POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 38(1): 31–42, Y. 2023

DOI: 10.31648/pjns.8607

DETECTION OF POTENTIALLY PATHOGENIC BACTERIA IN BUTCHER SHOPS: FIRST REPORT FROM AL MANDAQ CITY, SAUDI ARABIA

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Key words: food safety, pathogenic bacteria, butcher shop, meat, local market, *E. coli*.

Abstract

Food-borne pathogens cause significant economic losses and affect the quality of life. Butcher shops act as a perishable commodity and contribute to the possible spread of food-borne pathogens. Due to the absence of knowledge and studies related to food safety in Al Mandaq city, Saudi Arabia, the main objective of this study was to initiate and establish a database about the possibilities of the existence of pathogenic bacteria in butcher shops in the city. From each local shop at the city (n = 6), 14 samples were collected from various spots, and the potential bacterial pathogens were identified on specific media (blood agar, MacConkey agar, Hicrome *Staphylococcus* selective agar, and Eosin methylene blue agar). Based on the number of presumed pathogens, Shop 1 was the most contaminated (n = 57), followed by Shop 2 (n = 51). Among the collected samples, lamb meat contained the highest number (25) of all pathogens, followed by beef, the floor, and the fridge (22 each). The prevalence of *Staphylococcus aureus* was 29%, followed by *Salmonella* spp. (26%) and *Escherichia coli* (24%) in all the samples of the six butcher shops. All 84 samples were less contaminated with *Salmonellae* spp. (10%) compared with other isolated pathogens. The increased frequency of these potential pathogens in meat shows the appallingly unhygienic and unsanitary techniques used, from the slaughterhouse, during transportation to butcher shops, and during processing at the butcher shops. As a result, the current investigation, which is the first of its type in Al Mandaq, demonstrates that meat is highly contaminated with potential bacterial pathogens. Minimizing meat contamination in markets and using good sanitation and inspection techniques are crucial.

Introduction

Food-borne diseases (FBDs) are one of the most critical global public health issues and should be addressed on a priority basis to ensure a healthy environment (KHAN et al. 2022). Around the world, one person out of 10 (600 million people) is a victim of FBDs. Each year, 420,000 peo-

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ple die due to food poisoning. It is reported that 70% of FBDs are caused by microorganisms (WHO 2015). Bacteria play a crucial role in the development of FBDs due to either the production of toxins or the colonization of these bacteria to the epithelial cells of the gastrointestinal tract (KIRK et al. 2015).

Meat is a perishable food and provides a favorable environment for the growth of pathogenic bacteria and microorganisms, which causes it to spoil (BIRHANU et al. 2017). Meat is exposed to a variety of contaminations, from the production stage to the point at which it is available to consumers (ARAFA et al. 2022). Such contamination is the prime cause of illness and, occasionally, death upon ingestion due to the persistence of pathogenic microorganisms (WHO 2007). Mainly, FBDs result from ingesting pathogenic bacteria and microbial toxins (BANNON et al. 2016).

Many reports depict the isolation of bacterial pathogens from fresh meat, which plays an important role in the onset of diseases in humans (BANNON et al. 2016, BANTAWA et al. 2018, CASTELLANO et al. 2008, MESHAAL et al. 2021, MOR-MUR and YUSTE 2009, UKUT et al. 2010). These pathogens include *E. coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, etc. The main sources of these pathogenic bacteria in butcher shops are slaughtered animals, workers' gloves, knives, meat storage, contaminated water, tables, cutting boards, and weighing scales (BIRHANU et al. 2017).

With prolonged favorable environments, such as acidity, moisture, temperature, and availability of nutrients, the microbial load in meat increases (NIYONZIMA et al. 2015). *E. coli*, *Listeria monocytogenes, Salmonella* spp., *Staphylococcus aureus*, and *Campylobacter* spp. are considered the main contaminants of meat and can cause not only diarrhea but also other gastrointestinal disorders (ORPIN et al. 2018). One of the mesophilic commensal microbes present in the digestive tract of both humans and animals is *Escherichia coli* (DUBREUIL 2012). Gastroenteritis and extra-intestinal infections such as urinary tract infections (UTIs) are caused by pathogenic strains of *E. coli* (BANNON et al. 2016).

Determination of the bacterial load in the meat indicates the hygienic quality of the meat. Factors such as poor facilities in butcher shops, slaughtering of diseased animals, poor handling of the remains after slaughtering, and contaminated environments contribute to the high bacterial count, which in turn is a threat to humans (BIRHANU et al. 2017).

Without a hygienic environment at butcher shops and abattoirs, the availability of pathogen-free meat is minimal. Control measures are taken to ensure the hygiene and quality of meat, particularly in the catering industry (TAVAKOLI and RIAZIPOUR 2008). Few reports are available on handling practices and hygiene status of the meat at butcher shops in

some parts of Saudi Arabia, keeping these gaps in view, the purpose of this study was to establish a study and to assess the potential existence of pathogenic bacteria in local butcher shops at Al Mandaq city, Saudi Arabia, particularly *Staphylococcus aureus*, *Staphylococcus epidermis*, *E. coli*, *Salmonella* spp., and *Shigella* spp.

Materials and Methods

Samples collection

Samples were collected from instruments/apparatus used in butcher shops at different locations in Al Mandaq, located in the southwestern region of Saudi Arabia. These samples were classified into two categories: Abiotic samples and biotic samples (Table 1). A total of 84 different samples were collected aseptically using cotton swabs from six butcher shops (14 samples from each shop). All samples were stored in an icebox and then brought to the lab in sterilized containers.

Table 1

Types of collected abiotic and biotic samples from each butcher shop

Biotic samples	Abiotic samples
butchers' hands	meat storage
lamb meat	cutting knives
beef meat	cutting boards
–	scales
–	sinks
–	masks
–	floors
–	gloves
–	fridges
–	doors
–	saws
–	mincers

Isolation and identification of bacterial strains

Swabs were streaked on different media using the protocol of MELEBARI et al. (2022). Used media were blood agar, MacConkey agar, Hicrome *Staphylococcus* selective agar, and Eosin methylene blue (EMB) agar, and

incubated at 37°C for 24 hours. After 24 hours of incubation, morphologically distinct colonies were picked and purified. All the isolates were identified using specific characters on a selective medium. Salmonella-Shigella agar medium was used to identify *Salmonella* spp. and *Shigella* spp. The *E. coli* strains were identified by producing a metallic sheen on the EMB medium. Hicrome *Staphylococcus* selective agar was used to identify *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Staphylococcus aureus* and *Staphylococcus epidermidis* were identified based on the production of green and blue colonies on said medium. Gram staining and catalase activity of the isolated strains were performed.

Basic confirmation tests

Gram staining of the isolated strain was performed using the protocol of SMITH and HUSSEY (2005). Catalase activity of the isolated strains was conducted using 3% H_2O_2 on the bacterium colony, and bubble formation was noted. The hemolytic potential of the isolated strains was checked on blood agar (nutrient agar + 10% sheep blood). Pathogens were streaked on blood agar and incubated at 37ºC for 24 hours. Hemolysis was checked based on the clear zone and color of colonies.

Statistical analysis

Frequencies and percentages were used to represent the data. To compare the groups, a chi-square test was performed using SPSS 26. A substantial impact was judged to be demonstrated by a p-value of >0.05.

Results

Six butcher shops were targeted for the isolation of pathogenic bacterial strains with standard procedure. Samples were collected from different tools and storage used in the butcher shop, including meat storage, cutting knives, cutting boards, scales, sinks, masks, floors, gloves, fridges, doors, saws, mincers, the butchers' hands, lamb meat, and beef meat. These strains were *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella* spp., *Shigella* spp., and *E. coli*, based on identification on respective media. The highest pathogenic load was found in shop 1, where the total number of pathogens was 57, followed by shop 2 (51), shop 3 (46), shop 6 (45), shop 4 (43), and shop 5 (37) – Figure 1.

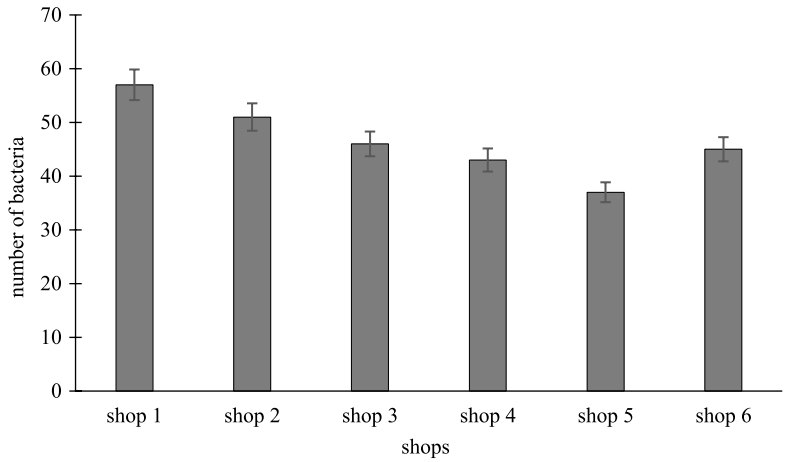

Fig. 1. Pathogenic load in collection site

Out of 57 bacterial strains from Shop 1 from all the samples, the number of each strain was ≥10, among which *S. aureus* was the highest (13), followed by *S. epidermidis* and *E. coli* (each 12), *Salmonella* spp. (10), and *Shigella* spp. (10). From Shop 2, 14 *S. aureus*, 13 *S. epidermidis*, 10 *E. coli*, and seven *S. aureus* were isolated. From Shop 3, 14 *S. aureus*, 12 *S. epidermidis*, 13 *E. coli*, two *Salmonella* spp., and five *Shigella* spp. were isolated. *Salmonella* spp. and *Shigella* spp. were isolated less in number (three each). From Shop 5, *S. aureus* (14) was found more than the others: *S. epidermidis* (10), *E. coli* (11), *Salmonella* spp. (1), and *Shigella* spp. (1). The highest number of pathogenic strains found in Shop 6 was *S. aureus* (14), followed by *S. epidermidis* (12), *E. coli* (10), *Shigella* spp. (5), and *Salmonella* spp. (4) – Table 2.

Table 2

Bacterial pathogens isolated from collection site

Shop	Pathogens [%]				
	S. aureus	*S. epidermidis*	*E. coli*	*Salmonella* spp.	*Shigella* spp.
Shop 1	23	21	21	18	18
Shop 2	27	25	20	14	14
Shop 3	30	26	28	4	11
Shop 4	30	33	23	7	7
Shop 5	38	27	30	3	3
Shop 6	31	27	22	9	11

Fourteen samples were selected for the isolation of bacterial strains. The collective number of bacteria from each sample was calculated from the total shops. Biotic samples contained higher numbers of pathogens than abiotic samples. The most contaminated was lamb's meat, from which 25 strains were isolated. Twenty-two strains were isolated each from beef meat, mincers, floors, and fridges. The bacterial count isolated from boards, scales, and gloves was 19 for each sample. From each sample of saws and sinks, 21 bacteria were isolated from all six shops. The lowest bacterial load was observed for masks, from which 14 bacterial isolates were recovered (Figure 2).

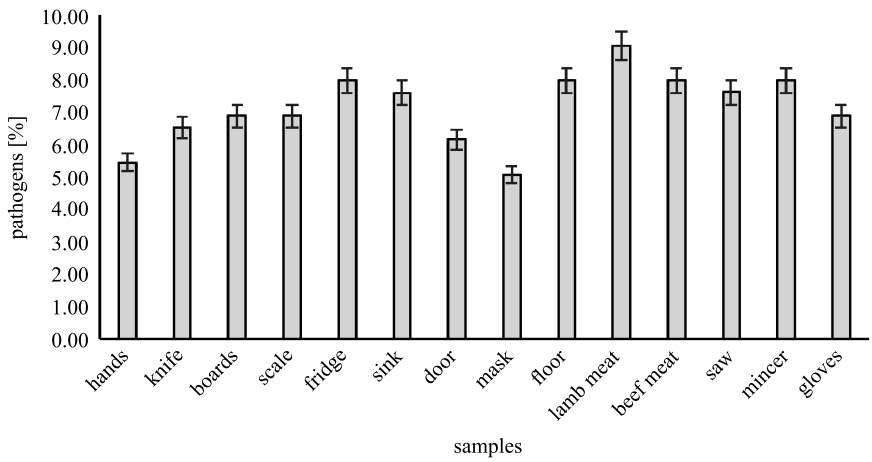

Fig. 2. Bacterial load with respect to samples from all the shops

Pearson chi-square analysis was performed to determine the association of the pathogens to the total observation (84) from all six butcher shops. From statistical analysis, a significant association between the sampling location and *S. aureus* ($p = 0.535$), *S. epidermidis* ($p = 0.353$), and *E. coli* ($p = 0.696$) count was not observed ($p > 0.05$). However, such association was found for *Shigella* spp. and *Salmonella* spp., where the p-values were 0.008 and 0.002 ($p < 0.05$), respectively (Table 3).

The total number of pathogens isolated from all the shops with respect to samples was calculated. The most isolated pathogen in all the shops was *S. aureus* ($n = 82$). Almost all 14 samples contained this pathogen. However, this pathogen was not present in the door sample of Shop 1 and the mincer sample of Shop 4 (Figure 3). This pathogen contributed 29% to the overall load of pathogens (Figure 4). The second most abundant pathogen in all the samples from all shops was *S. epidermidis* ($n = 70$).

Table 3

Chi-square analysis of the isolated pathogens from all the shops

Specification	*S. aureus*			*S. epidermidis*			*E. coli*			*Salmonella* spp.			*Shigella* spp.		
	value	df	*p*-value	value	df	*p*-value	value	df	*p*-value	value	df	*p*-value	value	df	*p*-value
Pearson Chi-Square	4.098[a]	5	0.535	5.544[a]	5	0.353	3.024[a]	5	0.696	18.830[a]	5	0.002	15.556[a]	5	0.008
Likelihood Ratio	4.493	5	0.481	6.810	5	0.235	3.331	5	0.649	19.347	5	0.002	16.582	5	0.005
Linear-by-Linear Association	0.694	1	0.405	0.434	1	0.510	0.506	1	0.477	10.211	1	0.001	10.979	1	0.001
N of Valid Cases	84	–	–	84	–	–	84	–	–	84	–	–	84	–	–
a) 6 cells (50.0%) have an expected count of less than 5; the minimum expected count is 0.88				a) 6 cells (50.0%) have an expected count of less than 5; the minimum expected count is 1.83			a) 6 cells (50.0%) have an expected count of less than 5; the minimum expected count is 2.83			a) 6 cells (50.0%) have an expected count of less than 5; the minimum expected count is 4.50			a) 0 cells (0%) have an expected count of less than 5; the minimum expected count is 5.00		

From Shop 1, this strain was isolated from all the samples except door and mask samples. From the hand sample of Shop 2 and mask and glove samples from Shop 3, *S. epidermidis* was not observed. All the samples from Shop 4 contained this strain. The fridge, sink, floor, and saw samples of Shop 5 were free of *S. epidermidis*. All the samples of Shop 6 contained these pathogens except mask and beef meat samples (Figure 3). This pathogen contributed 26% of the pathogens' total load from all the samples collected from the six butcher shops (Figure 4). *E. coli* was the third most abundant pathogen isolated from the samples (n = 67). Except for hand samples, all the other samples of Shop 1 contained *E. coli*. This pathogen was absent in samples of hands, knives, boards, and masks of Shop 2. *E. coli* was recovered from all the samples of Shop 3 except the mask sample. Knife, scale, and lamb meat samples for Shop 4 were not contaminated with the pathogen. The glove and mask samples of Shops 5 and 6 were free of *E. coli*. This pathogen was absent from the floor of Shop 5. Hands and fridges were contaminated with this pathogen in Shop 6 (Figure 3). This pathogen contributed 24% to the total load of the pathogens from all the samples collected from all six butcher shops (Figure 4). *Shigella* spp. was the fourth most abundant pathogen among the pathogens isolated from all the samples of all the shops (n = 31). Hands, board, door, and mask samples of Shop 1 were free of this pathogen. Scale, sink, door, floor, lamb meat, beef meat, and glove samples from Shop 2 were positive for the presence of *Shigella* spp. Board, fridge, sink, beef meat, and mincer samples from Shop 3 contained this pathogen. All the samples of Shop 5 were free of this pathogen except for the lamb meat. Samples of hand, floor, lamb meat, and saw from Shop 6 were contaminated with *Shigella* spp. (Figure 3). This pathogen contributed 11% to the total load of the patho-

gens from all the samples collected from all six butcher shops (Figure 4). *Salmonella* spp. was the fifth most abundant pathogen among the pathogens isolated from all the samples of all the shops (n = 27). Hands, sink, door, and mask samples from Shop 1 were free of *Salmonella* spp. Scales, sinks, floors, lamb meat, beef meat, mincers, and gloves from Shop 2 were positive for the presence of this pathogen. Fridge and sink samples collected from Shop 3 were contaminated with *Salmonella* spp. No sample from Shop 4 contained this pathogen except the fridge, sink, and mincer. All the samples from Shop 5 were free of *Salmonella* spp., except for lamb meat. Samples of the floor, lamb meat, and saws from Shop 6 showed the presence of this bacterium (Figure 3). This pathogen contributed 10% to the total load of pathogens from all the samples collected from all six butcher shops (Figure 4).

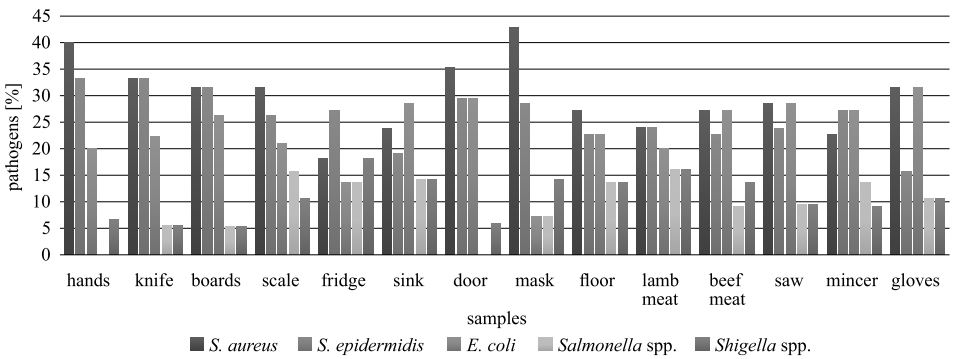

Fig. 3. Number of each pathogen from all the shops

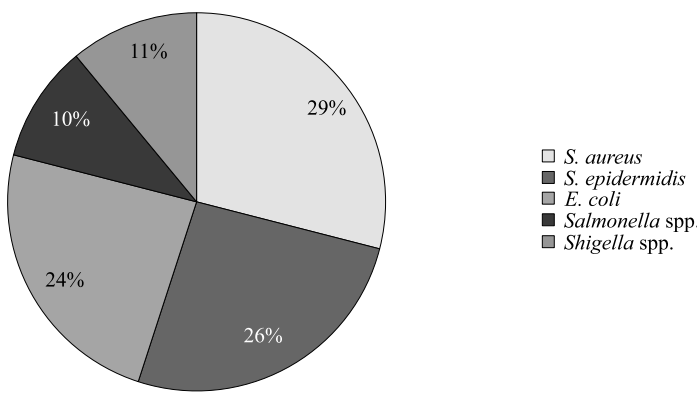

Fig. 4. Distribution of pathogens [%]

Discussion

Meat provides an excellent environment for pathogenic bacteria (DAS et al. 2019). Generally, meat is consumed cooked, but in some recipes, meat is used raw or partially cooked, which gives rise to the problem of food poisoning (HENNEKINNE et al. 2015). The butcher shop is a component of the food industry that can contribute to the spread of food-borne pathogens, toxins, and other contamination (BANNON et al. 2016). Using such contaminated food with pathogens or toxins results in diarrhea, which can lead to death. Each year, 3 million deaths occur worldwide due to food poisoning (WHO 2007).

An unhygienic butcher shop is considered a source of pathogens unless the SOPs of hygienic practices are employed (ROBERTS et al. 2009). From the current study, it is clear that such a load of pathogenic bacteria from all the samples results from a lack of SOPs and personal hygiene. Workers' hygienic conditions impact the possible contamination of the meat. Diseased and unclean workers, equipment, dressing type, and dressing process all accounted for pathogenic spread and storage (SAMUEL et al. 2011). A general recommendation for workers is to use contamination-free clothes, gloves, protective coats, and hair cover while processing the meat (MOAE 2010). In our study, *S. aureus*, *S. epidermidis*, *E. coli,* and *Shigella* spp. were recovered from the hands of the workers which is in agreement with the findings of BERSISA et al. (2019), who reported that cross-contamination could also happen while handling food with contaminated hands.

In the current study, gloves and masks are considered part of clothing. We isolated all the types of pathogens from these samples, indicating the unhygienic clothing of the workers. The workers themselves may be a potential source of contamination due to disease in addition to their clothing. It was advised that new hires be given a clinical and bacteriological examination before hiring and regularly afterward (BERSISA et al. 2019). The examination should include a medical history to ascertain any prior infections, focusing on venereal and skin diseases, dysentery, typhoid, and paratyphoid fevers (WHO 2004). Handling money and touching carcasses with the same unclean hands could be important contamination sources (BERSISA et al. 2019)). Another important consideration is that most abattoirs are located on the side of the road, where they are subjected to wind and vehicle-generated dust, which might contaminate them with the organisms prevalent there. The samples used, unclean methods of transportation, handling, and processing, an unhygienic atmosphere, and practices such as employing dirty cutting boards, knives, or utensils may

all account for the variation in the overall bacterial counts. Our results are in line with the results of ADEBOWALE et al. (2010), who reported that such variation in number might be due to cutting, cleaning, and storing practices. This study determined a possible variety of pathogens on knives and cutting boards. This is because of the contamination of meat with pathogens or contaminated water or previous persistence of the pathogens on the surface of other tools used in butcher shops. The same finding was reported by GURMU and GEBRETINSAE (2013). This study aimed to isolate bacterial pathogens, particularly *Salmonella* spp., *Shigella* spp., *E. coli, S. aureus*, and *S. epidermidis*. Our results align with KUMAR et al. (2014), who isolated the same pathogens from meat.

Conclusion

In summary, this study aimed to isolate bacterial pathogens, particularly *Salmonella* spp., *Shigella* spp., *E. coli, S. aureus,* and *S. epidermidis* using different kinds of culture media. From different local butcher shops, all locations showed the potential existence of pathogenic bacteria. The most isolated pathogen in all different shops was *S. aureus* (29%), and *Salmonella* spp. was the least (10%) among the pathogens isolated from all the samples of all the shops. The increased frequency of these pathogens in meat shows the appallingly unhygienic and unsanitary techniques used in the slaughterhouse, during transportation to butcher shops, and during processing at the butcher shops. Advanced techniques such as molecular identification of all the isolated strain-based 16S rRNA genes are recommended to know the exact taxonomic position in addition to antimicrobial susceptibility testing.

Accepted for print 7.03.2023

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