

Isolation of *C. Sakazakii* and Concentration of Enterobacteriaceae in Kitchen's Neonatal Intensive Care (NIC)



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Abstract

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). Among infants at greatest risk are infants less than 2 months of age. This research aimed to get more insight the habitat, to control the biological risk of the hospital kitchen's environment at the University Hospital of Obstetrics and Gynaecology "Queen Geraldin", where the powdered 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, the 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 to take measures during preparation and reconstitution of powdered 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. In order to identify potential environmental reservoirs we have analyzed 60 samples from the kitchen areas as well as personnel hands. At the end we have quantified Enterobacteriaceae and isolate *C. sakazakii* from the total of 60 samples. We have detected the presence of *C. sakazakii* in two environmental samples (3.3%) of the cases. Care should be taken while preparing storing and handling PIF and rules should be respected to meet the highest level of microbiological safety in hospital/nursery as defined in MRA Series 10 (FAO/WHO 2004).

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), its epidemiology 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.

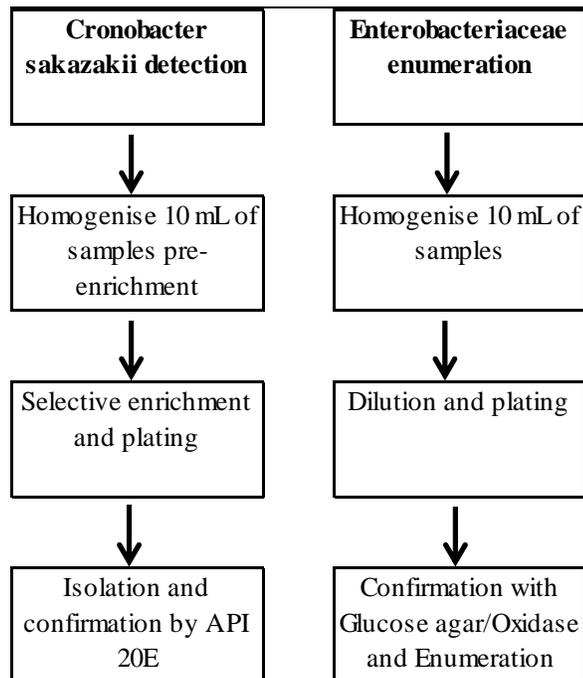
Microbiological investigation was carried out in kitchen`s facilities: working table, kitchen utensils, sponges, conditioning water pipe, sink drain area as well as hands of staff working with foods for infants [1,2].

The swabs were taken from the surface sites by swabbing vertically, horizontally, and diagonally using 100 cm² 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 that were transported to the Laboratory of Food Microbiological Control (FSVI) under chilled conditions 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 ± 1⁰C following the standard operating procedure.

Figure 2. Flow diarams. Detection of *C. sakazakii* and enumeration of *Enterobacteriaceae*Table 1 Positive samples with *C. sakazakii* and *Enterobacteriaceae* count in plate with VRBGA selective media.

<i>Source</i>	No. of samples 1 st round	No. of samples 2 nd round	Total sample/ source	Positive samples with (<i>C. sakazakii</i>)	CFU/ cm ² <i>Enterobacteriaceae</i> 1 st round	CFU/ cm ² <i>Enterobacteriaceae</i> 2 nd round
Filter conditioning grids	3	3	6	1	2,1E+02	7,1E+02
staff hands	3	3	6	0	5,4E+02	1,0E+01
Kitchen`s utensils	3	3	6	0	4,0E+01	1,5E+02
ktchen`s tables	3	3	6	0	5,0E+02	6,2E+02
refrigerator	3	3	6	0	9,0E+02	7,0E+02
sink drain area	3	3	6	1	1,7E+02	1,2E+02
sponge	3	3	6	0	3,2E+02	5,0E+01
storage cabinet	3	3	6	0	4,0E+02	2,3E+02
water pipe	3	3	6	0	4,0E+01	2,0E+01

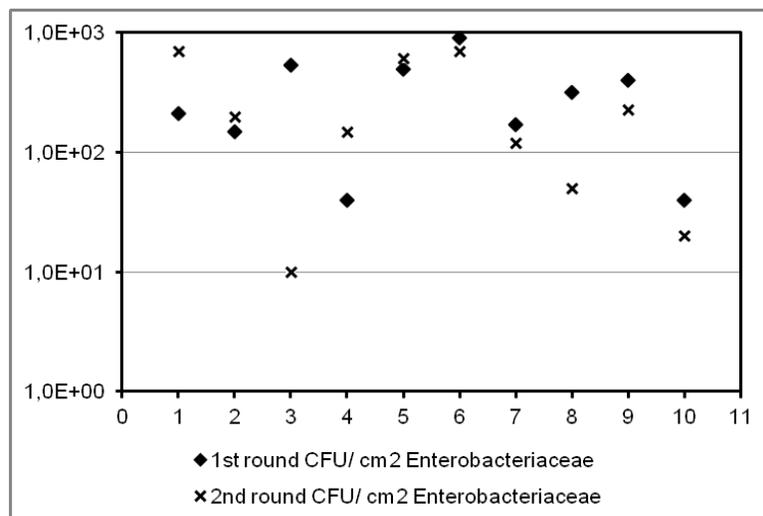
At the end of incubation, 0.1 ml of pre-enriched culture were transferred into 10 mL tubes with enrichment broth medium, Modified Laurylsulfate-Tryptose Vancomycin broth (mLST/v). The tubes were incubated for 24h ± 2h at 44 ± 0.5⁰C. Following incubations, a loop full from mLST/v, primary enrichment culture, was streaked on one selective solid plate *Enterobacter sakazakii* Isolation Agar (ESIA). Incubation of inoculated ESIA agar plates was done at 44 ± 1⁰C 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 $25 \pm 1^{\circ}\text{C}$ for $46 \pm 2\text{h}$. 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 *Enterobacteriaceae*, typical mauve colonies were isolated and identified with glucose agar and oxidase test.

3. Results and Discussion

Table 1, Figure 3 depicts values distribution of *Enterobacteriaceae* in different places of the kitchen and the presence/absence of *C. sakazakii* in CFU/cm². Counts obtained from two rounds showed minimum and maximum values respectively $1.0\text{E} +01$ and $9.0\text{E} +02$ CFU *Enterobacteriaceae*/cm². The refrigerator, personel hands and air conditioning filter were found to have higher concentrations with *Enterobacteriaceae*. Two(3%) samples were identified as positive with *C. sakazakii*. 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. Distribution of *Enterobacteriaceae* values in hospital kitchen areas



Lack of good hygiene practices used from food handlers has been reported to influence directly to microbial rates and could cause 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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