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Research Paper

Evaluation of an Environmental Monitoring Program for the Microbial Safety of Air and Surfaces in a Dairy Plant Environment

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ABSTRACT

Microbiological hazards can occur when foodstuffs come into contact with contaminated surfaces or infectious agents dispersed by air currents in the manufacturing environment. An environmental monitoring program (EMP) is a critical aspect of sustainable and safe food manufacturing used to evaluate the effectiveness of the microbial controls in place. An effective EMP should be based on risk analysis, taking into account previous sampling history to determine the selection of the sampling points, the scope of the test, and the frequency of analysis. This study involved evaluation of the environmental monitoring regime and microbiological status of a medium-sized dairy plant manufacturing food ingredients, e.g., proteins, milk powders, and dairy fats. The data specific to microbial tests ($n = 3,468$), recorded across 124 fixed sampling locations over a 2-year period (2014 to 2015) from air ($n = 1,787$) and surfaces ($n = 1,681$) were analyzed. The aim of this study was to highlight the strengths and weaknesses of the EMP in a select dairy processing plant. The results of this study outline the selection of sampling locations, the scope of the test, and the frequency of analysis. An analysis of variance revealed subsections of the manufacturing areas with high risk factors, especially the packaging subsection specified for bulk packaging, the atomizer, and the fluidized bed. The temporal and spatial analysis showed the potential to reduce or relocate the monitoring effort, most notably related to total coliforms and *Staphylococcus aureus,* across the dairy plant due to homogeneity across the sampling subsections with little or no deviations. The results suggest a need to reevaluate the current EMP and the corrective action plan, especially with regard to detection of pathogens. Recommendations for optimization of the EMP are presented to assist the dairy industry with reviewing and revising the control measures and hazard assessment with regard to existing contamination issues.

Key words: Dairy processing; Environmental monitoring program; Food safety

Out-of-specification results in a food product manufacturing plant are important indicators of problems, especially at a critical control point (CCP), as the quality of all products manufactured since the last demonstration of compliance is questioned. The economic costs of foodborne disease outbreaks include loss of productivity due to investigations to identify possible hygiene deficiencies, loss of product from recalls, costs of increased insurance, and loss of consumer confidence in product or even a served enforcement order. The growth of microorganisms can be limited by hygienic practices and well implemented cleaning and sanitizing practices *(1, 39)*. Although foodborne disease incidents are also caused by foods being improperly prepared or mishandled within a domestic or food industry environment *(13),* the primary responsibility lies with the food producers *(12, 40)*. Surfaces in a food manufacturing environment can support the growth of microorganisms and cause cross-contamination issues *(38)*. Airborne microbial contamination may occur as a result of unsatisfactory standards of hygiene or through contaminated environmental particles suspended in the air. Therefore, specific systems of microbial control and assurance activities should be established and maintained in a well-run food processing company. To evaluate the effectiveness of the microbial controls in place, the company needs to develop and maintain an environmental monitoring program (EMP), a crucial prerequisite program under the cover of a hazard analysis and critical control point (HACCP) program. However, there is no one-size-fits-all solution to establish sampling locations and frequency; the solution is dependent on several factors such as a large and diverse number of processes and products to which such hygiene guidelines must apply, the degree of implementation of automated manufacturing processes that limit human handling, the use of subsequent terminal sterilization, and historical profiles of the microbiological environmental data. Therefore, regulatory guidelines, international standards, and scientific advances related to the EMP indicate that a sampling plan should be based on the product and process risk evaluation to realize and demonstrate that the food manufacturing environment complies with food safety and hygiene standards, best practice guidance, and legal requirements and to identify and eliminate any potential contamination

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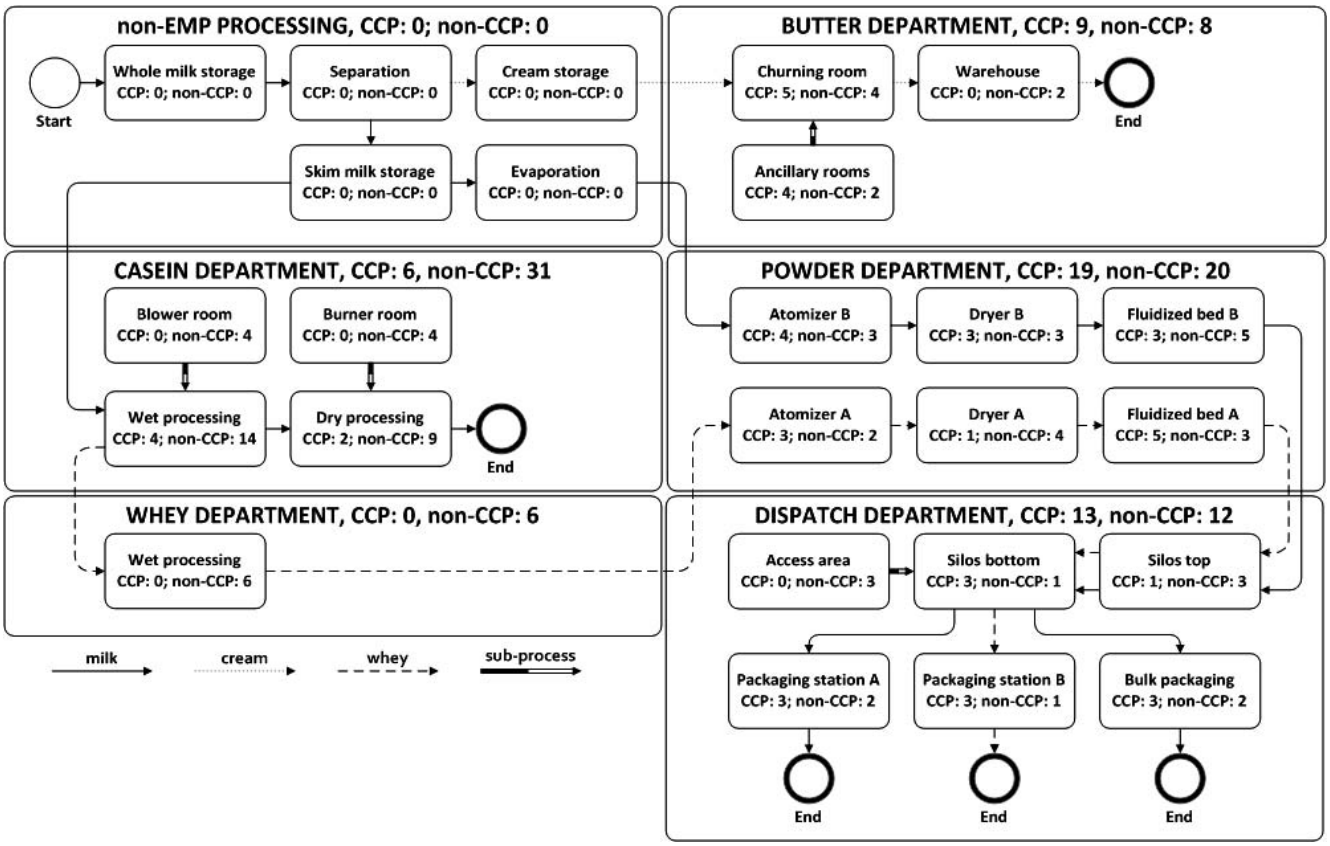

FIGURE 1. *General flow of dairy plant processes with CCPs and non-CCPs indicated.*

hazards *(9, 25)*. Consequently, selection of the sampling points, scope of the test, and frequency of analysis should be determined based on risk assessment taking into account previous sample histories. In addition, an environmental sampling plan needs to be adaptive in nature and be able to implement changes, whether temporary or permanent, depending on various factors, e.g., changes to the design of the process and manufacturing facilities, customer and legislation requirements, seasonal variations, and development of significant microbial trends *(32)*. Research shows that if an outcomes assessment was adopted, it would help to not only enhance a company's reputation but also maintain and expand it to new markets *(10, 15)*. Our study aims to apply statistical methods to show how the strengths and weaknesses of a current EMP can be reliably based and computed on routinely recorded data. We also examine any weaknesses encountered to provide recommendations for optimization of the EMP to assist the dairy industry with reviewing and revising the control measures and hazard assessment with regard to existing contamination issues.

MATERIALS AND METHODS

Study area. A project was undertaken by the Dairy Processing Technology Centre (Limerick, Ireland), an industry-academic collaborative research center, to determine the long-term growth opportunities for the dairy sector. This research was carried out in close cooperation with one of the industrial partners in a medium-sized dairy plant with European Union standards implemented *(12)* and awarded the British Retail Consortium (BRC) Global Standard for Food Safety certification, an internationally recognized benchmark for best practice in the food industry *(5)*. Identifying information was removed to preserve confidentiality. The facility comprises a milk processing area with an effluent plant, laboratories, warehouses, and administration offices. The dairy processing plant manufactures food ingredients, e.g., proteins, milk powders, and dairy fats (Fig. 1). Raw milk is transported to the milk intake bay on site by bulk road tankers. The raw milk is then pasteurized and separated into cream and skim milk. The butter production lines can be used to produce sweet, lactic, and salted butter from cream in a batch churning process. All dairy fats are stored in a cold store next to the production line. An ancillary area in the butter department is used when required, e.g., for lactic butter production. Depending on the process capacity and customer's requirements, some skim milk is dried to powder on dryer B, and the rest is directed to the casein department where casein is extracted and dried to powder. The by-product of the casein production process is milk permeate; the milk permeate goes to the whey department to extract protein followed by drying on dryer A in the powder department. Finally, all dairy powders are bagged and stored in the dispatch department. Irish dairy processing is seasonal, responding to the grass growing season, with a peak/trough ratio of 7:1 (May versus January). Therefore, standard manufacturing activities are suspended for winter, and normally the plant is closed from early December to mid-February.

Sample source. Over the 2-year period studied (2014 and 2015), a total of 3,468 microbiological examinations was conducted on the samples obtained during routine environmental sampling, as laid out in the standard operating procedures of the in-house EMP. Sampling was carried out during operational conditions, including the equipment operating and personnel present. Samples were examined for indicator microorganisms

TABLE 1. *Number of critical sampling points and total number of sampling points in areas of the dairy plant considered in the environmental monitoring program*

Department no. and name	Subsection	No. of sampling locations		
		CCP[a]	non-CCP	Total
1. Butter	A. Churning room	5	4	9
	B. Ancillary area and storage	4	4	8
2. Casein	A. Wet processing	4	18	22
	B. Dry processing	2	13	15
3. Whey	A. Wet processing	0	6	6
4. Powders	A. Dryer A	9	9	18
	B. Dryer B	10	11	21
5. Dispatch	A. Powder storage	4	7	11
	B. Powder bagging	9	5	14
Overall total		47	77	124

[a] CCP, critical control point.

(aerobic colony count [ACC], total coliforms, yeasts, molds, *Enterobacteriaceae, Staphylococcus aureus*) and pathogens (*Listeria* spp., *Salmonella, Cronobacter* spp.). Both qualitative and quantitative examinations were undertaken. The data regarding environmental contaminants were collected in conformance with current good manufacturing practices.

Sampling locations and frequency. For the purposes of the EMP, the dairy plant was divided into departments and subsections related to a particular service or group of products (Table 1 and Fig. 1). The sampling regime was composed of 124 sampling points across the dairy plant, with 47 points designated as CCPs based on in-house risk assessment and in accordance with the BRC Global Standard for Food Safety *(5),* European Union standards *(12),* and customer's requirements. These CCPs are locations where products are not processed in enclosed systems, e.g., pipework, and thus are exposed to the environment, e.g., product passing along conveyors. Sampling was performed on a rotating basis: 5 of the 124 locations sampled Monday to Saturday, with an additional 5 of the 47 CCPs sampled weekly.

Microbiology methodology. Microbiological results were assessed against the process hygiene criteria (Table 2) specified in the company's standard operating procedures, in accordance with the BRC Global Standard for Food Safety *(5)*. The number of airborne indicator organisms was evaluated using the settling plate technique for ACC, coliform count, yeasts, and molds. Open 90-mm petri dishes containing 20 mL of media were exposed for 45 min to the air. To monitor hygiene on surfaces, enumeration of indicator organisms was conducted for ACC, *Enterobacteriaceae,* and *S. aureus*. A 3M (St. Paul, MN) Swab-Sampler prehydrated with 10 mL of Letheen broth was used to swab a 100-cm^2 area (10 by 10 cm). One milliliter of the Letheen broth was transferred to a petri dish with medium applicable to detect the appropriate organism. Enumeration of ACC was conducted using milk agar (Oxoid milk plate count agar, Thermo Fisher Scientific, Basingstoke, UK) incubated aerobically at 30 ± 1°C for 72 to 74 h based on ISO 4833-2:2013 *(22)*. *S. aureus* counts were enumerated using Baird-Parker agar (Oxoid Baird-Parker agar base) with egg yolk tellurite emulsion (both Thermo Fisher Scientific) incubated aerobically at 37 ± 1°C for 24 to 26 h and then reincubated for a further 24 to 26 h based on ISO 6888-1:1999 *(17)*. Total coliform count was determined using violet red bile agar (VRB agar, Sigma-Aldrich, St. Louis, MO) incubated aerobically at 30 ± 1°C for 48 to 50 h based on ISO 4832:2006 *(20)*. *Enterobacteriaceae* counts were enumerated using VRB agar (Sigma-Aldrich) incubated aerobically at 37 ± 1°C for 24 to 26 h based on ISO 21528-2:2004 *(19)*. The numbers of yeasts and molds were determined using yeast extract glucose chloramphenicol agar (YGC agar, Sigma-Aldrich) incubated aerobically at 21 ± 1°C for 5 days based on ISO 6611:2004 *(18)*. Results of quantitative examinations were normalized to CFU per square centimeter. Detection of foodborne pathogenic organisms on surfaces was conducted for *Listeria* spp., *Salmonella,* and *Cronobacter* spp. with sterile sponges (3M Hydra-Sponge with Letheen broth). Detection of *Listeria* spp. and *Salmonella* was conducted using the Solus Scientific Solutions Ltd. (Mansfield, UK) enzyme-linked immunosorbent assay (ELISA)–based test system SOL 37/02-06/13 and SOL 37/01-06/13, respectively, validated to ISO 16140 *(23)*. Detection of *Cronobacter* spp. was conducted using a selective chromogenic agar (RAPID'Sakazakii medium, Bio-Rad, Marnes-la-Coquette, France), incubated aerobically at 44 ± 1°C for 24 to 26 h based on ISO/TS 22964:2006 *(21)*.

TABLE 2. *Hygiene criteria used by the dairy company examined in the present study to designate environmental samples*

Indicator or pathogenic microorganism	Microbiological parameter	Microbiological quality (CFU/cm^2)[a]	
		Satisfactory	Unsatisfactory
Indicators (air)	ACC[b]	≤4.72	>4.72
	Total coliforms	≤0.16	>0.16
	Yeasts	≤0.16	>0.16
	Molds	≤0.16	>0.16
Indicators (surface)	ACC	≤30.00	>30.00
	Enterobacteriaceae	≤1.00	>1.00
	S. aureus	≤1.00	>1.00
Pathogens (surface)	*Listeria* spp.	Not detected	Detected
	Salmonella	Not detected	Detected
	Cronobacter spp.	Not detected	Detected

[a] Criteria values can change depending on specific customer requirements. Microbial quality based on standards as outlined in "Materials and Methods."

[b] ACC, aerobic colony count.

Statistical analysis. Before analyses, all independent variables were evaluated for normality via Q-Q plots and Shapiro-Wilk tests. A one-way analysis of variance (ANOVA) was used to test for associations between nonparametric categorical variables, and the chi-square test was used to test a relationship between variables with two or more categorical, independent groups. A post hoc analysis was carried out using the Tukey honestly significant difference test to provide specific information on which means are significantly different from each other. Statistical analyses were carried out using SPSS version 22 (IBM, New York, NY), and the confidence level was set at 95% by convention.

RESULTS AND DISCUSSION

Assessment of spatial distribution of microbiological contamination in a dairy plant. A breakdown of the air and surface microbiology results of the manufacturing environment for five departments of the dairy plant is provided in Table 3. The mean microbial counts across

TABLE 3. *Quantitative and qualitative examinations by microbiological parameter undertaken on samples obtained for routine determination of the occurrence of indicator microorganisms and pathogens in the air and on the surfaces within the processing areas of the departments in a dairy plant*[a]

Microbiological parameter	Butter department		Casein department		Whey department		Powders department		Dispatch department		Total	
	u (%)	*n* (%)	*u* (%)	*n* (%)	*u* (%)	*n* (%)	*u* (%)	*n* (%)	*u* (%)	*n* (%)	*u* (%)	*n* (%)
Indicator (air)												
ACC	0 (0.0)	88 (100)	0 (0.0)	97 (100)	0 (0.0)	34 (100)	0 (0.0)	134 (100)	0 (0.0)	131 (100)	0 (0.0)	484 (100)
Total coliforms	0 (0.0)	55 (100)	0 (0.0)	70 (100)	0 (0.0)	34 (100)	0 (0.0)	81 (100)	0 (0.0)	78 (100)	0 (0.0)	318 (100)
Yeasts	1 (1.1)	90 (100)	0 (0.0)	96 (100)	0 (0.0)	34 (100)	0 (0.0)	135 (100)	0 (0.0)	138 (100)	1 (0.2)	493 (100)
Molds	0 (0.0)	90 (100)	0 (0.0)	96 (100)	0 (0.0)	28 (100)	5 (3.6)	140 (100)	3 (2.2)	138 (100)	8 (1.6)	492 (100)
Total (air)	1 (0.3)	323 (100)	0 (0.0)	359 (100)	0 (0.0)	130 (100)	5 (1.0)	490 (100)	3 (0.6)	485 (100)	9 (0.5)	1787 (100)
Indicator (surface)												
ACC	0 (0.0)	45 (100)	0 (0.0)	61 (100)	0 (0.0)	15 (100)	0 (0.0)	73 (100)	0 (0.0)	76 (100)	0 (0.0)	270 (100)
Enterobacteriaceae	0 (0.0)	67 (100)	0 (0.0)	60 (100)	0 (0.0)	20 (100)	2 (2.9)	68 (100)	0 (0.0)	76 (100)	2 (0.7)	291 (100)
S. aureus	0 (0.0)	64 (100)	0 (0.0)	60 (100)	0 (0.0)	20 (100)	0 (0.0)	65 (100)	0 (0.0)	76 (100)	0 (0.0)	285 (100)
Total (surface)	0 (0.0)	176 (100)	0 (0.0)	181 (100)	0 (0.0)	55 (100)	2 (1.0)	206 (100)	0 (0.0)	228 (100)	2 (0.2)	846 (100)
Pathogen (surface)												
Listeria spp.	0 (0.0)	54 (100)	0 (0.0)	64 (100)	0 (0.0)	13 (100)	0 (0.0)	81 (100)	0 (0.0)	62 (100)	0 (0.0)	274 (100)
Salmonella	0 (0.0)	54 (100)	0 (0.0)	64 (100)	0 (0.0)	13 (100)	0 (0.0)	81 (100)	0 (0.0)	62 (100)	0 (0.0)	274 (100)
Cronobacter spp.	1 (1.9)	54 (100)	10 (11.6)	86 (100)	0 (0.0)	13 (100)	1 (1.4)	71 (100)	1 (1.6)	62 (100)	13 (4.5)	287 (100)
Total (pathogens)	1 (0.6)	162 (100)	10 (4.7)	214 (100)	0 (0.0)	39 (100)	1 (0.4)	233 (100)	1 (0.5)	187 (100)	13 (1.6)	835 (100)
Overall total	2 (0.3)	661 (100)	10 (1.3)	754 (100)	0 (0.0)	224 (100)	8 (0.9)	929 (100)	4 (0.4)	900 (100)	24 (0.7)	3,468 (100)

[a] *u*, number of samples with unsatisfactory results; *n*, total number of samples analyzed; ACC, aerobic colony count.

TABLE 4. *Microbial counts of indicator microorganisms as determined by routine air and surface analysis within the processing areas of the departments in a dairy plant*[a]

Microbiological parameter	Counts (log CFU/cm^2), $\bar{x}$ ± SD (range)						Guideline (log CFU/cm^2)
	Butter department	Casein department	Whey department	Powders department	Dispatch department	Overall	
Indicator (air)							
ACC	0.13 ± 0.14 (0.02–0.64)	0.30 ± 0.35 (0.02–2.18)	0.41 ± 0.31 (0.09–1.54)	0.21± 0.19 (0.02–0.99)	0.17 ± 0.13 (ND–0.72)	0.22 ± 0.23 (ND–2.18)	≤4.72
Total coliforms	ND	ND	ND	ND	ND	ND	≤0.16
Yeasts	0.01 ± 0.03 (ND–0.16)	0.01 ± 0.03 (ND–0.09)	0.01 ± 0.01 (ND–0.03)	<0.01 ± <0.01 (ND–0.03)	<0.01 ± 0.01 (ND–0.05)	0.01 ± 0.02 (ND–0.16)	≤0.16
Molds	0.03 ± 0.03 (ND–0.14)	0.03 ± 0.03 (ND–0.14)	0.03 ± 0.04 (ND–0.14)	0.05 ± 0.05 (ND–0.25)	0.05 ± 0.12 (ND–1.35)	0.04 ± 0.07 (ND–1.35)	≤0.16
Indicator (surface)							
ACC	0.72 ± 0.78 (0.10–4.80)	1.56 ± 1.35 (ND–8.60)	1.76 ± 1.36 (0.30–4.60)	1.06 ± 1.00 (0.10–6.40)	0.83 ± 0.79 (0.10–4.30)	1.09 ± 1.08 (ND–8.60)	≤30.00
Enterobacteriaceae	ND	0.03 ± 0.13 (ND–0.90)	ND	0.12 ± 0.67 (ND–5.30)	ND	0.03 ± 0.33 (ND–5.30)	≤1.00
S. aureus	ND	ND	ND	ND	ND	ND	≤1.00

[a] ACC, aerobic colony count; ND, not detected.

departments are shown in Table 4. The supplemental tables present the one-way ANOVA results for each indicator microorganism analyzed across departments (Supplemental Table S1) and likewise across the subsections (Table S2) of the dairy plant. The results of the Tukey honestly significant difference test are available in Tables S3 and S4. In general, air analysis demonstrated that all departments are of good hygiene quality, with a small number of unsatisfactory results for yeast and mold counts in three departments: the butter department (one yeast), the powder department (five molds), and the dispatch department (three molds). Surface testing also showed good quality environments. The powder department surveillance showed two unsatisfactory results for *Enterobacteriaceae* over the 2 years of data analyzed (Table 3).

ACCs are useful for indicating the sanitary conditions under which the food is produced and processed. All the ACCs were satisfactory. In the butter department, this control can be associated with the use of a disinfectant fogger for routine sanitizing practice in the area. Chlorine dioxide use has a high level of efficacy against a range of pathogens *(3, 30)*. This effective decontamination method provides optimal penetration into complex areas with difficult access, e.g., complicated pipework *(29)*. Therefore, it is recommended to be implemented as a supplementary sanitizing strategy in the subsections with complex production lines, e.g., the wet processing of casein and whey departments and where considerably higher standard deviations (SDs) of ACCs (Table 4) were observed. Accumulation of milk residues on or near food processing equipment facilitates attachment and proliferation of bacteria. In addition, cross-contamination risk is raised with increased maintenance and repair operations. For example, we have observed the following: inappropriate cleaning and sanitizing before and after maintenance and repair operations; cleaning dirty equipment with pressure hoses that can cross-contaminate cleaned equipment in the same area due to aerosolized wet residues; food processing equipment repaired with unsanitized tools; maintenance teams or contractors missed some parts of the disposable safety clothing, e.g., hairnets, shoe covers, beard covers, gloves; unrestricted people or equipment traffic during maintenance and repair operations from outdoor to production area through backdoor with no footwear sanitation system in place; rough welds that can accumulate dirt; and use of duct tape to stop leaks that can cause considerable microbial buildup over time and can cause cross-contamination when dripping.

Strictly followed and validated sanitation procedures are particularly important to prevent or eliminate persistent nonconformities, e.g., biofilm development *(16, 28)*. Our findings emphasize the value of redeploying some of the ACC sampling efforts from the butter department to highlighted areas with higher mean ($\bar{x}$) and SD values (Table 4), e.g., the whey department.

Significant differences, at the $P < 0.05$ level, between departments in yeast count were calculated [$F(4,488) = 13.447$, $P < 0.001$]. Three sampling locations with the highest SD of yeast numbers were located next to each other

TABLE 5. *Number of environmental examinations taken over a 2-year period (2014 to 2015) with year-over-year growth rate, count means, and SDs of the numbers of indicator microorganisms as determined by routine air and surface analyses within the processing areas in a dairy plant*[a]

Microbiological parameter	n_{2014}	n_{2015}	YoY (%)	$\bar{x}_{2014} \pm$ SD	$\bar{x}_{2015} \pm$ SD	F	P
Indicator (air)							
ACC	242	242	0.0	0.21 ± 0.24	0.22 ± 0.23	0.25	0.617
Total coliforms	77	241	213.0	ND	ND		
Yeasts	250	243	−2.8	<0.01 ± 0.01	0.01 ± 0.02	21.61	<0.001
Molds	249	243	−2.4	0.04 ± 0.10	0.04 ± 0.04	0.29	0.591
Indicator (surface)							
ACC	126	144	14.3	0.86 ± 0.80	1.28 ± 1.25	10.51	0.001
Enterobacteriaceae	146	145	−0.7	0.06 ± 0.46	0.01 ± 0.07	1.84	0.176
S. aureus	140	145	3.6	ND	ND		

[a] *n,* number of examinations taken in a year; YoY, year-over-year growth rate; ACC, aerobic colony count; ND, not detected.

within the butter department, i.e., entrance to churning room ($\bar{x} = 0.04$, SD = ±0.06, range = not detected [ND] to 0.13), control panel ($\bar{x} = 0.03$, SD = ±0.05, range = ND to 0.16), and door to preparation area ($\bar{x} = 0.02$, SD = ±0.05, range = ND to 0.14). All three locations were characterized by high levels of personnel activities. These results, together with analysis of the temporal dynamic pattern of means (Supplemental Fig. S2), are indicative of recurrent nonconformities. This result may be associated with biofilm-dwelling yeast adhered to hard-to-reach process surfaces, e.g., conveyor tracks of a semiautomatic packaging line in this area. It is recommended to investigate potential sources of yeast contamination and hence develop targeted cleaning and sanitizing procedures.

Three unsatisfactory mold count results (Table 3) were recorded in the bulk packaging subsection ($\bar{x} = 0.13$, SD = ±0.27, range = ND to 0.13; $P < 0.001$). The additional five counts were recorded in the powder department, i.e., three beside the atomizer ($\bar{x} = 0.06$, SD = ±0.07, range = ND to 0.25) and two in an adjunct fluidized bed subsection ($\bar{x} = 0.07$, SD = ±0.05, range = ND to 0.02). Detection of molds should trigger careful visual inspection to identify any untreated condensation or dents in a pipeline to be causing leaks. Condensation can occur when warm moist air from the drying plant comes into contact with cold surfaces.

The two unsatisfactory *Enterobacteriaceae* results occurred within two subsections of the powders department, i.e., atomizer ($\bar{x} = 0.31$, SD = ±1.28, range = ND to 0.50) and fluidized bed ($\bar{x} = 0.14$, SD = ±0.41, range = ND to 1.50). Unsatisfactory air results for molds were also recorded within the same locations, together with surface ACC levels that were three times higher than those in adjunct locations (Table 3). Upon inspection, a mix of powder and grime was present on the pipework, railings, and floor. Moreover, powder bags labeled "waste" were left open to the air near the manufacturing equipment.

Total coliforms ($n = 318$) and *S. aureus* ($n = 285$) were not detected with the settling plate technique. These results are in accordance with those reported by Salustiano et al. *(36)* in a study of milk processing areas in a dairy plant. Due to customer requirements, a significant increase in total coliform sampling, from 77 to 241, was undertaken over the 2 years (Table 5), although no differences in quality outcomes were observed, demonstrating potential compliance with good hygiene practices. Notwithstanding, results of enumeration of microorganisms may differ with the test method used and may be affected by numerous factors, e.g., aerodynamic or flowing behavior of aerosols, humidity, temperature, ventilation and air-conditioning systems, personnel and manipulator activity in the sampling area, and media being inadequate for stressed or injured microorganisms *(41)*. The settling plate technique requires relatively long exposure time. This can lead to a dried media surface that can impact microbial growth and result in underestimation of the microbial counts. Active air sampling techniques are capable of detecting sufficiently low levels of microbial contamination in such dynamic environments, and they provide relatively shorter sampling time. Moreover, choice of sample locations should be based on environmental factors that favor microbial growth. Sampling the hot and dry environment near the dryers in the powder department is not likely to be an efficient way to find the target microbe because high temperature and low water activity generally decrease bacterial growth. The above-mentioned results suggest the need for continuously updating monitoring procedures and reviewing new methodologies, especially the rapid microbial methods emerging on to the market.

Assessment of temporal distribution of microbiological contamination. Results of statistical analysis and the year-over-year growth rate of the number of environmental examinations in each year are outlined in Table 5. A comparison over time, by department, on the means of microbial count is presented in Supplemental Figures S1 through S4. Our results (Table 5) show a significant correlation related to a 32.7% increase in year-over-year means of ACCs on the surfaces [$F(1,268) = 10.506$, $P < 0.001$] and yeast count [$F(1,491) = 21.611$, $P < 0.001$]. This can be associated with increased production in the examined period. Irish production of cheese, butter, and skim milk powder in 2014 stood at 425.4 thousand tons, whereas for 2015 it was 493.7 thousand tons, which is a 13.8% year-over-year growth rate *(7)*. Irish dairy processing is seasonal, responding to the grass growing season. According to the

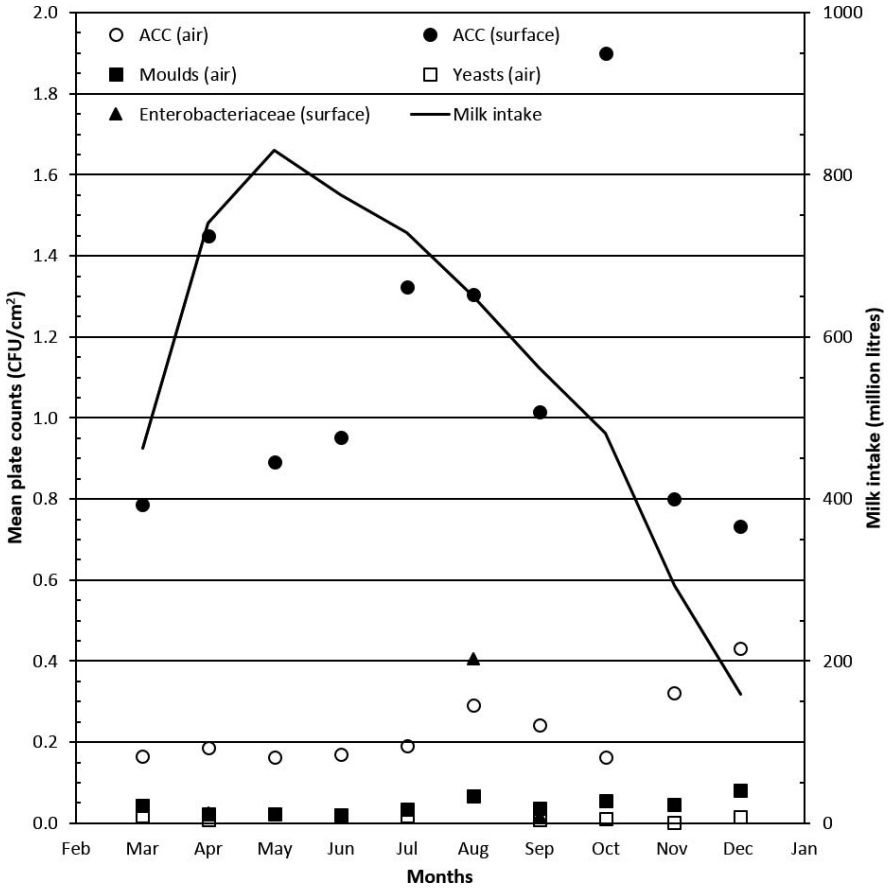

FIGURE 2. *Temporal distribution of ACC in the air, yeasts in the air, molds in the air, ACC on the surfaces, and* Enterobacteriaceae *on the surfaces over a 2-year time period (2014 to 2015) as determined by routine environmental sampling within the processing areas in a dairy plant plotted against average milk intake by creameries and pasteurizers (8).*

Central Statistics Office (Cork, Ireland), average milk intake by creameries and pasteurizers in Ireland for January 2014 and January 2015 was 123.4 million liters, whereas for May during the same time period it was 830.3 million liters, a 572.9% increase *(8)*. However, our results show that an association with highly seasonal milk supply patterns and differences in means of levels of microbial indicators between the months in the year (Fig. 2) cannot be inferred. However, the examined dairy plant uses groundwater from borehole-drilled water wells to perform routine sanitation practices. It is important to emphasize that groundwater contamination in Ireland is commonplace and subject to seasonal changes related to agricultural activity *(34)*. Further details of the fluctuation in the quality of the water source in a dairy plant in Ireland are also reported by Burke et al. *(6)*. These reports reinforce the assumption of potential correlations between changes in microbial levels in a dairy plant environment and the quality of the water used for sanitation practices. The increased levels of microbial contamination observed in December may be correlated with record-breaking rainfall, together with a relatively higher temperature in December 2015. Most weather-observing stations across Ireland reported double or triple their normal rainfall for December, and all mean temperatures for December were well above their long-term average *(31)*. For example, the weather station located near the dairy plant in Fethard Co., Tipperary, recorded 263.9 mm of rain (289% of 1981 to 2010 average) and 8.1°C mean temperature (3.1°C above the 1981 to 2010 average). Another factor that may account for increased variance in December is a holiday labor shortage that may impact cleaning and sanitizing operations. These results demonstrate that an EMP should be designed that allows detection of fluctuations in microbial counts due to possible seasonal variations.

Assessment of associations between occurrences of pathogens. *Listeria* spp. and *Salmonella* were not detected during the study period. Several studies conducted in a dairy industry have highlighted problematic areas concerning correlation between the levels of total coliforms and the presence and concentrations of pathogens, i.e., *Bacillus cereus, Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella (11, 24, 26)*. Because *Salmonella* was not detected in any of the 274 examined samples, the results of parallel sampling showed the presence of *Enterobacteriaceae*. A chi-square test was performed to examine whether detection of *Enterobacteriaceae* is likely to be associated with a detection of *Cronobacter* spp., and no significant relationship was found [χ^2 (1, $n = 178$) = 2.393, $P = 0.122$]. Phi and Cramer's V test results of 0.116 demonstrate that a strong positive relationship between the detection of *Enterobacteriaceae* and *Cronobacter* spp. cannot be inferred.

Cronobacter spp. were detected in 4.5% (13 of 835) of the samples analyzed from four of the five departments that were monitored. Twelve of 13 detections were related to dairy powders. Ten detections were obtained from the casein department (10 of 86, 11.6%), including the subsection for wet processing (6 of 37, 16.2%), i.e., the base of the decanter (2 of 8, 25.0%; CCP); access door on tank (2 of 6, 33.3%;

CCP); door handle near control room (1 of 8, 12.5%; CCP); ultra-osmosis loop (1 of 3, 33.3%); and the subsection for dry processing (4 of 36, 11.1%), i.e., the entrance to subsection (3 of 7, 42.9%; CCP) and dryer sieve (1 of 2, 50.0%; CCP). *Cronobacter* spp., except *C. condimenti,* have been associated with clinical cases of foodborne illness in infants from powdered formula and in immunocompromised adults from plant material, e.g., sprouts and fresh herbs *(4, 14, 27, 37)*.

A chi-square test was performed to examine whether detection of *Cronobacter* spp. is likely to be associated with any month in the year [χ^2 (9, $n = 287$) = 19.577, $P = 0.021$]. To assess the strength of association, Phi and Cramer's V tests were conducted. Both results were 0.021, demonstrating that correlation between months and *Cronobacter* spp. detection is negligible. Although *Cronobacter* spp. have been isolated from a wide range of sources, infant dairy powders have been recognized as the notable vehicle of transmission *(2, 33)*. The presence of *Cronobacter* spp. is not acceptable in a dairy manufacturing plant producing ingredients for infant formula powders, or for infant powders themselves. These results show that the implementation of alternative or supplementary strategies should be considered, e.g., the use of a disinfectant fogger in the production environment. Finally, the volume of testing aimed at the detection of *Cronobacter* spp. may be insufficient, particularly in areas of persistent nonconformities, i.e., the casein department. To improve spatial or temporal coverage of an area without increasing sample number, composite sampling should be considered. However, implementation of a compositing scheme should be first validated for the food matrix, the microorganism of interest, and the assay used *(35)*.

In summary, recurring detection of indicator organisms widely used to assess the efficacy of contaminating control programs demonstrates the need to review and revise the current hazard assessment with regard to existing contamination issues and to develop an efficient system of preventive and corrective actions with rigorous monitoring within the areas of high concern. An action level may be triggered based on results from individual sample locations, groups of related sample locations, or the maximum number of nonconformities per area. To provide confidence in the corrective action plan, it should include a step to validate that the detected nonconformity was in fact successfully removed, and the protocol for closing it should not be allowed until this validation step has been accomplished.

In conclusion, it is recognized that an environmental monitoring program plays an important role in the control of microbiological hazards. However, correct implementation and frequent reviews are critical. This study investigated the microbiological status and efficiency of an environmental monitoring regime in a medium-sized dairy processing plant. Evidence provided through statistical analysis assisted plant managers in revising accordingly the control measures and hazard assessment with regard to existing contamination issues. Significant mean differences between microbial levels distinguished the areas with inefficient cleaning and sanitizing operations, e.g., bulk packaging and subsections of the powders department such as the atomizer and fluidized bed. The results also demonstrate the potential to proceed with a reduction or relocation of the sampling effort, most notably related to total coliforms and *S. aureus,* due to homogeneity across the dairy plant with little or no deviations. The revised sampling plan can implement composite sampling and would be flexible to reflect trends of microbial contamination and possible seasonal variations. Appropriate preventive and corrective actions, along with efficient monitoring for *Cronobacter* spp., would contribute to a reduction in the dissemination of this pathogen in the food chain. Further recommendations include ensuring that procedures are in place for verification of results, identifying subsequent routes of dissemination, ensuring appropriate moisture control, and improving hygiene of the maintenance operations. The implementation of these recommendations is suggested so that managers may deliver effective food safety and quality decisions to grow consumer confidence and a sustainable business.

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SUPPLEMENTAL MATERIAL

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