessment estimated by frequency and concentration of *E. coli* throughout the production line from farm to consumer. The data obtained from the questionnaires, check lists, and swab samples were applied to estimate the risk of *E.coli* contamination in broiler food chain.

2.7 Overall risk assessment

The risk assessment of the risk contamination with *E. Coli* was based on the probabilities throughout the pathway. The overall risk was assessed by using risk estimation scheme given by Department for Environment Food and Rural Affairs(DEFRA) (OIE.2014). This was applied by multiplication of qualitative evaluations of risk throughout the risk

pathway (Fig.1). The risk assessment in each level was calculated by multiplication the frequency of the risk factors with their impacts as described by DEFRA (Fig. 2). The impact was defined as the degree of the effect of the risk factors on E. coli count. With respect to the level of slaughterhouse, the risk was estimated by E. coli count multiplied by the frequency of bacteria in both defeathering and prepackaging stages. Based on colony forming unit (CFU), the bacterial count was estimated as follows: low (zero to $> 5x10^4$ CFU), medium $(5x10^4CFU \text{ to } \ge 10x10^4CFU)$, and high (greater than $10x10^4$ CFU). The risk estimation at the slaughterhouse was based on the frequency of positive swab samples as follow: low (10%-40%) medium (41%-50%) and high (more than 50%). In the level of retailers and consumers, the risk estimation was established according to the frequency of risk factors.

Primary production

- ·Cleaning, Building, and Personal hygiene
- •Probability of chickens contamination at primary production



Slaughterhouse

- E. coli count in defeathering and prepackaging stages
- Probability of the contamination of chicken from slaughterhouse

Retailers

- · Refrigeration and storage
- · Probability of chicken's meat contamination at retailers level

Consumers

- · Source of chickens, cooking methods.
- Food safety awareness, washing of hands and dishes Probability of the consumption of contaminated chicken's meat

Overall risk assessment

Multiplication of (primary production x slaughterhouse x retailer x consumer)

Fig. 1: The risk pathway scenario of the contamination of E. coli in broiler food chain

	Н	M	M	Н	H*
	M	L	M	M	Н
	L	VL	L	M	M
Likelihood	VL	VL	VL	L	M
		VL	L	M	H
		Impact		\longrightarrow	

Fig.2: Risk estimation scheme as described by the Department for Environment Food and Rural Affairs (DEFRA).

Data Analysis

Statistical analysis was applied using Statistical Package for Social Sciences (SPSS, version 20.0). Univariate analysis using 2-tailed chi-square test was conducted to test the association between the investigated risk factors and the occurrence of E.coli. P-value ≤ 0.05 was considered statistically significant.

RESULTS

Risk estimation in primary production (farms) level

E. coli contamination was high in 16 farms (53.3%) out of the 30 farms investigated, whereas 7 farms(23.3%) showed medium contamination. The remaining seven farms revealed low contamination (Table 2).

Table 2. The level of *E. coli* contamination level in primary production

E. coli contamination level	Frequency	Percentage %
High	16	53.3 %
Medium	7	23.3%
Low	7	23.3%
Total	30	100.0%

As shown in table 3, more than 50% (16) of the farms did not clean poultry houses. This finding has demonstrated significant effect on the $E.\ coli$ count (p value 0.004) along with high estimation of risk. Nine farms (30%) had improper walls (cracks) and this was significantly associated with $E.\ coli$ count(p < 0.05) and estimated as medium risk. Sixteen (53.3%) farms were

careless about personal hygiene which significantly (p <0.01) reflected on $E.\ coli$ count with high risk estimation. High risk was also found in 50% of the farms that did not apply prerequisite program. The overall risk estimation of $E.\ coli$ in primary production was high.

Table 3: Risk factors associated with E. coli contamination level in primary production

Risk factor	Frequency % (likelihood)	P value (impact)	Risk Estimation
Cleaning not applied	16 (53.3%)	.004	High
Building not safe	9(30%)	0.03	Medium
Personal hygiene not applied	16(53.3%)	< 0.01	High
PRP not applied	15(50%)	0.01	High
Overall risk			High

PRP: Prerequisite program

Risk estimation in slaughterhouse level

Out of 30 samples examined ten (33.3%) were E. coli positive. Seven (46%) were positive in defeathering stage with mean bacterial count $17x10^4$ CFU, which was considered as high risk. Three (20%) samples were positive in prepackaging stage with mean count 5 x 10^4 CFU; the risk at this step was evaluated as low. The overall risk was medium.

Table4: Contamination of broiler meat with E. coli and the associated risk estimation in the slaughterhouse in Khartoum North, Sudan.

Source	Frequency %	Mean count CFU	Risk estimation
Defeathering	7(46.7%)	$17x10^4$	High
Prepackaging	3(20%)	5×10^4	Low
Overall risk	10 (33.3%)	-	Medium

Risk estimation in retailer level

More than eighty percent 26 of retailers did not monitor the refrigerator temperature, the risk estimated as high. Fourteen 46.6% retailers store the chicken with other food product, which regarded as medium risk, and 70% (21) disconnect the electric power at night this estimated as high risk. The retailer's risk was considered as high.

Table 5: Risk factors associated with E. coli infection in the retailer level of broiler food chain.

Risk factor	Frequency	Percentage	Risk estimation
Lack of temperature monitoring	26	86.6%	High
Mixed storage	14	46.6%	Medium
Disconnection of electric power at night	21	70%	High

Risk estimation in consumer level

Eighty percent of consumers purchased poultry meat from retailers which was considered as high risk. Ten consume the chickens without chilling which estimated as low risk.60% of consumer store the chicken with other food which is considered as high risk. 62% did not wash their hands before processing the chicken this will add high risk to *E.coli*

contamination. Low number of consumers (10%) did not wash dishes, the risk estimated as low. 56% of were careless about food safety awareness, with high risk evaluated. Most of the consumers (64%) prefer consumption of fried chickens which is more risky than other cooking method.

Table 6: Risk factors related to consumers and the associated risk estimation

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Risk Factors	Percentage	Risk Estimation	
Source(retailers)	80%	High	
Storage(not chilled)	10%	Low	
Mixed storing	60%	High	
Hand washing (not practiced)	62%	High	
Dishes washing(not practiced)	10%	Low	
Food Safety awareness	56%	High	
Improper cooking	64%	High	

Overall risk assessment

Table 7 shows the overall risk estimation. The overall risk assessment of *E. coli* in broiler food chain in Khartoum North is high. This means that the risky

event most likely appears either in the year during which the study was carried out, or in frequent intervals.

Table 7: Overall risk assessment of E. coli contamination in broiler food chain in Khartoum North, Sudan

Broiler Food chain	Risk estimation
Farms	High
Slaughterhouse	Medium
Retailers	High
Consumers	High

DISCUSSION

The present study revealed high frequency of risk factors related to contamination of broilers with *E. coli* in poultry farms as compared to the level of contamination reported by (Bodering *et al* 2018). The latter stated that about 30% of poultry farms were found positive to *E. coli* contamination. The increase in the frequency of risk factors in this study is most likely due to poor hygienic practices.

In the slaughterhouse, unlike poultry farms, the risk of contamination in the present findings was reduced to medium. *E. coli* was detected in 33.3% swab samples taken from chickens after defeathering and prepackaging stage. This was lower to what has been reported by(Mohammed –Noor *et al.*, 2012), that may be due to high frequency of risk factors at farm level, and higher as compared to earlier findings In addition, high contamination was detected at defeathering machine. Similar to the present result, contamination of *E. coli* at slaughterhouse was also concentrated in the defeathering machine. This was suggested to occur because of the firm attachment of the bacteria to the poultry skin and rubber fingers(Mohammed –Noor *et al.*, 2012).

Earlier study reported by (Hinton *et al.*,2000) indicated that broiler carcass can be contaminated by bacteria when contact with visceral content, in particular from alimentary tract, takes place during grow-out, transport to the processing plant holding or evisceration. The present results was in line with observation of (Iroha, *et al.*,, 2011) who reported that the method of slaughtering of animals is responsible for microbial contamination. They added that traditional method of butchering using knives and cutting line appears more capable of minimizing fecal contamination than modern machine systems. Overall, the reduction of risk contamination in slaughterhouse, compared to farm level, in this study may be due to application of effective control measures.

Concerning the retailer level in the current study, high risk was detected. This suggests that the risky event is likely to occur in the year of research or in frequent intervals. Likewise, the risk added by consumer in the present findings was also high. It is recommended that *E.coli* must be totally absent from poultry meat before it can be fit for human consumption (Adesiji *et al.*, 2011). It is plausible that these high levels of risk in this study as a result of the lack of food safety awareness.

The current study revealed overall high risk *E. coli* contamination in broiler meat. Such contamination might lead to cause infections related to such bacteria among consumers. Despite the fact that high risk estimation of *E coli* contamination in this study, it is difficult to assess the risk of getting a disease. This is mainly due to the lack of information on the dose response under Sudan conditions. It well known that the probability of disease occurrence depends factors other than bacterial load such as the immunity of the consumers and the method by which poultry meat is cooked, Further studies are required to assess the exposure dose of E. coli contamination in broiler meat.

CONCLUSION

It is concluded that the overall risk assessment of *E.coli* contamination in broiler food chain in Khartoum North was high. New strategies and proper food safety management system are needed in order to prevent/reduce the contamination of broiler meat with bacteria.

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