Detection and enumeration of *Enteric* bacteria associated with food handlers and surfaces of food manufacturing industry located in Hub city, Pakistan

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ABSTRACT

Foodborne microorganisms harbor and adheres itself to the food material and surrounding surfaces for a long time and influence the food quality and consumers health. Among these microbes the presence of *Enteric* indicator bacteria in food premises confers the indication of entero-pathogens, i.e. *E. coli, Salmonella, Shigella* and *Campylobacters* that could cause severe systemic infections in consumers. In this study, the hygienic status of confectionery and supplementary food processing facility was evaluated. A total of 10497 examinations were performed on 3499 swab samples collected from food premises and handlers for the analysis of *Enteric* indicator bacteria. From swabs, 1277 (12.2%) isolates were identified in which *Enterobacteriaceae* were found with higher frequency 604 (47.3%) followed by *Coliforms* 293 (30.8%) and *Escherichia coli* 280 (21.9%) respectively. The mean count (CFU/cm^2) was found maximum for plain surfaces (floors, walls and door), while the lowest was for equipment and machinery. Overall isolates percent prevalence was determined where *Enterobacteriaceae* were 47%, *Coliforms* 31% and *Escherichia coli* 22%. Majority of the floor surfaces were highly contaminated, where washing and sanitation practices were observed to be inappropriate. Worker hygiene status was lacking essential food safety and hygiene standards. In general, the *Enteric* bacteria were found with higher ratio, that could affect the food quality and quantity both to a greater extent with some influences on consumers health.

Keywords: *Enterobacteriaceae*; Foodborne microbes; Hygiene; Surface contamination; Food handlers; *E. coli*; Sanitation

1. INTRODUCTION

There are various types of foodborne micro-flora (pathogenic or non-pathogenic), that adheres itself to the food contents and surfaces for a long period of time. These microorganisms play vital role in food degradation, toxification and pathogenicity of consumers. This confers to affect the food quality and safety of food consumers [1,2]. Among these microorganisms, some foodborne pathogens cause serious health issues to humans, especially enteropathogenic *Entrobacteriaceae* family i.e. *Escherichia coli, Salmonella* and *Shigella* can cause severe infections [3,4]. The causative agents for foodborne diseases are the ingestion of microbial pathogens, chemicals or biotoxins produced by the microorganisms. The degree of disease can be accounted by the rate of mortality and morbidity outbreaks, considering the acute and chronic manifestations or severity that may lead to cause deaths, illnesses, health abnormalities and economic losses due to these foodborne agents [5,6].

Foodborne illnesses have been a major issue in public health for decades, and food handlers playing an important part in its transmission. Although there are various sources by which pathogens can contaminate food, multiply and cause infections in humans, but the persons who handle the food could be the possible cause of transmission. These food handlers contribute in food contamination through many ways i.e. serve as a vehicle, negligence or mishandling of food, incorrect food preparation, personal hygiene, skin, cuts, hair and mouth. More, improper sanitation of surfaces and equipments may influence the burden of foodborne microorganisms to a greater extent [5-9]. Several reports have shown that poor personal hygiene and handling of foodstuffs could lead to various illnesses. CDC identified over 400 food-related infections, in which 20% are due to food handlers [10,11]. Some more studies have reported that improper or poor handling of foods either in the manufacturing sector or homes can cause 97% of foodborne infections [12].

Foodborne diseases also affect developed countries and about one third of the world population has suffered from foodborne infections. Kaferstein and Abdussalam (1999) [27] reported that in industrialized countries about 10% of the population suffer from foodborne diseases. However, due to the broad spectrum of foodborne agents and common sharing of disease symptoms, study on many foodborne illnesses are under evaluation to understand the type and cause of illnesses. Identification of disease can be made possible by proper laboratory diagnosis for pathogens or toxins, and considering patients recent history for food consumption [13-15]. The burden of intestinal enteropathogenic bacteria in foods are affected by the GMP implementation in manufacturing industries. These microbes can be transmitted by direct contact with contaminated objects, equipments, raw materials, foods, water and fecal, or can be transmitted indirectly through surfaces, walls, air, machines, product carrying bags, buckets and trolleys. Importantly, poor hygienic status and food handlers working in production area(s) shares key roles in transmitting many intestinal enteropathogenic bacteria. Fecal-oral and human-to-human routes of transmission have direct impacts on either healthy individuals or food quality [13,16-19].

The factors or cautions that could play an important role in foodborne illnesses are the worker training, awareness of handling food and hygiene, correct techniques and implementation of quality standards in food premises [9]. To control foodborne contaminants, it is important to improve handlers practices during food manufacturing and processing, and implement GMP practices recommended by international standards. Generally, washing and sanitation of hands before handling food-stuffs, wear clean dresses and following

recommended CIP (cleaning-in-place) protocols for surfaces, machines and other equipments which have direct or indirect contact with food materials can reduce these contaminants [20-22]. The aim of this study is to evaluate the hygienic status of food handlers, machinery and food manufacturing surfaces inside production premises.

2. MATERIALS AND METHODS

2. 1. Sample collection

A total of 3499 microbiological swab samples were randomly collected and analyzed (Feb 2015 to March 2016) from handlers of food manufacturing industry (confectionery and supplementary foods) production plant, where they were in direct contact with processing foods. Other food contact surfaces selected for swab collection were production floors, walls, machines, doors, packing materials, buckets and bags (Table 1).

Table 1. Swabs samples collection frequency from food handlers and surfaces.

Categories	Variables	Collected swabs (n = 3499)
Plan surfaces	Floors, doors and walls	1175
Personals care/PPEs	Uniforms, hairnets, hands, papers and shoe covers	566
Machines/ Equipments	Ball mills, grinders, holding tanks, hoppers, weighing scales, Air conditions, products lining/ pipes	957
Product Carriers	RM (raw material) buckets, bags, cups, trolleys, packaging cartons and wrappers	801

Sample collection were performed on working days, and for the surety of workers normal daily routine practices and maintenance of hygienic condition of the production plant, the concerned department have no idea of planned sampling. Collected swab samples were analyzed for the detection and enumeration of *Enterobacteriaceae* (EB), *Coliforms* and *E. coli*. These samples were treated in an ISO accredited lab (ISO/IEC 17025:2005) with coordination of the department of Microbiology and Biotechnology, University of Karachi (Pakistan).

Swab samples from selected points or surfaces were collected according to the reference method ISO 18593 (2004) [26]. Sterile swabs (China) were removed from its coating, moistened tip in 10 ml sterilized neutralizing buffer peptone water (Oxoid, Hampshire, UK) in a tube and placed the tip of swab(s) on the surface to be investigated. The area was covered by a single swab was 20 cm^2 while rotating the swab clock and anti-clock wise in thumb verses forefinger against the selected area at right angles. Collected swabs were aseptically transferred in a cool box to the laboratory within two hours for further analysis.

2. 2. Sample preparation, inoculation and incubation

Swab samples in tubes were thoroughly mixed for 30 sec using vortex to make initial dilutions. These dilutions were serially diluted into further decimals. Each suspension was further treated on duplicate plates by pour plate method using 1 ml aliquots for the analysis of desired microorganisms according to the ISO suggested protocols for *Enterobacteriaceae*, *Coliforms* and *E. coli* respectively [23-25]. All media and reagents used for analysis were of Oxoid (Hampshire, UK) brand. Pure cultures for positive plating were obtained from the department of Microbiology University of Karachi.

2. 3. Counting and Identification of colonies

After completion of specified incubation, counting of colonies were performed as CFU/cm^2 of the surface area according to the ISO protocol [26]. Identification and further confirmation of *Enterobacteriaceae*, *Coliforms* and *E. coli* were performed through biochemical tests as mentioned in the respective protocols [23-25].

3. RESULTS AND DISCUSSION

The current study was conducted on a total 3499 swab samples collected from food handlers and production surfaces of the food industry for the presence and enumeration of *Enteric* bacteria. Three species of *Enteric* bacteria have been selected for the evaluation in swab samples (*Enterobacteriaceae*, *Coliforms* and *E. coli*). A total of 10495 examinations were performed on collected swabs in which 1277 (12.2%) *Enteric* bacterial species were identified. Out of all species isolated from swabs, *Enterobacteriaceae* were positive in 604 (47.3%) samples with the highest frequency, *Coliforms* in 393 (30.8%) and *E. coli* in 280 (21.9%) samples as shown in Table 2.

The average mean count for *Enterobacteriaceae* and *Coliforms* were found highest (33.6 and 22.6 CFU/cm^2) for plain surfaces (floors, walls and doors), followed by PPEs (25.6 and 22.6 CFU/cm^2) respectively. Average count for *E. coli* were observed maximum on PPEs (19.2 CFU/cm^2) and plain surfaces (16.5 CFU/cm^2) as shown in Figure 1. Standard deviation was observed with highest value for *Enterobacteriaceae* (39.9 CFU/cm^2) on plain surfaces and lowest for *E. coli* (5.3 CFU/cm^2) on product carriers.

Highest count for a single swab was observed for *Enterobacteriaceae* with 290 CFUs for category-1, 160 CFUs for category-4 and 120 CFUs for category-3 variables respectively (Figure 2). The lowest detectable count per swab was observed 10 CFU/cm^2 for all species in the study (data not shown). Further, percent count for all detected isolates per each category variables were also measured as illustrated in Figure 3. Maximum percent count was observed on floors (29.5%) and raw material bags (22.5%) respectively, while paper sheets, split ACs and packaging wrappers were found with no detectable count(s). More, overall percent prevalence of all the three isolates were also determined in positive swabs, where *Enterobacteriaceae* were found most frequent 47%, *Coliforms* 31% and *E. coli* 22% respectively as shown in Figure 4.

Table 2. Prevalence of *Enteric* isolates (*Enterobacteriaceae, Coliforms* and *E. coli*) associated with food handlers and surfaces of food industry.

Categories	Variables	No. of examinations	No. of detected isolates/ Percent	No. of individual detected isolates/ Percent		
				Enterobacteriaceae	*Coliforms*	*E. coli*
Plan surfaces	Floors	1350	398/ 29.5	195/ 49.0	123/ 30.9	80/ 20.1
	Doors	1275	252/ 19.8	132/ 52.4	74/ 29.4	46/ 18.3
	Walls	900	105/ 11.7	51/ 48.6	31/ 29.5	23/ 21.9
Personals care/ PPEs	Uniform	375	26/ 6.9	11/ 42.3	8/ 30.8	7/ 26.9
	Hair net	360	14/ 3.9	6/ 42.9	4/ 28.6	4/ 28.6
	Hands	370	52/ 14.1	20/ 38.5	17/ 32.7	15/ 28.8
	Papers sheets	220	0/ 0.0	0/ 0.0	0/ 0.0	0/ 0.0
	Shoe cover	370	42/ 11.4	18/ 42.9	14/ 33.3	10/ 23.8
Machines/ Equipments	Ball mill machines	675	13/ 1.9	6/ 46.2	4/ 30.8	3/ 23.1
	Grinder machines	450	22/ 4.9	9/ 40.9	7/ 31.8	6/ 27.3
	Holding tanks	300	4/ 1.3	2/ 50.0	1/ 25.0	1/ 25.0
	Machines hoppers	450	6/ 1.3	3/ 50.0	2/ 33.3	1/ 16.7
	Utensils	280	4/ 1.4	2/ 50.0	1/ 25.0	1/ 25.0
	Weighing scale	140	4/ 2.9	2/ 50.0	1/ 25.0	1/ 25.0
	Split ACs	152	0/ 0.0	0/ 0.0	0/ 0.0	0/ 0.0
	Pipe lines	425	1/ 0.2	1/ 100.0	0/ 0.0	0/ 0.0
Product Carriers	RM Buckets	460	80/ 17.4	36/ 45.0	25/ 31.3	19/ 23.8
	RM Bags	880	198/ 22.5	82/ 41.4	64/ 32.3	52/ 26.3
	Oil buckets	155	6/ 3.9	3/ 50.0	2/ 33.3	1/ 16.7

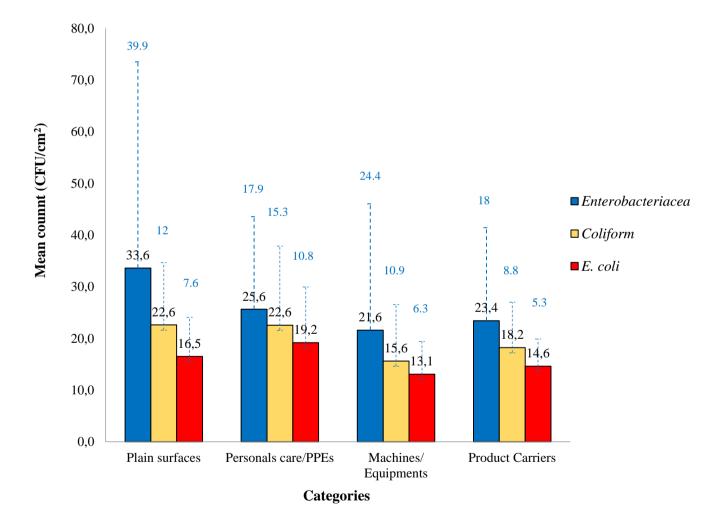|					
Oil cups	78	4/ 5.1	2/ 50.0	1/ 25.0	1/ 25.0
Trolleys	375	45/ 12.0	22/ 48.9	14/ 31.1	9/ 20.0
Packaging carton	225	1/ 0.4	1/ 100.0	0/ 0.0	0/ 0.0
Packaging wrapper	230	0/ 0.0	0/ 0.0	0/ 0.0	0/ 0.0
Sum	10495	1277/ 12.2	604/ 47.3	393/ 30.8	280/ 21.9

Fig. 1. Mean count and standard deviation of isolates per category. Bars represent mean count and doted-lines (blue) represents SD (+).
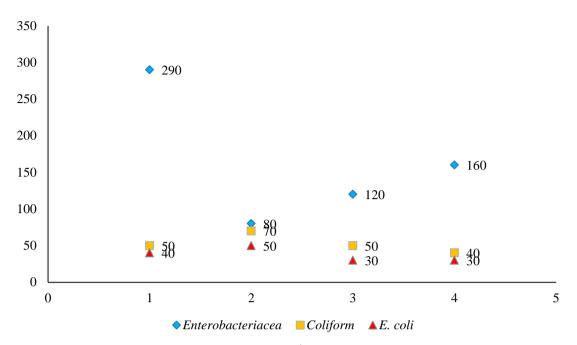
Fig. 2. Maximum observed count (CFU/cm^2) for each category: (1) Plain surfaces (2) Personals care/ PPEs (3) Machines (4) Product carriers.

In discussion, our current study revealed that the majority of floor surfaces were highly contaminated (29.5%) with *Enterobacteriaceae*. Physically, practices for floor washing, cleaning and sanitation were observed insufficient without any set frequency or standard. Floors were rarely washed with hot water, and very low quality domestic sanitizers were used for disinfection processes. Gibson *et al.* (1999) [28] found that the cleaning and washing stage of sanitation remove 1 log order of total surface microbes. Importantly, Dunsmore *et al.* (1981) [29] reported that cleaning phase may remove 99.8% of surface adhered bacteria. Improper cleaned surfaces having soil and food residues, could inactivate disinfectant action against bacteria that are present in these particles.

These practices may not remove adhered food residues on surfaces that have been sourced during production from process materials and can act as a vehicles for bacterial growth and formation of biofilms on surfaces. At the same time these bacteria could become the part of processed food and may spoil food or pathogenic for consumers. Our finding for higher microbial load on surfaces strongly agree with those of previously reported [30,31]. It was also observed during production that flow of handlers were free without any restriction within different production premises. So, these workers were playing a major role in cross contamination of segregated areas. We also reported raw material bags having second highest percent bacterial count (22.5%) after floors. These bags were residing on the surfaces of production areas and contributed equally in surface contamination during its inter-departmental flow while carrying raw food materials for processing. Interestingly, we found that machines and equipments were contaminated with very less bacterial counts that agrees with the results obtained by Lehto *et al.* (2011) [32]. These low counts showed good clean-in-place (CIP) procedures applied for these premises.
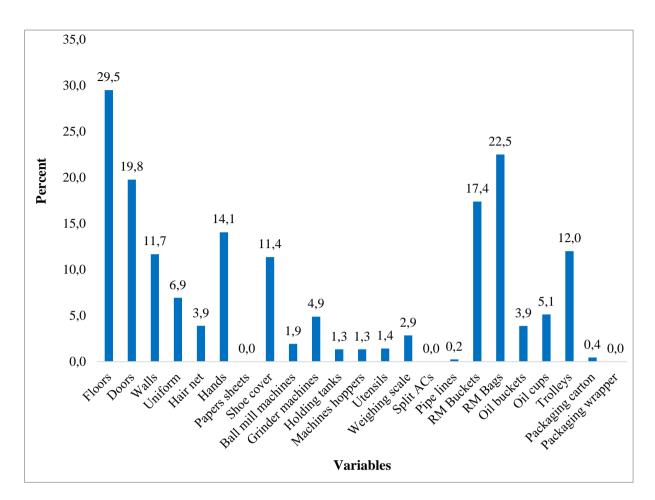
Fig. 3. Percent prevalence of positive isolates with respect to each variable.

The presence of *Coliform* counts (32.7%) on hands as shown in Table 2, indicates the fact that these bacteria have been brought while using the hands as a vehicle from contaminated materials or fecal, and thus could be the potential source of product contamination (Oranusi et al., 2013).

Coliforms bacteria also indicates that other highly pathogenic bacteria like *Salmonella, Shigella* or *E. coli* species, i.e. *E. coli O157:H7* could also be present and may cause severe systemic infections [12]. Many other researchers highlighted the role of food handlers as a threat in transmitting pathogenic bacteria that could contribute in public health illnesses [5,12,13,33,34].

These problems of handler contamination could be improved while following good hygienic practice (GHP) standards, proper training and awareness of workers general hands washing hygiene practices. More, it is also the responsibility of the department head to improve workers hygiene habits and educate personally through oral speech or posters before working in food premises.
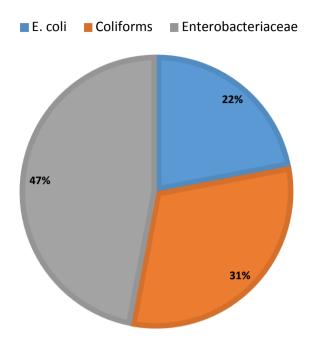
Fig. 4. Overall percent prevalence of positive isolates.

4. CONCLUSION

It is concluded from the present study that the hygienic status of the food processing surfaces, equipments and handlers plays an essential part in microbial contamination of food that has a direct influence on food quality and consumer health. Further, the findings of *Coliform* bacteria indicate the presence of other systemic pathogens like *Salmonella, Shigella* and *E. coli O157:H7* that could cause severe infections. The inadequate cleaning and sanitation procedures could not remove adhered food residues on surfaces that can help in formation of microbial biofilms. So, the reformulation of hygienic policy may require to improve the product quality and minimize the contamination and consumer health risk. Moreover, worker training and supervision by qualified professionals is required to ensure the microbiological free products.

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