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PREVALENCE OF SOME PATHOGENS IN SOME MEAT PRODUCTS AND THEIR CONTACT SURFACES IN A MEAT PRODUCT FACTORY

(With 3 Tables)

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(Received at 11/3/2008)

مدى تواجد بعض الميكروبات الضارة فى بعض منتجات اللحوم والاسطح الملامسة للغذاء فى احد مصانع اللحوم

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من خلال هذه الدراسة التى استمرت لمدة ثلاثة شهور وعلى مدى ثلاث زيارات لاحد مصانع منتجات اللحوم، حيث يطبق نظام الهاسب، تم تجميع عدد ٥٣١ عينة من كل من العمال والأسطح الملامسة للغذاء والأغذية قبل وأثناء العمل من ثلاث مناطق هى منطقة التجهيز ومنطقة التصنيع ومنطقة المنتج النهائى، وقد دلت النتائج أن المادة الخام هى المسبب الأكثر احتمالا للتلوث، حيث زاد العد البكتيرى من صفر قبل بدء العمل الى $٨,٨ \times ١٠^{٢}$ ، ٢×١٠^{٣}، ٢×١٠^{٣}، $٨,٧ \times ١٠^{٣}$ خلية/جم بالنسبة لكل من العمال والاسطح الملامسة وعينات الأغذية على التوالى فى منطقة التجهيز، بينما زاد الى ٦×١٠^{٣}، $٩,٢ \times ١٠^{٣}$، ٤×١٠^{٤} فى منطقة التصنيع. من جهة أخرى كان لكل من المعالجة بالحرارة وبرامج النظافة والتطهير القياسية تأثيرا محسوسا فى خفض العد البكتيرى الى $٢,٢ \times ١٠^{٢}$، $١,٥ \times ١٠^{٢}$ و ٢×١٠^{٢} خلية/جم فى منطقة المنتج النهائى. وقد لوحظ أنه لم يمكن عزل أى من الميكروبات محل الدراسة قبل بدء العمل بينما أمكن عزل ميكروب الليستريا مونوسيتوجينز من الماكينات فى كل من منطقتى التجهيز والتصنيع (٢ بنسبة ٣,٧% لكل منهما)، ومن اللحوم الخام (٢ بنسبة ٨,٣%) ومن منتجات اللحوم قبل التسوية (٣ بنسبة ١٢,٥%). وكان ميكروب المكور العنقودى الذهبى (ستاف اوريس) هو الميكروب الوحيد الذى أمكن عزله من أيدى العمال (٣ بنسبة ٤,٢%) بينما أمكن عزل ميكروب الأيشريشيا القولونية من الماكينات فى منطقة التجهيز (٢ بنسبة ٣,٦%) واللحوم والدواجن الخام (٣ بنسبة ١٢,٦%) من منطقتى التجهيز والتصنيع. أما ميكروبات السالمونيلا فلم يمكن عزلها وكذلك لم يمكن عزل أى من الميكروبات محل الدراسة من أى من المنتجات النهائية. وقد أوصت الدراسة بالتحقق من سلامة المواد الخام المستخدمة وزيادة عدد مرات النظافة والتطهير أثناء العمل للتقليل من خطر التلوث العرضى بالمصنع.

SUMMARY

Over a period of three months, throughout three visits to a meat product plant where HACCP system is in place, a total number of 531 workers,

surface swabs and food samples were collected pre- and during work from three areas (preparing, processing and packaging). Raw materials were the most probable cause of contamination. The aerobic plate counts increased from zero before working to $8.8x10^2$, $2.0x10^3$ and $8.7x10^3$ cfu/g or cm^2 in workers, meat contact surfaces and food samples, respectively in preparing area and to $6x10^3$, $9.2x10^3$ and $4x10^4$ cfu/g, in production area. Meanwhile the proper heat treatment and sanitary standard operating procedures significantly decreased the count to $2.2x10^2$, $1.5x10^2$ and $2x10^2$ cfu/g in the packing area. None of the tested microorganisms was isolated prior to food preparation. However, after preparation and processing *L. monocytogenes* was isolated from machines of preparation area (2, 3.7%), machines of production area (2, 3.7%), raw meat (2, 8.3%) and processed meat (3, 12.5%). *S. aureus* was the only isolated pathogen from workers hands (3, 4.2%). *E. coli* could be isolated from machines of preparation area (2, 3.6%) and raw meat and chicken (3, 12.6%) in preparation and processing areas, but *Salmonella* couldn't be isolated. None of the tested microorganisms could be isolated from any of the final products. For such plant, it's recommended to verify the quality of purchased raw material and to decrease the intervals of sanitization program to decrease the risk of cross contamination.

Key words: *Meat products, meat contact surfaces, L. monocytogenes, S. aureus, Salmonella*

INTRODUCTION

Ready to eat (RTE) foods including red meats, poultry and seafood have been documented to serve as vehicles for several bacterial pathogens and foodborne outbreaks have been associated with the consumption of contaminated RTE foods (Guerra *et al.*, 2001; Borch and Arinder, 2002; Gudbjornsdotttir *et al.*, 2004).

HACCP is becoming established and has been successful in the food industry, such as its association with reduction in pork carcass contamination (Bolton, *et al.*, 1999) and improving the control of food production in catering establishments (Martinez-Tome *et al.*, 2000).

Significantly more unsatisfactory samples were obtained from premises where no HACCP system was in place, as compared with those which had an undocumented or documented hazard analysis system in place (Gillespie *et al.*, 2000).

Commercially food production, catering and industrial food preparation must adhere to legislation regarding food safety. Analysis of the hazards were recognized through the world by WHO, FDA, FAO, European commission, and is becoming increasingly applied in developing countries (Gorman *et al.,* 2002).

L. monocytogenes can cause listeriosis in pregnant women, immuncompromized and eldery (Slutsker and Schuchat, 1999). Listeriosis has a mortality rate of 20-30% and thus represents a serious public health concern (Schuchat *et al.,* 1991). When present in the environment, *L. monocytogenes* can contaminate finished products through employee contact surfaces (such as hand tools, gloves and aprons), food contact surfaces and non food contact surfaces (Tompkin *et al.*, 1999). The prevalence of *Listeira* spp. in RTE meats have been variable ranging from 1.8% to 48% (Gibbons *et al.,* 2006). There are several reports of listeriosis associated with the consumption of RTE meats (Faber and Peterkin, 1991; Anon, 2001). Some of these epidemics resulted in mortalities, as well as large scale recalls of implicated RTE foods (Rouquete and Berche, 1996; Norrung, 2000; USDA, 2002).

Other enteric pathogens have been isolated form RTE foods including *E. coli* and *Salmonella* emphasizing the risk posed by consumption of these foods (Tsuji *et al.,* 2002; Faustini *et al.,* 2003; Lee and Middleton, 2003; Haeghebaet *et al.,* 2003). Pathogens such as *Listeria* spp., *Salmonella* spp. and *E. coli* have all been recovered from raw meats (Adesiyun, 1993). In many countries *S. aureus* is the second or third common cause of foodborne disease outbreaks behind *Salmonella* (Rosec *et al.,* 1997). *S. aureus* was the predominant microorganism found on hands of food preparators (Ryan *et al.,* 1996). Scott and Bloomfield (1990) identified the ability of *S. aureus* to cause cross-contamination for up to 24 h via the fingertips.

This study was conducted to evaluate the aerobic plate count as well as prevalence of *Listeria* spp. *Salmonella* spp., *E. coli* and *S. aureus* in raw processed and finished meat and chicken products together with the different premises, meat contact surfaces and employee at different processing stages and areas in a plant where HACCP system is implemented.

MATERIALS and METHODS

Sampling procedure:

Over a period of 3 months through three visits to a meat product plant where HACCP system is in place, 225; 72 workers and 153

surfaces swabs from workers and contact surfaces were collected pre-work directly after cleaning and disinfection. Another 306 samples of 72 workers, 153 food contact surface swabs and 81 food samples were collected during at least midway through a shift and towards the end of the working day from various processing stages. All swabbed surfaces were in direct contact with food. The plant was divided into three main areas representing raw material preparing area, processing area and packaging area. Each area was sampled for workers (gloves and aprons), surfaces [machines (breaker, grinder, mixer, cutter, filler and slicer), utensils (tables, knives, containers and cutting boards)] and food (raw meat, poultry and their products). Swabs from workers' hands and 25 cm^2 from food contact surfaces using sterile swab moisten with neutralizing buffer and 25 grams of food were collected. All samples were kept in an icebox and transported without delay to the laboratory.

Bacteriological analysis:

- Preparation of samples

On arrival to the laboratory, samples were homogenized with buffer peptone (1:10). Ten-fold serial dilutions were prepared using buffer peptone for the following analysis:

1. Aerobic plate count: using pour plate technique onto plate count agar and incubating at 35 °C for 48 h according to APHA (1992).
2. Isolation of *Salmonella*: samples were pre-enriched on buffer peptone for 24 h at 37 °C, enriched on Rappaport Vassiliadis for 24 h at 41.5 °C and tetrathionate broth at 37 °C for 24 h then plated on XLD and Brilliant green agar at 37 °C for 24 h, according to APHA (1992).
3. Isolation of *E. coli*: using lauryl sulphate broth incubated at 35° C for 24-48 h followed inoculation of loopful from positive tubes on *E. coli* broth incubated at 45.5° C for 24-48 h then plated on eosin methylene blue agar and incubated at 35° C for 24-48 h according to APHA (1992).
4. Isolation of *S. aureus*: using selective enrichment procedure, by enrichment on brain heart infusion broth at 37 °C for 24 h, then streaking on Barid Parker agar at 35° C for 48 h according to APHA (1992).
5. Isolation of *L. monocytogenes* : using *Listeria* enrichment broth at 30° C for 48h and plating on Oxford agar at 35° C for 24-48 h according to FAO (1992)

The results were statistically analysed using SPSS for Windows version 10. "SPSS Inc. Headquarters, Chicago, Illinois USA."

RESULTS

Table 1: Mean APCs and incidence of isolated microorganisms in different areas for workers samples

Area	Samples	No.		APC		Isolates No. (%)				
		B	D	B	D	B	*E. coli*	*L. monocytogenes*	*S. aureus*	*Salmonella*
Preparing area	Workers	24	24	-ve	$8.8X10^2$ **A**	-ve	-ve	-ve		-ve
	Gloves				$7.7X10^2$				1(4.2%)	
	Aprons				$1.0X10^3$					
Production area	Workers	24	24	-ve	$6.0X10^3$ **A**	-ve				-ve
	Gloves				$5.0X10^3$				1(4.2%)	
	Aprons				$7.0X10^3$					
Packing area	Workers	24	24	-ve	$2.2X10^2$ a	-ve	-ve	-ve		-ve
	Gloves				$1.4X10^2$					
	Aprons				$3.0X10^2$				1 (4.2%)	

B: before work D: during work There is significance difference between means containing the same capital and small letter

Table 2: Mean APCs and incidence of isolated microorganisms in different areas for surfaces samples

Area	Samples	No.		APC		Isolates No. (%)				
		B	D	B	D	B	*E. coli*	*L. monocytogenes*	*S. aureus*	*Salmonella*
Preparing area	Surface	54	54	-ve	$2.0X10^3$ **A**	-ve			-ve	-ve
	Machines				$2.0X10^3$					
	Breaker				$3.0X10^3$		1(1.8%)	1(1.8%)		
	Grinder				$2.0X10^3$			1(1.8%)		
	Utensils				$3.0X10^3$			-		
	Tables				$2.6X10^2$			-		
	Knives				$3.2X10^2$			-		
	Containers				$4.0X10^3$			-		
	Cutting boards				$1.5X10^3$		1(1.8%)	-		
Production area	Surface	54	54	-ve	$9.2X10^3$ **A**	-ve	-ve		-ve	-ve
	Machines				$1.3X10^4$			-		
	Mixer				$8.5X10^3$			2 (3.7%)		
	Cutter				$1.9X10^4$			-		
	Filler				$1.0X10^4$			-		
	Utensils				$4.8X10^3$			-		
	Tables				$4.0X10^3$			-		
	Knives				$5.5X10^3$					
Packing area	Surface	45	45	-ve	$1.5X10^2$ a	-ve	-ve	-ve	-ve	-ve
	Machines				$1.4X10^2$					
	slicer				$2.2X10^2$					
	Tables				$1.9X10^2$					
	Trolleys				5.0X10					
	Filler				$1.0X10^2$					
	Utensils				$2.0X10^2$					

There is significance difference between means containing the same capital and small letter

Table 3: Mean APCs and incidence of isolated microorganisms in different areas for food samples.

Area	Samples	No.		APC		Isolates No. (%)				
		B	D	B	D	B	*E. coli*	*L. monocytogenes*	*S. aureus*	*Salmonella*
Preparing area	Food	-	24	-ve	$8.7X10^3$ A	-ve				-ve
	Meat				$5.4X10^3$		-	2 (8.3%)	-	
	Poultry				$1.2X10^4$		1 (4.2%)	-	2 (8.3%)	
Production area	Food	-	24	-ve	$4.0X10^4$ A	-ve				-ve
	Meat				$2.0X10^4$		1 (4.2%)	3 (12.5%)	-	
	Poultry				$6.0X10^4$		1 (4.2%)	-	1 (4.2%)	
Packing area	Food	-	33	-ve		-ve	-ve	-ve	-ve	-ve
	Meat products				$2.0X10^2$ a					
	Poultry products				$1.5X10^2$					
					$2.5X10^2$					

There is significance difference between means containing the same capital and small letter

DISCUSSION

It was evident in this study that following a thorough cleaning and disinfection of the plant (pre-working) all food contact surfaces and workers swabs for the 3 sampled areas were proved to be uncontaminated with the aerobic bacteria (Table 1, 2 and 3), an indication that the cleaning and disinfection exercise instituted by the plant successfully eliminated potential contaminants on the workers and food contact surfaces.

Results from the APCs (Table 1) illustrate a significant increase in the bacterial load for workers swabs sampled prior to food handling, nil / hand compared to each of those sampled during preparation ($8.8x10^2$ cfu/hand) and processing ($6x10^3$ cfu/hand). That was also true when applied to the equipment swabs (including machines and utensils) as they were free from the APC prior to preparation of the raw food materials while showed $2x10^3$ cfu/cm^2 and $9.2x10^3$ after preparation and processing of the raw food, respectively (Table 2).

These results were unsurprised considering that HACCP system is established at the plant; food hygiene training of the staff, physical separation of raw food materials and unwrapped cooked RTE foods by means of separate refrigerators, equipment, utensils, serving counters, handling areas and surfaces), the use of separate staff, adequate temperature control of heat treatments and of freezers and refrigerators. In this concern, Gillespie *et al,* (2000) stated that significantly unsatisfactory or unacceptable samples were obtained from premises where no HACCP system was in place as compared with these which had an undocumented or documented hazard analysis system.

Results highlighted that raw materials were the most probable contributory factor of causing contamination of both the workers (hands and aprons) and the machines and utensils. That was proven by observing the bacterial count (Table, 3) of the raw meat and chicken ($8.7x10^3$ cfu/g). This proportionally high bacterial load could contaminate both workers and the used machines and utensils of the preparing area. The condition that emphasize the spread of bacteria in the working environment when preparing meat and chicken. This result agrees with Gorman *et al.* (2002) who observed that chicken samples as raw material showed contamination values in the upper ranges test ($>10^5$ cfu/g) in 84% of the samples. The case which is contributed to an increase in the contamination level of worker's hands from 60% to 88% at the frequency range 10^3-$<10^5$ cfu/g. That is also the case in the counter tops and draining boards before and after chicken preparation. The raw food materials in addition to their additives (spices, salt,...) spread the contaminations to the workers' hands and the machines (mixer, cutter, filler) of the production area. That is the cause of increasing the mean bacterial counts to $6x10^3$ and $9.2x10^3$ cfu/g, respectively resulting in prepared food with mean counts of $4x10^4$ cfu/g.

The effect of the proper temperature / time and the good manufacturing practice (GMP) in the plant was shown through the significant ($P<0.05$) bacterial decrease of the finished products ($2x10^2$ cfu/g) (Table 3).

Results also highlighted the significant ($P<0.05$) increase of the contamination rate of workers handling the raw food materials in both of the preparing area ($8.8x10^2$ cfu/g) and the production area ($6x10^3$ cfu/g) than those handling the final products ($2x10^2$) (Table 1). The same observation was achieved throughout comparing the contamination rate of equipment utilizing the raw materials of both preparing are ($2x10^3$) and processing one ($9.2x10^3$ cfu/cm^2) compared to those utilizing the cooked finished products ($1.5x10^2$ cfu/cm^2) ($P<0.05$) (Table 2).

Prior to food preparation, all workers' hands, machines and utensils were free of all the tested microorganisms. However, after preparation and processing *L. monocytogenes* was the most isolated microorganisms in both preparation area [(meat breaker and grinder, 2 (3.7%)] and the production area [mixer 2 (3.7%)] (Table 2). The pathogen could also be isolated from 2 (8.3%) of raw meat samples and 3 (12.5%) of processed meat products (Table 3).

The ability of foodborne microorganisms as *Salmonella* and *Listeria* spp. to become disseminated from naturally contaminated foods

(such as chicken) to various hands and food contact surfaces is well known (Gorman *et al.*, 2002).

Throughout the present study we observed that in spite of the studied plant applied restrict sanitary programs according to HACCP system *L. monocytogenes* could be isolated from various machines and utensils. The same findings were achieved by several studies which have shown that *L. monocytogenes* is capable of contaminating food processing machines with bacterial attachment and biofilm formation representing a source of potential contamination of food products (Autio *et al.* 1999). Once attached to a surface, *L. monocytogenes* appear to be more difficult to be removed (Autio *et al.*, 1999, Aase *et al.*, 2000 and Beresford *et al.*, 2001). A study led by Lunden *et al.*, 2002) showed that such equipment with poor hygienic properties design is susceptible to persistent *L. monocytogenes* contamination. It was observed that remnants on the machines grooves and surfaces were associated with *L. monocytogenes* detection. This conclusion was confirmed by a study led by Chasseignaux *et al.* (2001) (7.4%) raw pork meat plant.

In the present study, the incidence of *L. monocytogenes* contaminated the surfaces of the premises was 2.6% which is more or less in agreement with the incidence of the pathogen in meat processing plants in Nordic countries (0-15.1%) (Gudbjornsdottir *et al.*, 2004). While it was lower than the contamination levels of the equipment of a meat plant (37%) recorded by Chasseignaux *et al.* (2002) and extremely lower than that observed by Thevento *et al.* (2005) (50.9%).

The incidence of *L. monocytogenes* in raw and processed meat (prior to heat treatment) was 5 (20.8%). The pathogen couldn't be isolated from any of the final products (post heat treatment). In this concern the incidence of *L. monocytogenes* in raw meats is largely variable, from a low incidence as in our findings to high incidence which may reach to 38.9-80% as recorded by Gibbons *et al.* (2006).

It was observed through this study that *S. aureus* was the only isolated pathogen from two workers hands (4.2%) after preparation and processing the raw food materials. In this concern, Gorman *et al.* (2002) achieved the same result. They found that *S. aureus* was the predominant microorganism found on hands of food preparators following food preparation. Food preparator hands were recorded as the main factor contributing up to 39% of domestic food poisoning outbreaks (Ryan *et al.*, 1996). The pathogen could be isolated also from 3 (6.25%) of raw chicken, prepared and processed (precooked). Scott and Bloomfield (1990) identified the ability of the *S. aureus* to cause cross

contamination for up to 24 hours via fingers' tips. It's important to mention that only one (4.2%) of the workers aprons in the packing area (final product area) was contaminated by the pathogen, but none of the final products proved to harbor the pathogen. The researchers informed that it was an exceptional case as the worker doesn't belong to the final product area. Due to the over work he was accidentally called from the preparation area to support the other workers. It seems that the worker didn't follow the proper sanitary rules and thus represented a potential risk of cross contamination.

E. coli could be isolated from 2 (3.6%) of the meat breaker and the cutting boards (preparation area) and from 3 (6.25%) of raw prepared, processed and precooked meat and chicken. Gorman *et al.* (2002) reported that *E. coli* was isolated from 7 (28%) chicken samples following their preparation, four of which were found to cross contaminate one or more of the premises surfaces such as counter top and draining boards.

Salmonella has been found to survive on dry surfaces for long periods of time (Humphrey *et al.*, 1994). *Salmonella* failed to be detected in the examined raw food premises surfaces or final products. However, Gorman *et al.* (2002) reported that a small number of *Salmonella* infected chicken (8%) had the ability to cause 100% cross contamination with other sites in the preparing premises including the counter top and the dishcloth.

It was obvious in the present study that all the final cooked products were negative for all the tested pathogens although the raw food materials and some of the processing facilities were contaminated. The result which indicate the proper and efficiency of the products heat treatment (time/ temperature). On the other hand, such a plant may need to decrease the intervals between cleaning and sanitization item to increase its ability to eradicate pathogens such as *L. monocytogenes*.

From this study, it was undoubtful that the contaminated raw food material is the real cause of disseminating microorganisms and contamination of various facilities (equipments and utensils) and workers' hands. Therefore, it is recommended to verify the quality of purchased raw material and to increase frequency of cleaning and disinfecting of the premises and the hygienic practice of the workers to decrease the risk of cross contamination.

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