

Hygienic and Sanitary Condition of Environment and Meat Surfaces in the Restaurant of Sohag University Hospital

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Abstract

The microbiological quality of red meat produced from most of the food-processing plants in Egypt has always been questionable. This study aimed to examine the bacteriological quality of a restaurant in the Sohag University hospital environment (air, water; worker's hands, worker's clothes, and knives) beside the meat surfaces. Bacteriological examination was performed for air, water, worker's hands, worker's clothes, and knives, in addition to the meat surfaces. Mean total bacterial count; TBC, total *coliform* count; TCC, total fecal *coliform* count; TFCC, total *Escherichia coli* count, and total *Staphylococcus aureus* were carried out. The obtained results revealed that the TBC, TCC, TFCC, and total *E. coli* counts were higher than the recommended standard for sanitary practices. In addition, we observed that worker's clothes contain more bacterial count than the hands and knives. The knives' swabs contained less bacterial burden but still higher than the recommended guidelines. In addition, 9 bacterial isolates were consistently isolated during this study including; *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Enterobacter* spp, *Klebsiella* spp., *Proteus* spp, *Citrobacter* spp, and *Serratia* spp. with varying percentages of frequency across the sampling points. Whereas, none of *Salmonella* spp. were isolated. In conclusion, the presence of pathogenic microorganisms in this study is of special concern and meat hygienists should be fortified to review the processes involved in the environment surrounding the meat as well as meat processing of university restaurants in Egypt.

Keywords:

Bacteriological Examination, Restaurant Environment, Meat Surface Contamination.

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Introduction

The hygienic state of the University hospital restaurant in Sohag was evaluated from the bacteriological point of view. The continuous drive to increase the hygiene standards carries many challenges for meat hygienists especially in developing countries (Adesokan and Raji, 2014). Preventing foodborne illnesses remains a major public health concern. All over the world, millions of people get sick from the food they eat (world health organization; WHO, 2015). Unsafe food harboring pathogens causes over 200 diseases in the world (WHO, 2020). Most of these illnesses can be prevented. The restaurant's environment is including air, walls, floors, workers' hands and clothes, contact surfaces, water, soils, and used instruments. These environments may represent the main sources of meat contamination (McLaughlin and Mineau, 1995; Sinha, 1997; Bridges et al., 2000; Boadi and Kuitunen, 2003; Amisu et al., 2003; and CDC, 2019). Pathogenic microorganisms detected in animal carcasses or shed in animal wastes may be a considerable source of hazards with many pathogens including *Salmonella*, *Escherichia coli* 0157:H7, *Yersinia enterocolitica*, *Campylobacter*, *Cryptosporidium parvum*, and *Giardia lamblia* (Ferragut et al., 2015).

Meat and meat by products were contaminated by microorganisms including airborne bacteria and molds is a major commercial problem in meat manufacturing (Rawat, 2015). Unhygienic environmental conditions in food processing plants can arise due to suspended particles in the air (Oliveira et al., 2020). These particles are microscopic, carrying microbes, and are suspended in the air as an aerosol (Fernandez et al., 2019). Airborne impurities are considered biological and known as bio-aerosols, and include bacteria, viruses, fungi, and pollen grains (Lou et al., 2021). These particles are existing in the air as solid (dust) or as a liquid. Some time ago it was thought that

food products get contaminated when they encountered unclean surfaces but now it is known that additional product contamination arises from contact with airborne pathogens (Kornacki, 2014; and Bintsis, 2017). Tap water used for cleaning, and washing may be a significant source of contaminating meat and its products (WHO, 1970; Hegazi, 1995; and Milios et al., 2014). Moreover, meat contact surfaces as worker's hands, worker's clothes, and knives used in the food processing areas carry many pathogens, which transferred directly to meat (Chan and Mowad 1998; Smith, 2000; and Levin and Warshaw, 2008). Therefore, meat processing plants should equip workers with the correct kinds of utensils and basic equipment. Such utensils and tools must be subjected to simple routine examination and maintenance to be carried out by the persons in charge on a regular basis (Schmidt, 2019). This does not include the checking of more sophisticated equipment, which must be undertaken by specialized technicians usually obtained from the supplier companies.

Several species of pathogenic bacteria and fungi have been isolated from food-processing plant's environments such as *Staphylococcus*, *E. coli*, *Streptococcus*, *Salmonella*, *Aspergillus*, *Mucor*, *Saccharomyces* and *Penicillium* species (Amisu et al., 20003). These pathogens might threaten public health by contaminating the meat (Meadows, 1995, Gauri, 2004, Raheem and Morenikeji, 2008). Pathogenic airborne and spoilage microorganisms can be presented to meat in several pathways. It is known that contamination can occur at several points during transportation from the abattoir, processing, and storage.

Although coliforms were easy to detect, their association with fecal contamination was questionable because some coliforms are found naturally in environmental samples (Caplenas et al., 1984). This led to the introduction of the

fecal coliforms as an indicator of contamination. Fecal coliform, first defined based on the works of Eijkman (Eijkman, 1904) is a subset of total coliforms that grows and ferments lactose at elevated incubation temperature, hence also referred to as thermo-tolerant coliforms. Fecal coliform analyses are done at 45.5°C for food testing, except for water, which use 44.5°C (Neufeld, 1984; and American Public Health Association (APHA), 1998). The Most Probable Number (MPN) method is a statistical, multi-step assay consisting of presumptive, confirmed and completed phases. Feng et al., (1995) indicated that presence of *E. coli* in food or water became accepted as indicative of recent fecal contamination and the possible presence of some other pathogens as *Citrobacter*, *Klebsiella* and *Enterobacter* that can also ferment lactose and are similar to *E. coli* in phenotypic characteristics, so that they are not easily distinguished. The term "coliform" was coined to describe this group of enteric bacteria. Coliform is not a taxonomic classification but rather a working definition used to describe a group of Gram-negative, facultative anaerobic rod-shaped bacteria that ferments lactose to produce acid and gas within 48 h at 35°C. In 1914, the U.S. Public Health Service adopted the enumeration of coliforms as a more convenient standard of sanitary significance.

Consequently, this work was performed to investigate the degree of bacterial contamination of environmental components of the restaurant of Sohag University Hospital and meat surface.

Materials and methods

1. Sampling:

The environmental samples were collected from the restaurant of Sohag University Hospital. Twenty-eight samples were collected from each item including air, water, worker's hands, worker's clothes, and

knives, beside the meat surfaces as described below:

1.1. Workers' hands, clothes, and knives: Twenty-eight swabs were collected from the hands' palms, fingers and nails, 20 cm² template was used to mark the area of sampling. Pre-moistened swabs with peptone water 0.1% were used to swab the marked area (AOAC Official Methods of Analysis, 2019). The collected swabs were dipped in sterile screw-capped bottles containing 10 ml peptone water 0.1% (Vuia-Riser et al., 2018).

1.2. Air: Twenty-eight air samples were collected using liquid impinger (Sigma Aldrich, USA) at the mid-day during working hours (Santl-Temkiv et al., 2017). Sterile phosphate-buffered saline (25 ml) was used for the collection of suspended dust particles. The liquid impinger was adjusted at a rate of 5 L/min. During sampling, the liquid impinger was moved all around the processing area of the restaurant to trap the finely suspended dust particles. The suspension in the liquid impinger was thoroughly shaken in order to obtain a homogenous distribution of its bacterial content.

1.3. Water samples: A total of 28 water samples of tap water used in food processing were collected in sterile transparent 500 ml capacity glass bottles (WHO, 2006). The bottles were fitted with sterile ground glass stoppers.

All samples were sent to the laboratory in an icebox within the minimum delay for further bacteriological examination.

2. Bacteriological Examination

2.1. Total colony count (TCC), total fecal coliform count (TFCC), total *E. coli* count, and total *Staph. aureus* counts were performed. Isolation and identification of pathogenic bacteria were conducted according to previous methods (Cruickshank et al., 1980; American Public

Health Association (APHA), 2005; Paul and Janet, 2008; WHO, 2010; and AOAC, 2019).

2.2. Plate count method was used to enumerate the presence of aerobic plate count, coliform, *E. coli* and *S. aureus* (Cruickshank et al., 1980). Plate count agar (PCA; Merck, Germany), violet red bile dextrose agar (VRBD; Merck, Germany) and eosin methylene blue agar (EMB; Merck, Germany), and Mannitol salt agar (HiMedia Laboratories, LLC, India) were used respectively. Ten-fold serial dilutions were conducted on the thoroughly homogenized samples. Plating 0.1 mL aliquot from each dilution on the specified media. The plates were then incubated aerobically at 37°C for 24 h. The countable plates were selected where the colonies were counted and recorded.

2.3. Ten tube Most Probable Number (MPN) coliform test - Presumptive and Confirmed procedures. The MPN method confirmed and completed phases. In the assay, serial dilutions of a sample were inoculated into broth media. Analysts scored the number of positive tubes (acid and gas) as indication of lactose fermentation. With 10 mL of undiluted of each were inoculated into 10 tubes of 2x Lauryl tryptose (LST) broth (10 mL of medium). Incubate tubes at 35°C. Then tubes were examined at 24 ± 2 h for growth and gas formation. If they were negative at 24 h, re-incubated for an additional 24 h and examined again for gas. Perform a confirmed test on all presumptive positive (gassing) tubes as follow:

2.4. MPN - Confirmed test for coliforms: From each gassing LST or lactose broth tube, transfer a loopful of suspension to a tube of BGLB broth. Incubate BGLB tubes at 35°C ± 0.5°C and examine for gas production at 48 ± 3 h. and record. Calculate MPN using 10 tube MPN Table, Standard Methods for the Examination of Water and Wastewater (American Public Health Association. 1998).

2.5. MPN - Confirmed test for fecal coliforms and *E. coli*: From each gassing LST or Lactose broth tube from the Presumptive test, a loopful of each suspension were transferred to a tube of EC broth, incubate for 24 ± 2 h at 44.5°C and examined for gas production. If negative, re-incubated and examined again at 48 ± 2 h. The results of this test were used to calculate fecal coliform MPN.

2.6. MPN - Completed test for *E. coli*: each gassing EC tube was gently agitated. A loopful of broth was streak on Levine's eosin-methylene blue (L-EMB) agar and incubated for 18-24 h at 35°C ± 0.5°C. Examine plates for suspicious *E. coli* colonies (dark centered and flat, with metallic sheen. Transfer up to 5 suspicious colonies from each L-EMB plate to Plate count agar (PCA) slants, incubate them for 18-24 h at 35°C ± 0.5°C and use for further testing.

2.7. Bacterial colonies were further identified based on Bergey's Manual of Determinative Bacteriology (Williams, 2000) and Gram staining, Mannitol fermentation, hemolytic activities. Presumptive *E. coli* colonies detected on EMB agar were then subjected to standard biochemical tests (indole, methyl red, Voges-Proskauer, and citrate tests, urease, oxidase, and growth on triple sugar iron agar (da Silva et al., 2013).

Statistical analyses

Statistical analyses were conducted by GraphPad prism software (Version: 8.0.1.244). Correlation analyses for environmental variables (air, tap water, worker's hand clothing, and knives' swab) set against raw meat surface were performed by using Pearson's correlation. Correlation coefficient [r] is significant at $P < 0.05$.

Results and Discussion

The production of food with high quality and safe for human consumption is the main target for all meat hygienists. Unfortunately, the bacteriological load of meat, air, and tap water used in the food-processing plants was higher than the recommended standard for sanitary practices. The mean total bacterial count of exposed meat surface was 4.5×10^5 CFU/cm² (Table 1). This bacterial burden is higher than those previously recorded (Lee and Fung, 1986; and Fliss et al., 1991). The high bacterial count could be due to many reasons such as the bad handling of carcasses during evisceration and meat transportation from the abattoirs to the consumers (Roberts, 1980; and Gauri, 2004). Concerning the total colony count in air and tap water, results revealed that the mean count was 8.4×10^3 and 8.2×10^2 , respectively (Table 1). These results

indicate that both air and water may be considered vehicles for meat contamination (Raheem and Morenikeji, 2008). The bacterial isolates from air samples were *Staph. epidermidis* (27.3%), *Staph aureus* (24.2%), *Klebsiella* species (12.1%), *Citrobacter* spp. (9.1%), and *Serratia* spp. (9.1%). *E. coli* was representing 6.2% of the isolates. A contamination of beef products processed in abattoirs could be contributed to various factors especially during processing and manipulations procedures such as skinning, evisceration, storage, and distribution at slaughterhouses and retail establishments (Doxon et al., 1991; and Milios et al., 2014). The food-processing hall's air may become major sources of meat contamination by these pathogenic and potentially pathogenic bacteria (Bintsis, 2017). Fortunately, in this study, most of the bacterial colonies isolated from the meat surfaces were non-pathogenic.

Table 1. The logarithm₁₀ of mean total bacterial, coliforms, fecal coliforms, *E. coli* and *staph. aureus* counts for samples collected from raw meat surface, air, water, worker's hands, clothes, and knives in the restaurant of Sohag University Hospital.

Sample	Range	TBC	TCC (MPN)	TFCC (MPN)	Total <i>E. coli i.</i> count (MPN)	Total <i>Staph. aureus</i>
Meat surface (CFU/cm ²)	Minimum	2.84	1.08	0.00	0.00	1.58
	Maximum	9.38	4.46	2.79	1.91	3.83
	Mean	5.65	2.46	2.16	1.00	2.41
Air (CFU/m ²)	Minimum	2.47	0.48	0.00	0.48	2.12
	Maximum	5.72	3.18	2.42	2.00	3.32
	Mean	3.92	2.31	2.09	1.48	2.71
Tap water (CFU/m ²)	Minimum	5.45	3.47	3.48	3.47	4.62
	Maximum	7.41	3.46	3.45	3.46	6.58
	Mean	5.91	3.46	3.45	3.47	4.76
Worker's hands (CFU/cm ²)	Minimum	1.83	0.48	0.48	0.48	1.86
	Maximum	2.71	2.55	1.80	1.15	2.36
	Mean	1.89	1.68	1.45	0.90	2.38
Worker's clothes (CFU/cm ²)	Minimum	1.99	0.48	0.48	0.48	1.73
	Maximum	4.49	2.99	1.93	1.34	3.42
	Mean	2.45	2.08	1.42	1.26	2.53
knives' (CFU/cm ²)	Minimum	1.36	0.00	0.48	0.00	1.58
	Maximum	4.79	2.63	1.95	1.43	3.25
	Mean	2.52	1.49	1.11	1.00	2.51

TBC; total bacterial count, TCC; total coliform count, TFCC; total fecal coliform count, total *E. coli* count; MPN; most probable number, CFU; colony-forming unit. *Permissible limits of TBC for fresh meat (10^6) according to the Egyptian Organization for Standardization and Quality (2005). *Permissible limits of *Enterobacteriaceae* and *staphylococci* (10^2) according to Egyptian Organization for Standardization and Quality (2005).

The mean total bacterial count (TBC), total colony count (TCC), total fecal coliform count (TFCC), total thermotolerant *E. coli* count (T. *E. coli* count) and T. *Staph. aureus* in the worker's hands/m² were 7.8×10, 4.8×10, 2.8×10, 0.8×10 and 2.4×10², respectively. Moreover, the mean TBC, TCC, TFCC, T. *E. coli* count, and T. *Staph. aureus* in the worker's clothes /m² were 2.8×10, 1.2×10², 2.6×10, 1.8×10, and 3.4×10², respectively. On the other hand, the mean TBC, TCC, TFCC, T. *E. coli* count, and T. *Staph. aureus* of the knives swabs/m² were 3.3×10², 3.1×10, 1.3×10, 1.0×10, and 3.2×10², respectively. The obtained results indicated that the contact surfaces may play a vital role in the contamination of meat

during processing (Mead, 1989; and Schlegelova et al., 2010).

The interactions between the microbial populations in the environment and those on the surface of raw meat were represented in Table 2. So, our result demonstrated that there are positive correlations between TBC in environmental samples and those on the raw meat surfaces. In addition, positive correlations were found between swabs taken from knives used in beef cutlets and beef surface in terms of TBC, TCC, Total thermotolerant *E. coli* count, and total *Staph. aureus* count (Table 2). Moreover, a positive correlation was found between worker's clothing and meat surfaces in terms of total *Staph. aureus* counts.

Table 2. Pearson's correlation coefficients [r] for total bacterial, coliforms, fecal coliforms, *E. coli*, and *staph. aureus* counts for samples collected from raw meat surface versus environmental samples in the restaurant of Sohag University Hospital.

Meat surface versus Environmental sample	TBC	TCC	TFCC (MPN)	Total <i>E. coli</i> count (MPN)	Total <i>Staph. Aureus</i> count
Air	0.86**	0.27	0.38	0.05	0.33
Water	0.79*	-0.08	0.46	0.08	0.31
Worker's hands	0.69*	0.34	0.33	0.07	0.47
Worker's clothes	0.98**	0.35	0.34	0.31	0.72*
Knives' swab	0.84**	0.74*	-0.02	0.62*	0.75*

TBC; total bacterial count, TCC; total *coliform* count, TFCC; total fecal *coliform* count, total *E. coli* count; MPN; most probable number, CFU; colony forming unit. Each value represents Pearson's correlation [r] < 0.7, strong correlation, [r] = 0.5–0.7, moderately to strong correlation, and [r] = 0.3–0.5, weak to moderate correlation. * is significant if $P < 0.05$, while ** is significant if $P < 0.01$.

Concerning the bacterial isolates, (Fig. 1) revealed 9 isolates. No *Salmonella* spp could be isolated from the meat surfaces, air, and tap water. The percentages of isolates were greatly different. *Staph. epidermidis* represent the main bacterial isolated from the meat surfaces, air, and tap water followed by *staph. aureus*. The enteropathogenic bacteria that could be isolated from the meat surfaces were *E. coli* (13.8%); *Pseudomonas aeruginosa* (10.3%); *Klebsiella* species (6.9%); *Proteus* species (6.9%) and *Citrobacter* species (3.4%). This could be attributed to the contamination of beef during processing and handling of meat during processing and

storage (Doxon et al., 1991; and Bartram et al., 2003). On the other hand, bacterial isolates of the contact surfaces of the knives, handler's hands, and clothes, Fig. 1. showed that the most prominent isolates were *Staph. epidermidis* and *staph aureus* in all examined samples followed by other enteropathogenic organisms such as *E. coli*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Proteus* species, and *Serratia* species. No *Salmonella* or *E. coli* could be isolated from these samples except in handler's clothes (Schlundt, 2002; and Othman, 2015).

Microorganisms that are implicated in foodborne diseases may contaminate meat

directly and indirectly especially from animal manure in slaughterhouses (Heredia and García, 2018). In addition, they can be transmitted to meat from the contact surfaces, utensils, and other slaughtering equipment (Yen, 2003; and Diyantoro and Wardhana, 2019). Contamination of meat surfaces constitutes a major problem in most developing countries' meat processing plants where they are considered potential sources of infection. In fact, microbial contaminations of carcasses have been repeatedly reported to play a significant role in the meat shelf life of beef (Khalafalla et al., 2016). Therefore, when all meat-processing steps are carried out within a facility specifically prepared for meat processing, sources of contamination should be much more simply and sufficiently controlled. In most developing countries, the traditional methods of meat manipulation, processing, and presentation are the main cause of poor sanitation, which

in turn leads to considerable loss of product as well as the risk of food-borne disease (Garcia, 2007; and Schmidt, 2019). Hence, fecal matter per se is a major source of contamination and could reach carcasses through direct contamination as well as indirectly by other vehicles and equipment as knives, worker's clothes, and hands (Abdalla et al., 2009). Furthermore, contaminations with pathogens such as *Salmonella*, *E. coli*, and other enteric bacteria that can reach the meat may cause severe health problems for the public (Kibret and Abera, 2012). Environmental contaminations with pathogenic bacteria remain to have a major concern for contaminating raw meat in developing countries. Raising the meat handlers' health awareness about spreading foodborne diseases in food processing plants is important in limiting outbreaks of these diseases (Adesokan and Raji, 2014).

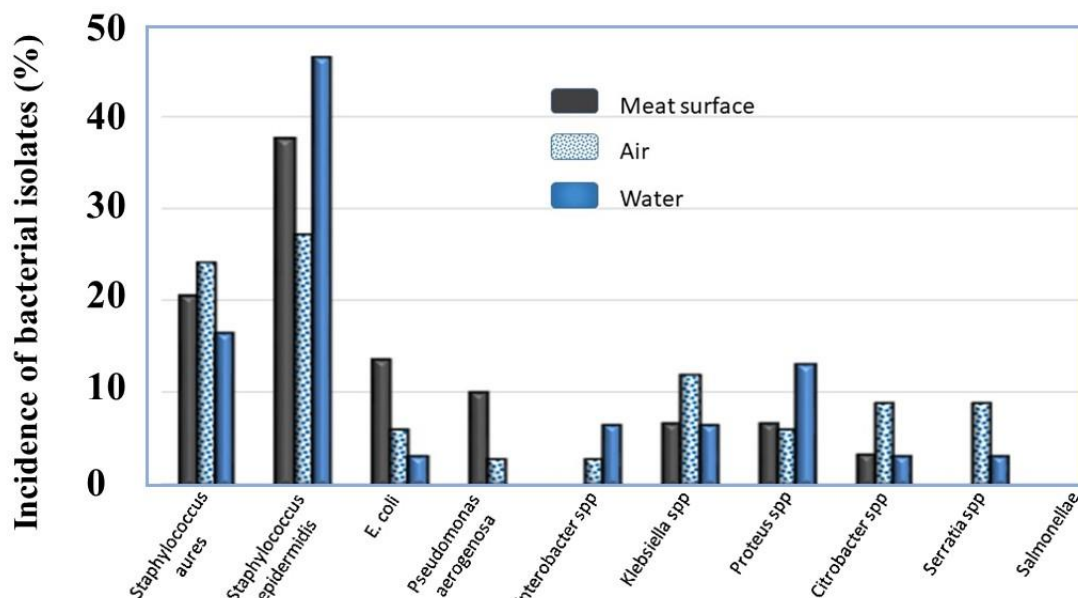


Figure 1. Incidence of bacterial isolates (%) from raw meat surface, air, and water in the restaurant of Sohag University Hospital.

Conclusion

In conclusion, contamination of air and water, and unclean surfaces of tools equipment may be a potential source of microbial continuation of meat surface. Therefore, it is worthy to enhance the health

awareness of personnel in food-processing plants about sanitary and hygienic measures and control practices that prevent microbial infections.

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Conflicts of interest

All authors disclose the absence of any type of interest conflicts.

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