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Efficacy of Cleaning Agents against Bacterial Isolates from Raw Meat Sold in Market Places in Lahore

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Abstract:
Foodborne diseases are caused by the ingestion of foodstuffs contaminated with microorganisms or chemicals and are considered a growing public health problem worldwide. Contaminated meat is one of the main sources of food-borne illnesses and death caused by agents that enter the body through ingestion. The present study was conducted to evaluate the effect of cleaning agents on bacteria isolated from raw meat sold in market places in Lahore. The bacterial contaminants were isolated and identified using specific culture techniques and the effect of dettol, lemon juice, vinegar and safeguard on bacterial isolates was determined. The predominant bacterial pathogen isolates were *Escherichia coli* 25(50%) followed by Salmonella spp. 15(30%), *Staphylococcus aureus* 6(12%), and Pseudomonas spp. 4(8%). Among the cleaning agents, lemon juice, vinegar, safeguard, and dettol were effective in killing and or reducing the bacteria attached to the meat. Lemon juice was more effective against bacteria than other agents. The raw meat is heavily contaminated with the high incidence of bacterial pathogens, and different pathogens may acquire resistance to different cleaning agents. Therefore, there is an urgent need to minimize the contamination of raw meat sold in market places by the implementation of necessary measures.
Keywords: Foodborne diseases, meat, cleaning agents, bacterial pathogens.

INTRODUCTION

Meat is animal flesh derived from mammalian species that is used as a food for human consumption. Its high nutritive value having both essential macro and micronutrients makes it an important part of balanced diet for most people (Steele and Galton, 1967). Microbial contamination of meat leads to spoilage, resulting in economic losses (Komba *et al.*, 2012). Typically, the meat of the healthy animal is sterile; however, contamination may occur during the various stages of slaughter, preparation, and transportation (Ercolini *et al.*, 2006). A variety of microbes can contaminate meat although different species may become dominant depending on factors that include pH, oxygen, water availability, and storage temperature (Wiegand *et al.*, 2007). The contaminated meat and meat products readily cause a variety of biological, chemical, physical, and particularly microbial food hazards (Kim *et al.*, 2016). The extent and composition of microbial flora reflect the standard hygiene of meat (Blaser, 1997).

The contaminants may also be present due to diseased animals, unhygienic environments (polluted water, air, etc.), unhygienic butchers habits/processing methods, faulty slaughtering procedures, post-slaughter handling, and storage, etc. (Mawia *et al.*, 2012). An additional source of cross-contamination exists in the slaughtering process, such as tools, equipment, human contacts, and carcass to carcass contact (Huffman, 2002).

Unfortunately, meat is a suitable medium for the growth of different microorganisms (WHO, 2007). The presence of microbial contamination in food can reduce the shelf life of food and promote foodborne illness. Foodborne pathogens originating from the animal during slaughter such as *Salmonella spp.* and *Escherichia coli, Campylobacter,* and *Staphylococcus aureus* (Dhama *et al.,* 2013), contaminate the carcasses and spread to cut or raw meat intended for further processing causing a major public health problem. Other important foodborne pathogenic bacteria include *Listeria monocytogenes*, *Clostridium perfringens, Yersinia enterocolitica, Bacillus cereus, Escherichia coli,* and *Vibrio parahaemolyticus* (Finstand *et al.,* 2012). Drug-resistant bacteria can and do travel on meat (Maripandi and Al-Salamah, 2010).

The most commonly used cleaning agents in food applications are chlorine gas, sodium or calcium hypochlorite, and organic chlorine (Sodium Dichloroisocyanurate) (Yedeme *et al.*, 2017). Potential benefits of clean meat include sustainability, environmental friendliness, animal welfare, food safety, and novel foods (Cassiday, 2018). There is an increasing interest in applying natural antimicrobial compounds in the food industry. Consumers are increasingly avoiding the consumption of foods treated with chemicals. Natural alternatives are required to achieve a high level of safety concerning foodborne pathogenic microorganisms (Rauha *et al.*, 2000). The natural sanitizers, such as organic acids, have been investigated because of their bactericidal activity (Uyttendaele *et al.*, 2004).

The comprehensive knowledge of the current status of meat shops, including the bacteriological analysis of food and environmental samples, risk assessment, and handler training could improve the microbiological quality of meat sold at meat shops. Microbiological analysis at the verification step helps to determine the impact of improvement actions. Besides, educational campaigns targeting food workers and consumers may play an important role in the prevention of foodborne illness (Phang and Bruhn, 2011).

The present study aimed to perform a comprehensive evaluation of meat shops, including risk quantification, determination of the bacteriological quality in raw white meat samples, and evaluate the effect of cleaning agents on bacterial isolates from meat samples.

MATERIALS AND METHODS

Sample collection

A total of 50 specimens of white meat (chicken) were collected from various meat shops of Harbanspura pull Lahore. About 100 grams of meat samples were collected in clean, dry, and sterile polythene bags and transported to the laboratory for microbiological analysis within one hour or refrigerated at 4°C till further analysis and processed no later than 96 hours after purchase.

Primary culture

Samples were centrifuged at 6000 rpm for 5 minutes after the sediments settled into the bottom of tubes and supernatant was discarded. Primary sediments obtained by centrifugation of meat were cultured on Blood agar, MacConkey agar, Nutrient agar, and CLED agar by spread out technique. Then these culture plates were incubated at 37°C for 24 hours.

Purification of Bacterial Isolates

Bacterial colonies having different morphology were selected for purification by multiple streaking (Iqbal *et al.*, 2015). The bacterial colonies with different morphological characteristics were picked by a loop from primary culture plates and cultured on Blood agar, MacConkey agar, and Nutrient agar plates. The pure cultured plates were labeled and incubated at 37°C for 24 hours.

Identification of bacteria

To identify unknown pure bacterial culture on Petri plates, grams staining, colony morphology, and biochemical tests following Bergey's Manual of Determinative Bacteriology were performed (Bergey *et al.*, 1994).

Serial dilution method

Five clean test tubes were taken. Pipette out known volume (usually 1ml) of cleaning agent and place it into a known volume of distilled water (usually 9ml), this produces 10ml of dilute solution. This dilute solution has 1ml of extract /10ml of solution producing 10 folds of dilution i.e. the amount of cleaning agent in each ml of diluted solution is 0.1ml. This process can be repeated 4 times to make successive dilutions of 0.1ml, 0.01ml, 0.001ml, and 0.0001ml. Four cleaning agents, namely Dettol, safeguard, vinegar, and lemon juice (Figure 1); and four pathogenic bacteria *E. coli*, *Salmonella*, *Staphylococcus,* and *Pseudomonas* were used in this experiment. For each test, 100ml of Luria broth was inoculated with the few colonies of a pathogenic bacterium and incubated at 37°C for 24 hours on the rotatory shaker at 120rpm. After incubation, 1ml of broth culture was spread uniformly on a nutrient agar plate with a sterile glass spreader. The plate was air-dried for few minutes. Sterile filter paper discs were soaked with 10 fold dilutions of different cleaning agents. Then the discs were placed on inoculated nutrient agar plates (Figure 2) which were incubated at 37°C for 24 hours. After incubation, clear zones around the discs were measured and recorded (Iqbal *et al.*, 2016).

Fig. 1. Cleaning Agents used in the experiment.

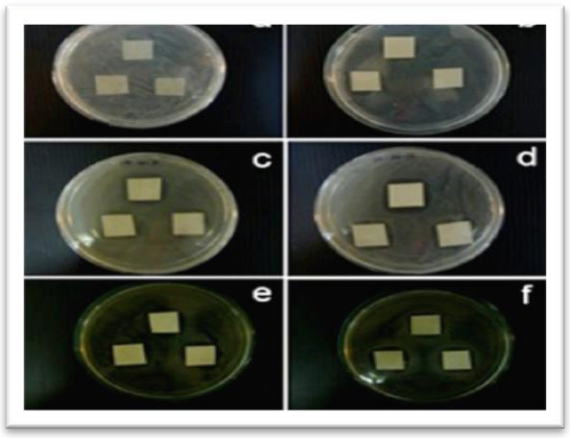

Fig. 2. Application of cleaning agents on agar plates.

Optical density of bacteria

To investigate the effect of different dilutions of cleaning agents on bacterial growth, the optical density of *E.coli*, *Salmonella*, *S. aureus,* and *Pseudomonas* growth in culture was measured. Growth curves were measured on each instrument by determining the optical density at 600nm (OD600) (Bernardez and de Andrade Lima, 2015). The optical density of the control group was 0.

RESULTS

Biochemical identification of bacterial isolates

All of the purified bacterial isolates (n=50) were identified based on culture characters, microscopic morphology with gram's reaction (as shown in table 1), and biochemical profiles (as shown in table 2).

Prevalence of bacteria

Out of biochemically identified bacterial isolates (n=50), the highest number was of *E.coli* 25(50%) followed by *Salmonella spp.* 15 (30%), *Staphylococcus aureus* 6 (12%), and *Pseudomonas species* 4 (8%) (Figure 3).

Table 1. Microscopic and Colonial characteristics of pathogenic bacteria of meat.

Sr. No.	Bacterial species	Colony characteristics			Morphological characteristics		
		Color on agar	Color on MacConkey Agar	Color on Blood Agar	Gram Staining	Motility test	Oxygen Requirement Test
1	*E.coli*	Opaque large yellow colonies and non-mucoid colony elevation	Pink to rose-red colonies may be surrounded by a zone of precipitated bile.	Slightly convex, grey	-Ve rods	Motile	Aerobe or facultative anaerobe
2	*Salmonella*	Translucent, opaque, smooth colonies	Pale, colorless smooth, transparent raised colonies	Red colonies some With black centers	Grams –ve short rod-shaped singly arrange	Motile	Aerobe
3	*Staphylococcus aureus*	Uniform opaque and deep yellow colonies	No growth to sight growth (pale pink)	Yellow to cream or white colonies	+Ve cocci	Non-motile	Facultative anaerobe
4	*Pseudomonas*	Pale blue-green with irregular edges	Colorless to pink.	Slightly opaque colony	-Ve rods	Motile	Aerobe

Table 2. Biochemical identification of meat pathogens.

Biochemical test		*E.coli*	*S. aureus*	*Salmonella*	*Pseudomonas*
Oxidase test		_	_	+	+
Catalase Test		+	+	+	+
Indole Production Test		+	_	_	_
Methyl Red Test		+	+	+	_
Vogues Proskaur Test		_	+	_	_
Lactose Fermentation Test		+	+	_	
Mannitol Salt Agar		+	+	+	+
Citrate Utilization Test		_	+	+/_	+
Eosin Methylene Blue		+	_	_	
Urease Production Test			_	_	+
Triple Sugar Iron Test	Slant	A	K	K	K
	Butt	A	A	A	K
	Gas	+	_	+	_
	H2S	_	_	+/_	_

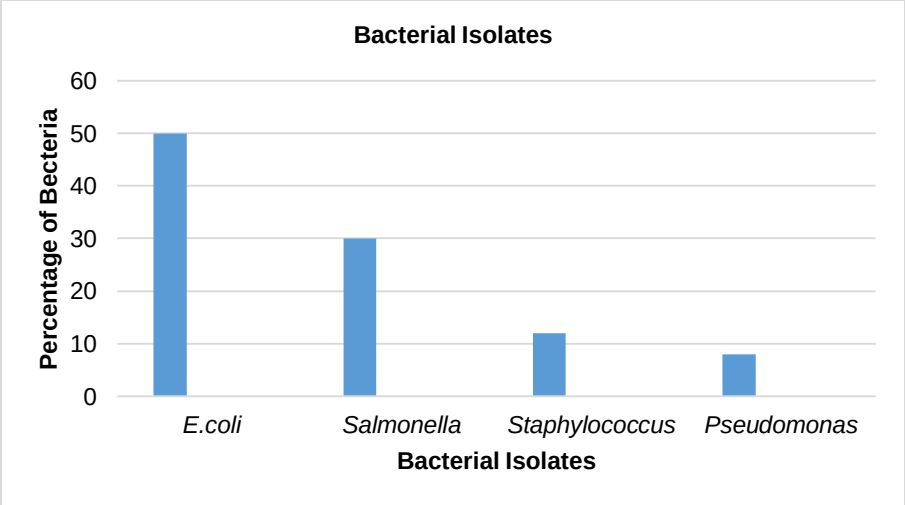

Fig. 3. Prevalence of bacteria in meat samples.

Optical Density of Organism

The data plotted for all the organisms showed overall similarity in the shapes of the curves. There were some initial shoulders before the exponential phase of death depending on used dilution of Dettol and other cleaning agents and the species of organisms considered. For each of the organisms, there was little or no decline in the number of bacteria after 24 hours of exposure to cleaning agents at 0.1ml, 0.01ml, 0.001ml, and 0.0001ml concentration. The loss of bacteria was more at 0.1ml than other dilutions. However, there was a rapid decline in bacterial growth after 24 hours of treatment. Higher the number of bacteria, the higher the optical density of the culture. While optical density is inversely proportional to dilution of

solution more diluted the solution lower the optical density of the culture.

Dettol showed the highest optical densities at a smaller concentration which is 0.1ml and the lowest optical density at 0.0001ml concentration. Moderate optical density was observed at 0.001ml concentration (Table 3).

The lemon juice showed the highest optical density at 0.1ml concentration and the lowest optical density at 0.0001ml concentration because more concentrated solutions have more optical densities (Table 4).

The growth of all strains examined was inhibited by 0.0001ml concentration of vinegar and optical density was low at this concentration (Table 5). The pH of agar containing 0.1% acetic acid was 5.1. The growth of the tested strains was not inhibited in the culture medium at the same pH prepared using hydrochloric acid. Vinegar act as bactericidal.

Similarly safeguard showed high optical density at 0.1ml concentration and low at 0.0001ml dilution (Table 6).

Table 3. Optical density measurement of bacterial culture against Dettol.

Bacterial isolates	Concentration of Dettol (ml)			
	0.1	0.01	0.001	0.0001
E.coli	2.5	1.9	0.4	0.3
Salmonella	2.4	1.7	0.3	0.2
S. aureus	2.1	1.6	0.2	0.1
Pseudomonas	2.0	1.4	0.3	0.2

Table 4. Optical density measurements of bacterial culture against safeguard.

Bacterial Isolates	Concentration of Lemon juice (ml)			
	0.1	0.01	0.001	0.0001
E.coli	3.2	2.0	0.7	0.4
Salmonella	3.1	1.9	0.6	0.4
S. aureus	3.0	1.8	0.5	0.3
Pseudomonas	2.9	1.7	0.4	0.2

Table 5. Optical density measurement of bacterial culture against vinegar.

Bacterial Isolates	Concentration of vinegar (ml)			
	0.1	0.01	0.001	0.0001
E.coli	2.4	1.4	0.8	0.4
Salmonella	2.3	1.2	0.6	0.3
S. aureus	2.0	0.9	0.3	0.2
Pseudomonas	1.9	0.6	0.1	0.1

Table 6. Optical density measurement of bacterial culture against lemon juice.

Bacterial Isolates	Concentration of Safeguard (ml)			
	0.1	0.01	0.001	0.0001
E.coli	1.9	0.6	0.3	0.3
Salmonella	1.4	0.8	0.4	0.3
S. aureus	1.2	0.7	0.3	0.2
Pseudomonas	1.1	0.5	0.2	0.1

The results showed that different types of microorganisms vary in their response to different types of cleaning agents. Vinegar was the least effective against all the pathogens under study. None of the four pathogens was sensitive to vinegar. On the other hand, dettol

was found highly effective against all pathogens. Lemon juice was also highly effective for all the pathogens. All four pathogens were sensitive to dettol and lemon juice at different concentrations. Antibacterial effect of Dettol and lemon juice was better against *S. aureus* and *E. coli* than against *Salmonella* and *Pseudomonas.* Safeguard also showed antibacterial activity against the above four types of bacteria.

DISCUSSION

Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs (Fratamico PM *et al.*, 2005). Changes in eating habits, mass catering complex, and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors. Contaminated raw meat is one of the main sources of food-borne illness (Bhandare *et al.*, 2007).

Recent increase in the consumption of meat and its products arises from reasons including high protein contents, vitamins, minerals, and lipids. The meat was being chopped on dirty wooden logs (tree trunks), which are rarely washed and dried in the sun. The butchers are not accustomed to wear gloves and the majority of today`s diseases are foodborne arising due to contamination by bacteria. Most of us buy meat from local slaughter shops which pose a high risk of contamination. Larger meat shops have modern cutting and processing machines that are cleaner, but they are mostly for export purposes only (Bhandare *et al.,*, 2007).

The contamination of meat is the main source for the spread of foodborne diseases. Therefore, the detection of appropriate concentrations of antimicrobial agents for the disinfection of contamination is of great practical value. The main objective of the present study was to evaluate suitable concentrations of cleaning agents to inhibit and eliminate the growth of bacteria to avoid contamination and the spreading of diseases. At the right concentration, cleaning agents such as Dettol, safeguard, lemon juice, and vinegar are used to kill bacteria and microbes. However, the bacteria can survive and become resistant to treatment if lower levels are used.

Resistance against antibiotics by pathogenic bacteria is a major concern in antimicrobial therapy for both humans and animals (Iqbal and Ashraf, 2018; Shahzad *et al.*, 2017). Serious concerns about bacterial drug resistance from nosocomial, community-acquired, and food-borne pathogens have been growing for several years and have been raised at both national and international levels. The results of this experiment indicate that different pathogens acquired resistance to different cleaning agents. The results also suggest that the antibacterial effects of cleaning agents are not only dependent on the types of cleaning agents but also on their concentrations. Similar results were found in a previous study (Shaker *et al.*, 1986). They demonstrated that many biocides are bactericidal or bacteriostatic at low concentrations for nonsporulating bacteria, but high concentrations may be necessary to achieve a sporicidal effect. By contrast, even at high concentrations dettol, lemon juice, vinegar, and safeguard lack sporicidal effect.

In the present experiment, dettol was effective against all the bacteria at concentration of 0.1ml and showed no efficacy at 0.00001ml concentration. Similarly, vinegar, lemon juice, and safeguard were more effective at 0.1ml concentration, moderate effectiveness at 0.01ml concentration, and show no effectiveness at 0.00001ml concentration. This is in agreement with (Milhaud and Balassa, 1973), who reported that the development of resistance during sporulation to cleaning agents was an early event but depend to some extent on the concentration of the cleaning agent used.

In our present study, the predominant bacterial pathogen isolated was *Escherichia coli*

(50 %). It is quite similar to the previous study (Stephan *et al.*, 2004). In our study, the percentage of *Salmonella* isolates was 30%. *Salmonella spp*. remains amongst the most important food-borne pathogens worldwide. Outbreaks of Salmonella have been linked to a wide range of foods including poultry, eggs, beef, fish, dairy products, and chocolate (Izat *et al.*, 1990). *S. aureus* was found to be predominant after *E.coli*. A previous study demonstrated the occurrence of *S. aureus* in meat (Wu *et al.*, 2018). Our present study is quite contrasting to that of the previous study where the percentage of *Pseudomonas* isolates was only 8%. Species of the genus Pseudomonas are recognized as major food spoilers and the capability to determine spoilage can be species- as well as strain-dependent (Stellato *et al.*, 2017).

CONCLUSION

Since food safety is a major concern to the food industry and the consumers, research is ongoing constantly to find more effective methods to reduce or kill foodborne bacterial pathogens. The present study reveals the cleaning agent's action pattern against bacteria isolated from meat shops which are heavily contaminated with bacterial pathogens. This states the role of raw food as a reservoir of bacteria that can be transferred to humans thereby causing gastrointestinal disorders and foodborne illness which can be life-threatening. Basic hygienic practices must be incorporated in abattoirs and retail meat outlets to ensure food safety. Training should be given to meat handlers and butchers regarding food safety practices and proper inspection procedures should be strictly adhered to minimize the contamination of raw meat and meat products sold in market places. Among all cleaning agents, lemon juice was more effective against bacteria than other agents. Research is now underway to determine the efficacy of cleaning agents on other pathogenic and spoilage bacteria on chicken and other meats.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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