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Isolation and Identification of Food-Borne Bacteria Associated to Beef Carcass from Different Slaughter Houses in Khartoum State Sudan

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A B S T R A C T

In this investigation a total of 570 bacterial isolates were obtained from 450 beef carcass swab samples collected from different slaughter houses of Khartoum State. According to the cultural characteristics, bacterial morphology and biochemical reactions the identified bacteria were: *Escherichia coli* (32.3%), *Klebsiella pneumoniae* (27.2%), *Proteus spp* (21.2%) and *Pseudomonas aeruginosa* (19.3%) respectively. *E.coli* represented the predominant bacteria (32.3%) isolated from swab of beef carcass compared to other bacteria *Klebsiella pneumoniae* (27.2%), *Proteus spp* (21.2%), *Pseudomonas aeruginosa* (19.3%). The percentage of *E.coli* isolated from slaughterhouses of Omdurman locality is 65%, Khartoum 10% and Bahri 25% respectively. The *E. coli* represents the predominant Bacterial spp isolated from slaughterhouses of Omdurman locality compared to other localities of Khartoum state. *Proteus spp* also recorded a numerical increase in Omdurman, similar to *E. coli* but with lowest percentage of it followed by *Klebsiella spp* and *Pseudomonas spp* respectively.

Introduction

Meat is a source of animal proteins for human being nutrition, constituting a suitable medium for the growth of many pathogens (Williams, 2007). Meat and meat products can be contaminated in different stages of the food chain, from the abattoir during evisceration to the processing stage (Rega *et al.*, 2022). It can be contaminated during slaughtering and dressing, initially dressed carcasses may be exposed to contamination by some microorganisms which may survive during meat processing and

storage (Ercolini, 2009; Rouger, 2017). Coliforms are the most frequently identified group on meat, especially *Citrobacter freundii* and *E.coli*, while other microorganisms are less frequent such as *Klebsiella, Salmonella, Shigella sonnei, Proteus spp and Staphylococcus aureus* (Turtura and Lorenzelli, 1994).

Assessment of the hygienic risk in a beef slaughtering process should involve enumeration of organism indicative of fecal contamination, such as *E.coli* at specific points in the process, the contamination and/or cross contamination of carcasses, during slaughtering operations were demonstrated and the results indicated presence of bacteria of potential public health significance (Doyle, 1991; Biss and Hathaway, 1995).

Shiga toxin-producing *Escherichia coli* (STEC) causes high frequencies of foodborne infections worldwide and has been linked to numerous outbreaks each year (Blankenship *et al.,* 2023). *E.coli* O157: H7/NM has been globally recognized as an important food-borne pathogen since the outbreak was first reported in the United States in 1982 (Riley *et al.,* 1983; Kim *et al.,* 1998; Cordovez *et al.,* 1992; Allerberger *et al.,* 1996 and Tamura *et al.,* 1996).

Escherichia coli are both pathogenic and commensal bacteria that are considered to be in food hygiene an important sign of fecal contamination, they are also used as sentinel bacteria to assess the presence of antimicrobial resistance phenomenon in animals and in human, *E.coli* can thus be isolated from a variety of sources, such as the feces, manure, water and foods of animal and plant origin, and consequently, they can easily survive in various environments and spread (Sacher-Pirklbauer *et al.,* 2021). Enterohemorrhagic *E.coli* EHEC infections may be sporadic, in small clusters, or manifest as larger outbreaks, transmission is via the fecal-oral route and frequently occurs through ingestion of contaminated food or water, direct contact with infected animals, humans, objects or rarely inhalation (Grant *et al.,* 2008). It is widely acknowledged that controlling *E.coli* O157: H7 within the bovine population would be an effective method of reducing transmission to humans (Stevens *et al.,* 2002).

Pseudomonas aeruginosa bacteria are emerging causes of food spoilage and foodborne diseases. Raw meat of animal species may consider a reservoir of *Pseudomonas aeruginosa* (Poursina *et al.,* 2023). *Pseudomonas spp* has been recognized as a unique meat spoiling organism. The proliferation of these spoilage organisms might influence the organoleptic meat quality; therefore, the current investigation is being carried out to detect *pseudomonas spp* associated with meat displayed in Mosul city retails (Jawher *et al.,* 2022). *Pseudomonas spp* has been recognized as a unique meat spoiling organism, the proliferation of these spoilage organisms might influence the organoleptic meat quality, therefore, the current investigation is being carried out to detect pseudomonas associated with meat displayed in Mosul city retails (Tahr and Hasan, 2022).

During the last few decades, extensive attention has been paid on the management of food alteration and contamination caused by spoilage organisms such as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* (Meliani and Bensoltane, 2015). *Pseudomonas aeruginosa* is important cause of meat corruption, it may act as foodborne diseases after the consumption of contaminated food samples (Poursina *et al.,* 2022).

Members of the genus *Klebsiella spp* belong to the family Enterobacteriaceae, they are Gram-negative, non-motile, usually capsulated, facultatively anaerobic bacteria. They are found in different environmental sources such as water and soil (Melo-Nascimento *et al.,* 2018). It is also found in a variety of environmental sources such as soil, water, and vegetation (Wareth and Neubauer, 2021). It is often present in a wide range of domestic and wild mammals as well as in insects and has been also recovered from foods (Guo *et al.,* 2016). *Proteus spp.* are Gram negative rods measuring 1-3µm in

length and 0.4-0.8µm in diameter, motile by peritrichous flagella, facultative anaerobic, non-spore forming and non-capsulated with most isolates having fimbriae. *Proteus species* are frequently found in soil, water and the intestinal tract of many animals and humans (Drzewiecka, 2016). *Proteus mirabilis* is an opportunistic pathogen often associated with a variety of human infections acquired both in the community and in hospitals (Sanches *et al.,* 2019). *Proteus mirabilis*is ubiquitous, Gram-negative rod found in poultry, soil, sewage, water, fecal matter and considered as the normal micro flora of gastro intestinal tract of human and animals (Sanches *et al.,* 2020).

The main of this research to Isolation and identification the most important bacteria associated with beef carcasses with special reference to *E. coli* O157: H7.

Materials and Methods

Area of Study

This study was conducted out in Khartoum State during the years 2019 and 2022.

Source of samples

A total of 450 beef carcass swab samples collected from different slaughter houses in three localities of Khartoum State. 150 samples were collected from Omdurman, 150 from Khartoum locality and 150 from Bahri locality (Table.2).

Primary isolation

Three loop full from swab sample were streaked on McConkey's 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 37^{o}C 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, then the same colonies were sub cultured on nutrient agar and EMB agar.

Biological and biochemical identification

All the biochemical tests were performed according to Smith *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, coaggulase 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 investigation a total of 570 bacterial isolates were obtained from 450 beef carcass swab samples collected from different slaughter houses of Khartoum State (Table1). According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 4) the identified bacteria were: 184 *E. coli* (32.3%), 155 *Klebsiella pneumoniae* (27.2%), 121 *Proteus spp* (21.2%)110 *Pseudomonas aeruginosa* (19.3%) respectively.

E.coli represented the predominant bacteria percentage (32.3%) isolated from beef carcass samples compared to other bacterial isolated *Pseudomonas aeruginosa* (19.3%), *Klebsiella pneumoniae* (27.2%), *Proteus spp (*21.2%) (Table 5, Fig2).The number of bacteria isolates in Omdurman locality increased significantly from Bahri and Khartoum localities respectively (Table. 4).

In this study a total of 570 bacterial isolates were obtained from swab of beef carcass samples collected from different slaughter hosing of Khartoum State. It was shown the bacteria isolated were: *E.coli* (32.3%), *Klebsiella pneumoniae* (27.2%), *Proteus spp* (21.2%), *Pseudomonas aeruginosa* (19.3%) respectively.

Escherichia coli represented the predominant bacteria percentage (32.3%) isolated from beef carcass samples compared to other bacteria. This finding is agree with Abdalla *et al.,* (2009) who reported that, *E.coli* (8.8%), *Pseudomonas spp* (14.76%), *Klebsella spp* (10.12%) and Proteus spp (7.17%). Similar study investigated by Awatif *et al.,* (2015) who showed that, *Proteus mirabilis*13.5%, *Klebsiella pneumoniae* 6.7%, *Citrobacter spp* 1.9%, and *Vibrio spp* 1.9% from contaminating beef carcasses in Khartoum State.

Wafa (2004) found that, *S.aureus*, *Bacillus cereus*, *Corynebacterium pseudotuberculosis*, *E.coli*, *Salmonella spp and Klebsiella pneumoniae* were isolated from fresh beef meat before and after processing, this finding is in line with my study. Schlegelova *et al.,* (2004) who isolated the *E.coli* from beef carcass contamination in slaughter house and prevalence to antimicrobial drugs in isolates of selected microbial species, this result was agrees with the current study.

Other studies reported by Nazik (2017); Abdelwahed and Abdelgadir (2019) who isolated the *Pseudomonas spp* from bacterial contamination of beef related to hygiene practices inslaughter houses in Khartoum State, this finding is accord with my study.

Uzoigwe *et al.,* (2021) who reported that isolated the *E.coli* spp and *S.aurues* from assessment of bacterial contamination of beef in slaughterhouses in Owerri zone, Imo state, Nigeria this finding is in line with my current study. Bersisa and Negera (2019) who showed the *E. coli* was the dominant bacterial species isolated (35.2%) followed by *S.aureus* (22.5%) and *Salmonella spp* (9.9%) from investigation of bacteriological quality of meat from abattoir and butcher shops in bishoftu, Central Ethiopia, this finding is in line with the present study.

The percentage of *E.coli* isolated from slaughterhouses of Omdurman locality is 65%, Khartoum 10% and Bahri 25% respectively. The *E. coli* represents the predominant Bacterial spp isolated from slaughterhouses of Omdurman locality compared to other localities of Khartoum state. *Proteus spp* also recorded a numerical increase in Omdurman, similar to *E. coli* but with lowest percentage of it followed by *Klebsiella spp* and *Pseudomonas spp* respectively.

Table.1 Bacterial isolated from beef carcass swab samples collected from different localities of Khartoum State

Bacteria spp	Slaughter houses of Bahri locality			Slaughter houses of Omdurman locality			Slaughter houseof Khartoum locality	Total of +ve Bacteria
Slaughter houses	Shargalnile	Sabloqa	Kadro	boqaa	Huda	Salam	Sahafa	
E.coli	25	20	1	41	35	44	18	184
Pseudomonas spp	14	18	9	11	13	24	21	110
Protus spp	9	27	25	15	13	21	11	121
Klebsiella spp	13	14	20	15	24	35	34	155
No of bacteria	61	79	55	82	85	124	84	570

Table.2 Sources of carcass swab samples

Locality	Number of carcass swab samples
Khartoum	150
Omdurman	150
Bahri	150
Total	450

Table.3 Bacterial isolated from carcass swab samples collected from different localities of Khartoum State.

Bacterial isolated	Khartoum locality	Omdurman locality	Bahri locality
E. coli	18	120	46
Pseudomonas spp	21	48	41
Protues spp	11	49	61
Klebsiella spp	34	74	47
Total	84	291	195

Fig.1 Percentage of *E.coli* collected from different localities of Khartoum State.

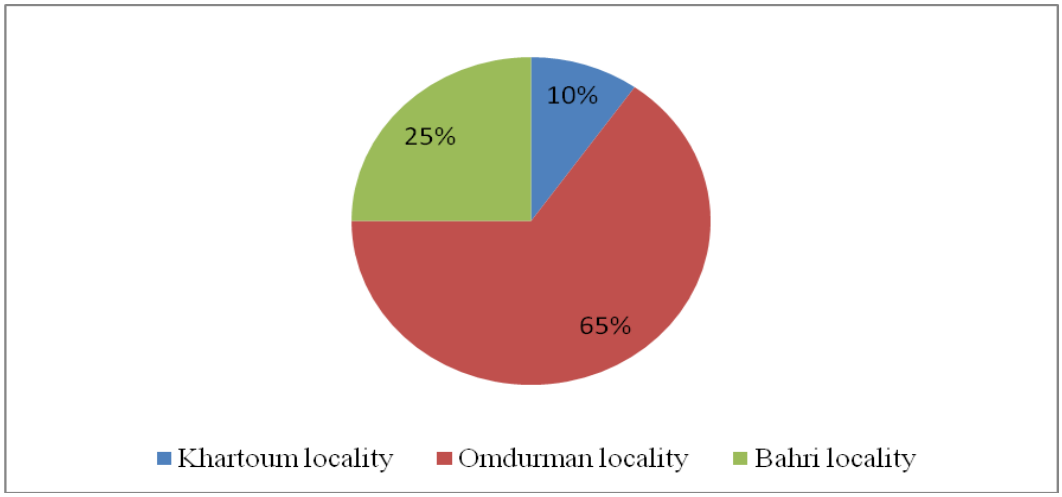


Table.4 Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria

Test	*E. coli spp*	*Pseudomonas aeruginosa*	*Proteus spp*	*Klebsiella pneumoniae*
Aerobic growth	+	+	+	+
Colonies on MacConkey	Bright Pink	Bright Pink	pail	Pink
Haemolysis on blood agar	+	+	+	+
Gram-reaction	-	-	-	-
Shape	Rods	Rods	Rods	Rods
Motility	+	-	-	-
Catalase	+	+	+	+
Oxidase	-	+	-	-
Indole	+	+	-	+
Methyl red	+	-	+	-
VP	-	-	-	-
Citrate	-	-	+	+
H2S	-	-	+	-
O/F	+	+	+	+
Glucose	+	-	+	+
Lactose	+	-	+	+
Maltose	+	-	+	+
Inositol	-	-	-	-
Sucrose	+	-	+	-
Mannitol	+	-	+	+
Xylose	+	-	-	+
Raffinose	-	-	-	+
Sorbitol	+	-	-	+
Trehalose	+	-	+	+
Dulcitol	-	-	-	+
Cellobiose	-	-	-	+

Table.5 Total number and percentage of bacterial isolated from beef Carcass swab samples collected from Khartoum State

Bacterial spp.	Number	Percentage
E. coli	184	32.3%
Pseudomonas	110	19.3%
Proteus	121	21.3%
Klebsiella spp	155	27.1%
Total	570	100%

Fig.2 Percentage of bacterial isolated from beef Carcass swab samples collected from Khartoum State.

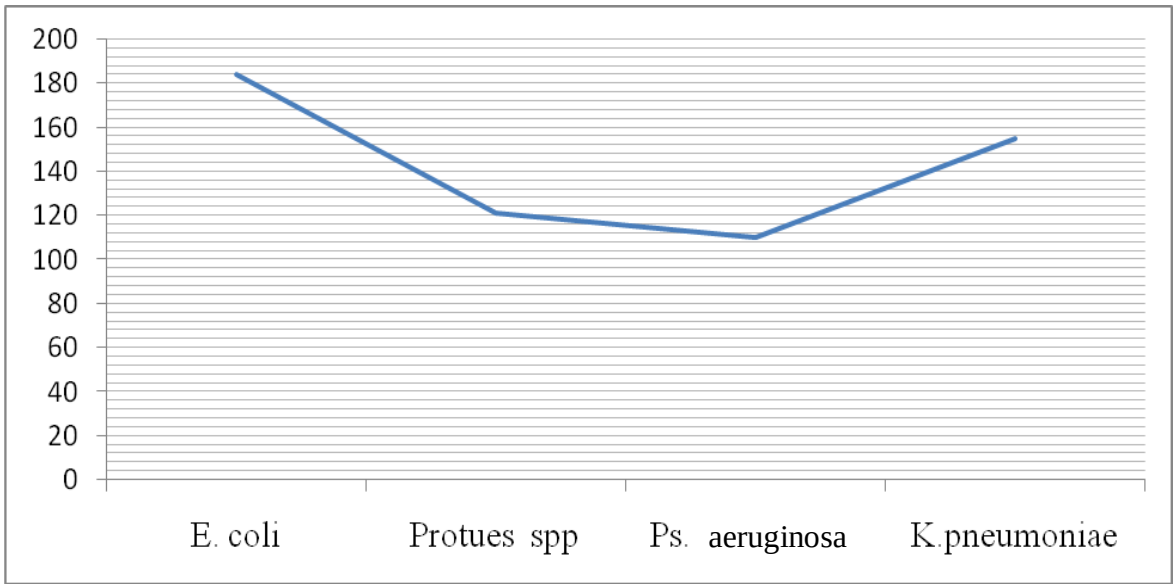
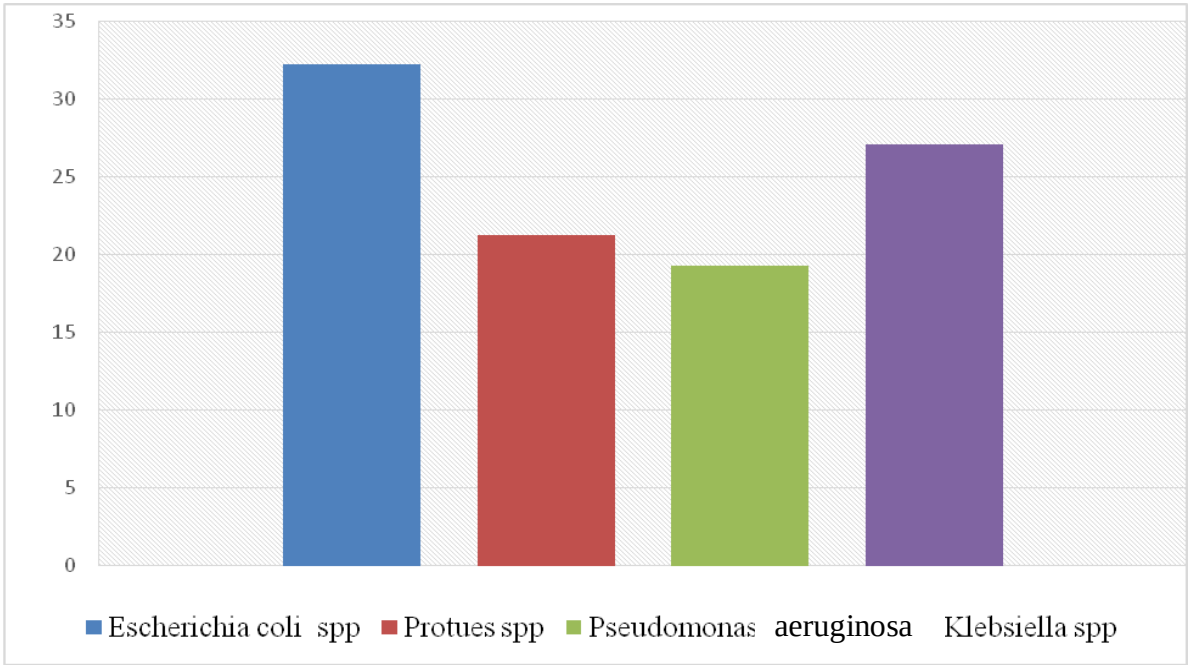


Fig.3 Bacterial isolated from beef Carcass swab samples collected from Khartoum State.



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