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Isolation Frequency of *Escherichia coli* in Slaughter Houses and Slabs in Khartoum State, Sudan

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A B S T R A C T

A cross sectional study was conducted from 2019 to 2022 at nine slaughter houses and slabs from three different localities in Khartoum state, Sudan, to isolate *Escherichia coli* from different parts of beef carcasses. A total of 432 dry swab samples from the neck, shoulders, rump, and flanks during pre and post wash, 12 dry swab samples (6 samples prewash and 6 samples post wash) were collected from each slaughter houses and slabs. All *Escherichia coli* isolated were identified using biochemical reactions and sugar fermentation tests. The mean frequency of *Escherichia coli* isolated during prewash were: (3.44±2.24, 3.44±1.74, 2.78±1.99 and 3.22±1.48) and (1.89±1.45, 2.44±1.81, 2.00±1.41, 2.44±1.94) during post wash of neck, shoulders, rump and flanks respectively, from all nine slaughterhouses and slabs in Khartoum state, (n=108). Using univariate ANOVA there was significant differences in the frequency of isolates between the four sites of sample collection with $p \leq 0.05$. The mean frequency of *Escherichia coli* isolated in Khartoum in pre wash was3.50 and 2.08 in post wash, in Bahri the frequency was 2.41 and1.33 in pre wash and post wash respectively and in Omdurman the mean in prewash was 3.75 and in post wash was 3.167. There were no significant differences in the frequency of *Escherichia coli* isolation between the three areas of the state, but when the post-hock multiple comparisons was applied there was a statistically significant difference between Bahri and Omdurman slaughterhouses with ($P<0.05$). In conclusion, the frequency of isolation of *Escherichia coli* is significantly lower in modern slaughter house that follow the good hygienic practices.

Introduction

Food borne diseases are a great threat to public health and can be regarded as the main concern and challenge in food industry (Devleesschauwer *et al.*, 2018). *Escherichia coli* are Gram-negative,

facultative anaerobic rods that belong to the family Enterobacteriaceae, these organisms were identified and confirmed by their colony morphology and biochemical characteristics (Singh and Prakash, 2008).

Serological differentiation is based on three major surface antigens: somatic, flagella and capsule. The capsule antigen descriptor has been dropped often and only the flagella and somatic are commonly employed as descriptors of serotypes (Singh and Prakash, 2008).

Escherichia coli is common in both humans and animals as part of the regular flora and is generally harmless to humans; However, the development of virulence factors causes some strains of *Escherichia coli* to become pathogenic, and as a result, they rank among one of the most prevalent foodborne pathogens associated with food safety issues (Ma *et al.*, 2021). *Escherichia coli* infection is highly prevalent in abattoir environment, and can pose a major threat to human health in underdeveloped communities (Abu Elnaga *et al.*, 2014).

Escherichia coli O157 is recognized as particularly as virulent food borne bacterial pathogen which only requires a small number of bacterial cells to cause disease in the host (Pakbin *et al.*, 2021). The World Health Organization declared that more than 1.5 million people died from the acute diarrheal diseases caused by different food borne pathogens around the world (Pires *et al.*, 2021).

The presence of various bacteria on meat is an indication of low standard levels of handling of meat from pre-slaughter to post-slaughter, abattoir facilities and sales of meat (Obeng *et al.*, 2013). Unhygienic practices both at the slaughterhouses and retail shops that can predispose the public to meat borne infections, could be improved through training and implementation of quality control systems (Gutema *et al.*, 2021). Meat hygiene and safety is usually less controlled in many developing countries where meat for human consumption is approved based on visual inspection, if at all, without routine microbiological testing (Cook *et al.*, 2017). Cattle slaughter operations, such as bleeding, dressing and evisceration, expose sterile muscle to microbial contamination that were present on the skin, the digestive tract and in the environment (Bacon, 2002).

The aim of this study was to isolate and identify *Escherichia coli* from beef carcasses in pre and post washing steps at slaughterhouses and slabs in Khartoum state, Sudan using conventional bacteriological methods.

Materials and Methods

Area of Study

This study was carried out in Khartoum State during the years 2019 to 2022.

Source of samples

A total of 432 swab of beef carcass samples collected from three localities of Khartoum State, 144 swab samples were collected from each locality from different sites of the carcasses (neck, shoulders, rump and flanks) pre and post washing. In Omdurman locality samples were collected from ElHuda (Slaughterhouses), Alsalam and Albuqea (slabs). In Bahri locality samples were collected from Elkadro (Slaughterhouses), Eleilafon and Shergelnile (slabs). In Khartoum locality samples were collected from Alsahafa, Gabalawlia and Soogalmashia (slabs).

Primary isolation

Three loop full from swab sample were streaked on MacConkey, agar, EMB agar then the streaking over the plate was completed using the wire loop.

Incubation of culture

All inoculated solid and liquid media were incubated aerobically at 37C^{o} for 18-24 hours.

Examination of cultures

Cultures on semi-solid media were examined grossly for colonial morphology and haemolysis whereas, broth media were checked for turbidity, change in colour, accumulation of gases in Carbohydrates media and for sediment formation.

Preparation and staining of smears

From one colony on each plate one half was taken with a sterile loop, emulsified in a drop of normal saline on a clean microscopic slide, the smear was allowed to dry and then fixed by passing the slide over a flame, the slides were placed on the rack and flooded with crystal violet stain for one minute and rinsed with water.

They were then covered by iodine for a minute and rinsed with water, alcohol was poured and immediately the slides were rinsed with water, the slides were counter stained with neutral red for two minutes and rinsed with water again and allowed to dry by blotting with filter paper, a drop of immersion oil was added to each slide and examined under microscope, colonies which showed Gram positive cocci. Gram-positive bacilli and Gram-negative bacilli were sub cultured on nutrient agar.

Sub culturing and purification

Purification was based on the characteristics of colonial morphology and smear, discrete colonies were picked, smeared, fixed, and Gram-stained, and then the same colonies were sub cultured on nutrient agar.

Biological and biochemical identification

All the biochemical tests were performed according to Sneath *et al.*, (1986) and Barrow and Filtham (1993), the identification include: Gram's reaction, presence or absence of spores, shape of organism, motility, colonial characteristics on different media, aerobic and anaerobic growth, sugars fermentation ability and biochemical tests (staining of smear, catalase test, oxidase test, coagulase test, oxidation fermentation test, motility test, glucose breakdown test, fermentation of carbohydrates, urease activity, citrate utilization, gelatine hydrolysis test, nitrate reduction test).

Results and Discussion

In this study a total of 195 *Escherichia coli* were isolated from 432 swab samples of beef carcass collected from different slaughterhouses and slabs in Khartoum State. According to chemical characteristics, bacterial morphology and biochemical reactions (Indole, Methyl red, VP, H2S, Aerobic growth, Colonies on MacConkey, Haemolysis on blood agar, Gram-reaction Shape, Motility, Catalase, Oxidase and sugar fermentations test).

The percentage of *Escherichia coli* isolates for all localities were: 83(43%), 67(34%) and 45(23%) from Omdurman, Khartoum and Bahri localities slaughterhouses and slabs respectively Fig (1).

There were no significant differences in the frequency of *Escherichia coli* isolation between the three areas of the state using ANOVA, but when the post-hock multiple comparisons was applied there was a statistically significant difference between Bahri and Omdurman slaughterhouses with ($P<0.05$) Table 1.

Concerning carcass sites, the mean frequency of *Escherichia coli* isolated during prewash were: (3.44±2.24, 3.44±1.74, 2.78±1.99, 3.22±1.48) and (1.89±1.45, 2.44±1.81, 2.00±1.41, 2.44±1.94) during post wash of neck, shoulders, rump and flanks respectively, in all slaughterhouses and slabs of Khartoum state, (n=108). Regarding the mean frequency of isolates in both slaughterhouses and slabs in prewash (3.22) and post wash (2.19) samples, there was statistically significant difference with ($P<0.05$) (Table 3), (Fig 2). The findings of this study demonstrated that there was a significant difference at ($P<0.05$) between Albuqea slab compared to Elkadro Slaughterhouse, Alaielafoon and Soogalmashia slabs (Table 2.)

The frequency of the isolation of *Escherichia coli* in Elkadro Slaughterhouse was significantly lower compared to all Slaughterhouses and slabs in the three localities with (P<0.05) (Table 2). For all other slaughterhouses and slabs, the differences in the frequency of *Escherichia coli* isolates were of no statistical significant differences (Table 2).

When comparing the frequency of isolates between the different sites of samples in pre and post wash, there was a significant difference in the frequency of isolates in the four sites of sample collection **(**Table 4), (Fig 3). But when comparing the sample type with the slab together there were no significant differences in post and prewash samples (Table 5).

R Squared is 0.49 which indicates that about 50% of the change in the frequency of the isolates was due to the slaughterhouses and slabs and the area of the samples (Table 5).

When comparing the *Escherichia coli* contamination between pre and post wash from different sites of carcasses (neck, shoulders, rump and flanks) at different Slaughterhouses and slabs of Khartoum state there was significant difference (P < 0.05) in the frequency of isolates, this result in agree with Ali (2007) who recorded high contamination level on flank site and lower contamination level on rump sites during skinning.

Results obtained from the present study indicated that *Escherichia coli* isolated from the Bahri locality was lower compared to Omdurman and Khartoum localities. These differences could be due to the good hygienic status in Elkadro slaughterhouse. On the other hand, study reported by Yousif and Mustafa (2020) indicated that, Khartoum locality scored less positive *Escherichia coli* isolates as compared to Bahri and Omdurman in butcher shops in Khartoum State, Sudan.

Table.1 Comparison between Omdurman, Khartoum Bahri localities slaughterhouses and slabs.

Locality		Mean ± SE	Significance
Omdurman	Khartoum	0.67 ± 0.49	NS
Omdurman	Bahri	1.58 ±0.49	***
Khartoum	Bahri	0.91 ± 0.49	NS

NS: Not significant, * P<0.05

Table.2 Multiple Comparisons of *Escherichia coli* isolated from beef carcasses Slaughterhouses and slabs pre and post wash from three localities of Khartoum state.

Slaughterhouses		Mean ± SE	Significance
Albuqea	Alhuda	1.12 ± 0.77A	NS
	Alsalam	0.88 ± 0.77 A	NS
	Elkadro	4.00 ± 0.77B	***
	Shergelnile	1.23 ± 0.77 ^{A}	NS
	Alaielafoon	1.62 ± 0.77 ^{A}	*
	Alsahafa	1.23 ± 0.77 ^{A}	NS
	Gabelawlia	1.00 ± 0.77 ^{A}	NS
	Soogalmashia	1.88 ± 0.77 ^{A}	*

A, B: Mean values within the rows with different superscript capital are significantly different. NS: Not significant * P<0.05

Table.3 Comparison of *Escherichia coli* contamination pre and post wash at different Slaughterhouses and slabs of Khartoum state.

Slaughterhouses	Mean ± SD		Significance
	Prewash	Post wash	significance
Albuqea	4.00 ± 1.82	4.25 ± 1.70	***
Alhuda	3.50 ± 1.91	2.50 ± 0.58	***
Alsalam	3.75 ± 1.70	2.75 ± 1.50	***
Elkadro	0.25 ± 0.50	0.00 ±0.00	***
Shergelnile	4.25 ± 1.70	1.75 ± 0.96	***
Alaielafoon	2.75 ± 1.70	2.25 ± 1.50	***
Alsahafa	3.50 ± 1.70	2.50 ± 2.38	***
Gabelawlia	4.50 ± 1.70	1.75 ± 0.96	***
Soogalmashia	2.50 ± 1.29	2.00 ± 1.41	***

Table.4 Compared of *Escherichia coli* contamination pre and post wash from different sites of carcasses at different Slaughterhouses and slabs of Khartoum state.

Sites	Manipulation Mean ± SD		Significance
	Prewash	Post wash	
Neck	3.44 ± 2.24	1.89 ± 1.45	***
Shoulder	3.44 ± 1.74	2.44± 1. 81	**
Rump	2.78 ± 1.99	2.00 ± 1.41	*
Flanks	3.22 ± 1.48	2.44 ±1.94	**

Table.5 Multi comparison the slaughterhouses and slab and the sample type

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	112.125a	17	6.596	3.104	.001
Intercept	528.125	1	528.125	248.529	.000
Sample type	19.014	1	19.014	8.948	.004
Slab	77.250	8	9.656	4.544	.000
Sample type * slab	15.861	8	1.983	.933	.497
Error	114.750	54	2.125		
Total	755.000	72			
Corrected Total	226.875	71			
a. R Squared = .494 (Adjusted R Squared = .335)					

Fig.1 Percentage of *Escherichia coli* isolated from slaughterhouses and slabs of Khartoum state.

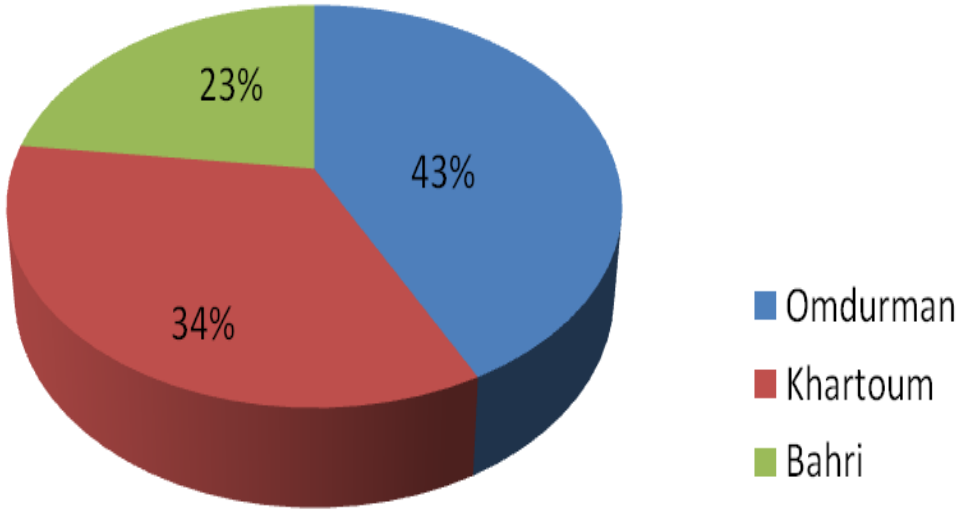


Fig.2 Comparison of *Escherichia coli* contamination in pre and post wash from different sites of carcasses at different Slaughterhouses and slabs of Khartoum state.

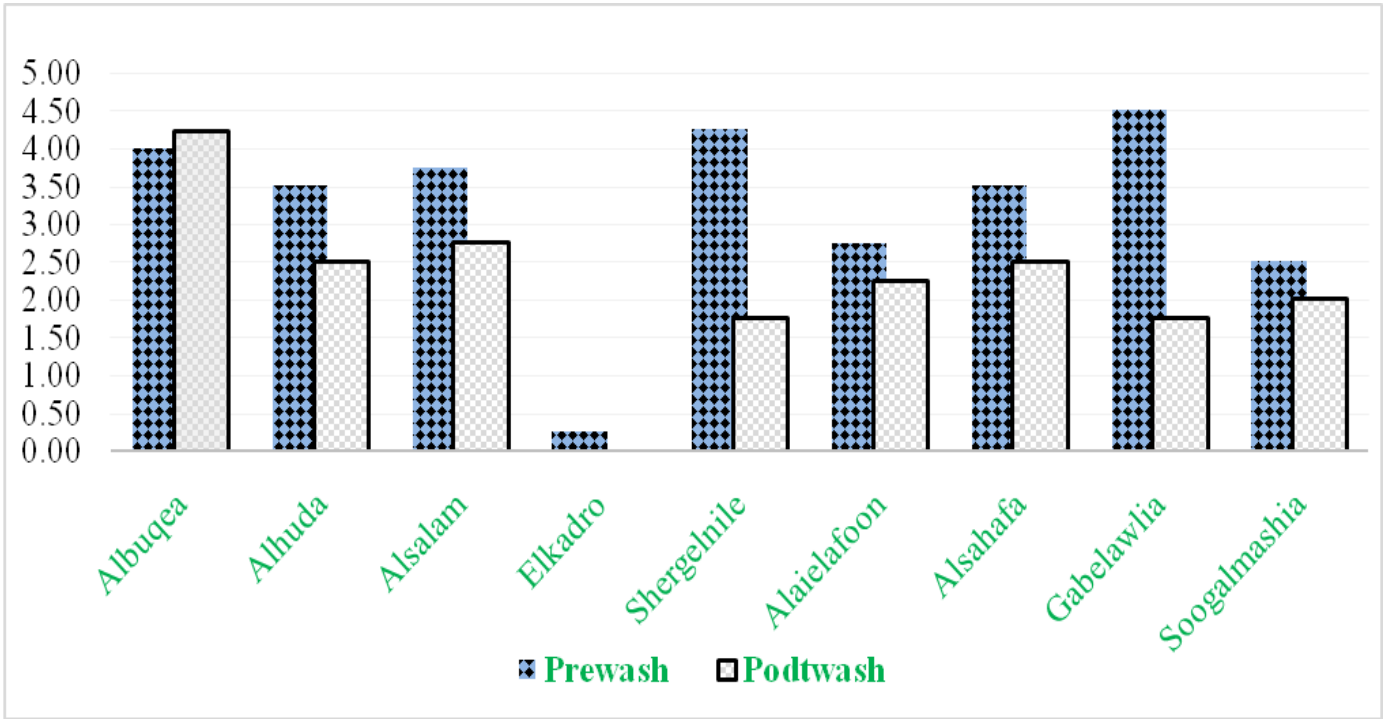
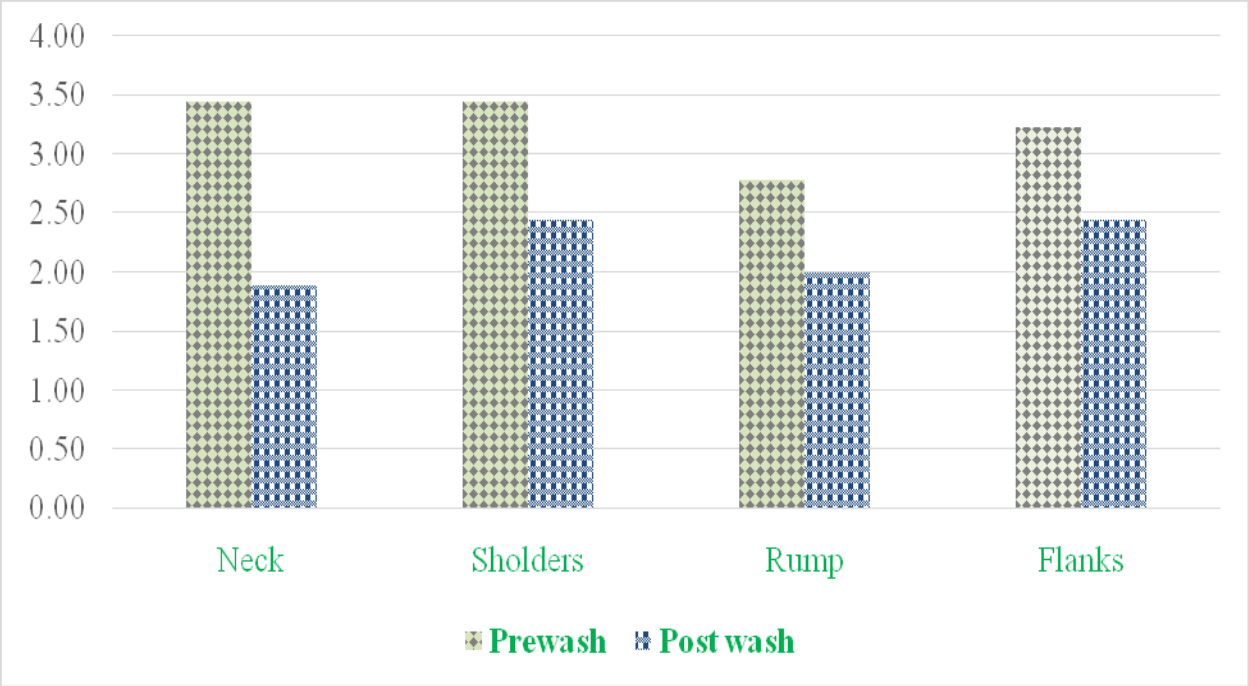


Fig.3 Compared of *Escherichia coli* contamination pre and post wash from different sites of carcasses at different Slaughterhouses and slabs of Khartoum state.



The finding of the current study showed that the frequency of *Escherichia coli* isolated during prewash was high in the four sites compared to post wash.

This finding is in agreement with (Gill and McGinnis, 2000; Bacon *et al.,* 2000; Abdalla *et al.,* 2009 and Abdalla *et al.,* 2010) who reported that the presence of high counts of *Escherichia coli* in meat in prewash stage was probably associated with the poor hygiene practices involved in meat processing that result into higher chances of fecal contamination after evisceration.

In the current research, the frequency of the isolation of *Escherichia coli* in Elkadro Slaughterhouse was significantly lower compared to all other slaughterhouses and slabs. These differences may be attributed to the fact that the hygienic standard of Elkadaro slaughterhouse was higher than that applied in Omdurman slaughterhouses where there was no clear demarcation between the clean and dirty operations in the latter, these findings in agree with (Elhassan, 2006 and Elhassan *et al.,* 2011).

Similar study reported by Abdelwahed and Abdelgadir (2019) who found that the level of bacterial contamination in beef at Elsabloga slaughter house was very high due to relatively poor hygienic practices in different slaughterhouses in Khartoum State, which constitutes a real public health hazard.

The frequency of *Escherichia coli* isolated of post wash in neck site was higher than shoulders, rump and flanks. These results are in agreement with the study of (Ali, 2007 and Abdalla *et al.,* 2009 and Chepkemoi, 2016), who reported that the highest contamination was detected at the point of washing, on different sites of bovine carcasses. The frequency of isolation of *Escherichia coli* is significantly lower in modern slaughterhouse that follow the good hygienic practices.

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