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The concentration and frequency of *C. sakazakii* in Queen Geraldin Hospital in Tirana

RENIS MAÇI[1]*, BIZENA BIJO[2], FATMIRA SHEHU[2],ADRIAN XINXO, HALIT MEMOÇI[1]

[1] Department of Food Microbiology, Food Safety and Veterinary Institute (FSVI), "Aleksandër Moisiu" No. 10, Tirana, Albania

[2] Department of Veterinary Public Health, Faculty of Veterinary Medicine (FVM), Kodër-Kamëz, Tirana, Albania

*corresponding author e-mail: renismaci@gmail.com

Abstract:

In the last years the International Commission for Microbiological Specification for Foods ranked *Cronobacter sakazakii* as "Severe hazard for restricted populations, life threatening or substantial chronic sequelae or long duration"(ICMSF, 2002). The objective of this study is to control the biological risk of the hospital kitchen`s environment at the University Hospital of Obstetrics and Gynaecology "Queen Geraldin" where the powered infant formula is prepared. Efficiency of risk control must be verified through the application of microbiological monitoring plan that provides application of microbiological environmental criteria, proper cleaning of equipments used in production lines, control of the final product during their shelf live, collection of samples from the raw material, surfaces and environment as well as control measures during preparation and reconstitution of powered infant formulae. This study was performed to determine the frequency distribution of *C. sakazakii* and the concentration of *Enterobacteriaceae* in different sampling areas of the kitchen. We performed 60 samples in total. The samples were collected from the kitchen areas, and from the personel hands. At the end we quantified for *Enterobacteriaceae* and identified for *C. sakazaki*i in 60 samples. We detected in two environmental samples (3.0%) the presence of *C. Sakazakii.* Rules that should be respected to meet the highest level of microbiological safety in hospital/nursery are defined in MRA Series 10 (FAO/WHO 2004)

Keywords: PIF; *Enterobacter sakazakii (Cronobacter sakazakii)*; food safety.

1. Introduction

Hospital surfaces, including those in food preparation areas, are some of the major contributing factors of food-borne illnesses outbreaks [HAI] [12].

Cronobacter sakazakii (*C. sakazakii*) is an emerging food-borne pathogen that has raised constantly the interest among the public community and food industry, especially in the production of powder infant formula [14].

Due to the dangerousness of *C. sakazakii* infections versus neonates is necessary to introduce rigorous control measures in order to reduce the risks contamination at various levels: industrial(to prevent food contamination from production to marketing; at domestic level (reducing the risk of contamination), during preparation, handling, and storage; and legislative level by establishing guidelines and recommendations issued by competent authorities [5].

Even though *Cronobacter sakazakii* is classified in category "A" because of well-established causes of illness in infants (e.g. systemic infection, necrotizing enterocolitis [NEC] and severe diarrhoea) and its epidemiology that is still unclear(FAO/WHO) [3].

Different studies has shown that this pathogen is not restricted to powdered infant formula. It can also be found in a broad range of foods and in water, in a variety of areas, including hospitals and houses [5].

Several outbreaks of *Cronobacter spp.* (*Enterobacter sakazakii*) have been described as food-borne illness in neonates and infants. Powdered infant formula has been recognized as a sources of infection, especially in hospital nurseries, where a bulk of formula nutrient is prepared for entire day and instructions for preparation are not always come after correctly [9].

Research has begun to characterize the virulence factors and pathogenic potential of Cronobacter. The survival of this foodborne pathogens is mainly attribute to: a) formation of biofilm and the putative production of cellulose as one of the components of the extracellular matrix; b) adherence to hydrophilic and hydrophobic surfaces; c) the production of extracellular polysaccharides; d)the ability of *E.*

sakazakii to produce cell-to-cell signalling molecules. [11].

Like other bacteria, biofilm formation of *Cronobacter* contributes to its persistence on food contact surfaces [8]. Biofilm forming ability of *Cronobacter* species is thought to be very important for its distribution behaviour in infant formula milk and environmental samples. Meanwhile, according to a series of reports, bacterial biofilm formation depends on the composition of medium where the biofilm is developed [7,10,13]. Cellulose was identified and characterized as an extracellular matrix component present in the biofilm of an *Enterobacter sakazakii* (M9) [6].

The ability to adhere (host surfaces) on mucous membranes, gastric and intestinal epithelial or endothelial tissue is necessary at the successful colonization and disease establishment. There are different studies that describe the adherence ability of *E. sakazakii* strains versus epithelial cell lines HEp-2 and Caco-2, as well as the brain micro-vascular endothelial cell line HBMEC.

The role of lipopolysaccharide (LPS) structure in the stability of outer membrane and the ability of biofilm formation in *Cronobacter sakazakii* has been study [16].

Some strains of *C. sakazakii* produce molecules that activate N-acylhomoserine lactone (AHL) sensor, which are responsible for the cell density regulation and quorum sensing [15].

2. Material and Methods

2.1. Sampling sites

The analytical samples were sampled (based on sandard operating procedure ISO 18593: 2004 (sampling technique from surface) at hospital kitchen facilities at the University Hospital of Obstetrics and Gynaecology "Queen Geraldin". On this study we collected a total of 60 samples surface swabs which were examined. The procedure was done twice over one working hour, during preparation of food in 10 different areas of the kitchen. Sterile Swabs were moistened prior to the collection of samples using sterile Buffer Peptone Warter. Microbiological investigation was carried out in kitchen`s facilities: working table, kitchen utensils, sponges, conditioning water pipe, sink drain area as well as hands of staff working with foods for infants [1,2].

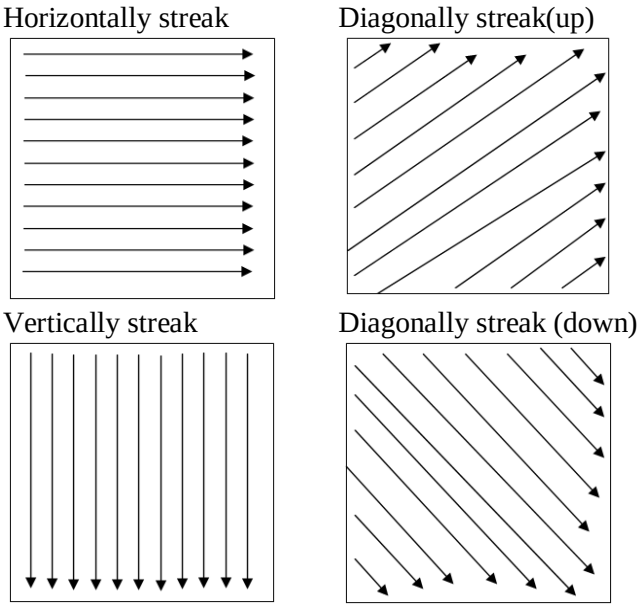

Figure 1. Surface swabbing patterns.

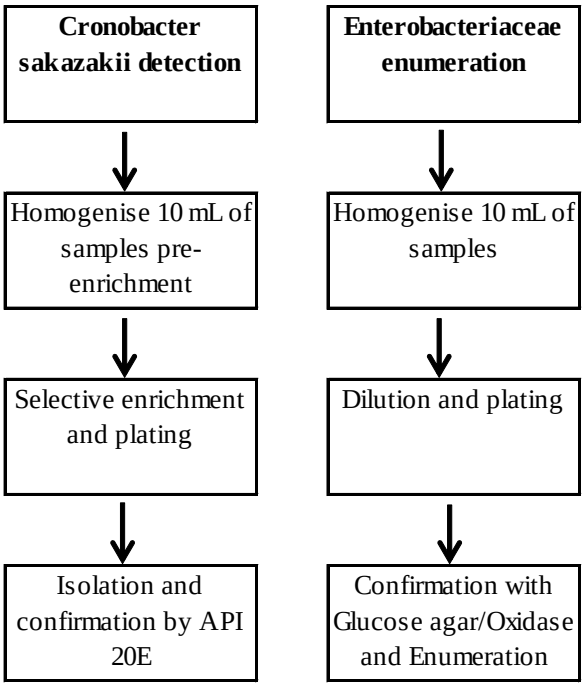

Figure 2. Flow diarams. Detection of *C. sakazakii* and enumeration *of Enterobacteriaceae*

The swabs were taken from the surface sites by swabbing vertically, horizontally, and diagonally using 100 cm^2 sterile square template. Each sample container was identified with a code. Samples with respective container were stored between 0-4 °C. At the collection time they were put in a cool box and after they were transported to the Laboratory of Food Microbiological Control (FSVI) under chilled conditions 0-4 °C the same day.

2.2. Sample preparation and incubation

A sample was considered the surface swab with 10 mL transport medium which was after homogenized with 90 mL primary enrichment broth medium, Buffered Peptone Water (BPW). The incubation was done for 18h ± 2h at 37 ± 1^0C following the standard operating procedure.

At the end of incubation, 0.1 ml of pre-enriched culture were transferred into 10 mL tubes with enrichment broth medium, Modified Laurylsulfate-Tryptose Vancomycin broth (mLST/v). The tubes were incubated for 24h ± 2h at 44 ± 0.5^0C. Following incubations, a loop full from mLST/v, primary enrichment culture, was streaked on one selective solid plate *Enterobacter sakazakii* Isolation Agar (ESIA). Incubation of inoculated ESIA agar plates was done at 44 ± 1^0C for 24 h ± 2h. Examination of the plates for the presence of potential colonies of *C. sakazakii* was carrying out by discriminate typical colonies (1-3 mm blue-green colonies) from other colonies (colourless, straw or purple colonies). At least five blue-green colonies were selected for confirmation and transferred on to agar plate Trypticase Soy Agar (TSA) at 25 ± 1^0C for 46 ± 2h. Typical colonies in TSA are yellow pigmented. Pure culture were tested further by oxidase test and identified by biochemical test using API 20E (Biomeriux, France).

Regarding confirmation of *Enterobacterieceae*, typical mauve colonies were isolated and identified with glucose agar and oxidase test.

Table 1 Positive samples with C. sakazakii and CFU count of Enterobacteriace

Source	***No. of samples 1st round***	***No. of samples 2nd round***	***Total sample/source***	***Positive samples with (C. sakazakii)***	***CFU/ cm2 Enterobacteriaceae 1st round***	**CFU/ cm2 *Enterobacteriaceae* 2nd round**
Filter conditioning	3	3	6	1	210	710
grids	3	3	6	0	150	200
staff hands	3	3	6	0	540	10
Kitchen`s utensils	3	3	6	0	40	150
ktchen`s tables	3	3	6	0	500	620
refrigerator	3	3	6	0	900	700
sink drain area	3	3	6	1	170	120
sponge	3	3	6	0	320	50
storage cabinet	3	3	6	0	400	230
water pipe	3	3	6	0	40	20

3. Results and Discussion

Table 1, Figure 3 and 4 depicts values distribution of *Enterobacteriaceae* in different places of the kitchen and dhe presence/absece of *C. cakazakii* in CFU/cm^2. Counts obtained from two rounds showed minimum and maximum values respectively 1.0E +01 and 9.0E +02 CFU *Enterobacteriaceae*/cm^2.

The refrigerator, personel hands and air conditioning filter were found to have higher concentrations with *Enterobacteriaceae*.

2(3%) samples were identified as positive with *C. sacazakii* . These samples belong to conditioning filter and sink drain area. These results are disturbing in front of microbiological standards applicable in developed countries.

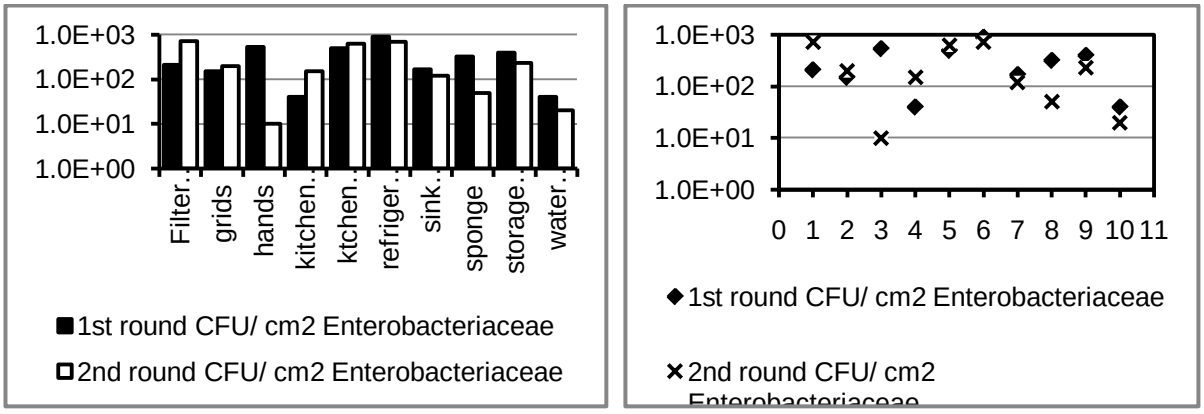

Figure 3(left) and 4 (right). Distribution of Enterobacteriaceae values in hospital kichen areas

Lack of good hygiene practices used from food handlers has been reported to influence directly to microbial rates and could couse food-borne illnesses, which can increase the statistical rates of deaths [4].

Also, the presence of dust, cracks in the ceiling, walls and floor that were found during the sampling may serve as a shelter for microorganisms, increasly the possibility of cross contamination and formation of biofilms.

4. Conclusions

The findings of this study indicate that by using swab surfaces techniques and standard operating procedures for detecton of pathogens as *C. sakazakii* and enumeration *of Enterobacteriaceae* emphasize the importance of monitoring plans implementation at the kitchen environment.

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