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Detection of *Enterobacter sakazakii* from Commercial Children Dry Milk

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Abstract

This study included isolation and identification of *E. sakazakii* from 51 different samples of powdered infant formula milk involved (Dialak 1 & 2, Celia 1 & 2, Primalac, Biomil 1 & 2, Similac, Nictalia 1 & 2, MAMi, Novolac (AD), Novolac (AR), Novolac (Allernova), S-26 AR, Nursoy, S-26 PDF gold. The results showed that one batch from three of the batches identified of Novolac and Dialak were contaminated and all the types of other infants were non-contaminated. The strains code given as (E1, E2, E3, E4); the bacteria showed resistance to antibiotics used was cephalosporin batch; the third generation showed sensitive to antibiotics life results through inhibition processes such as (Cefotaxime, Sifutetan and Siftadizim), where the diameters of inhibition zone for Siftadizim (18 mm), Sifutetan (22 mm) & Cefotaxime (25 mm) confirmed the bacteria by API and Vitek Compact-2 (biomero).

Keywords

***Enterobacter sakazakii*, Infant Dry Milk, Antibiotic & Vitek Compact-2**

1. Introduction

Enterobacter sakazakii is a gram-negative bacteria, motile, non-spore forming, facultatively anaerobic, bacillus forming yellow pigmented colonies after 24 - 48 hours at 37˚C incubation on a non-selective medium rod that was formerly known as "yellow-pigmented *Enterobacter cloacae*" until 1980 [1]. This bacterium is an emerging opportunistic pathogen predominantly associated with bacterial meningitis in immune compromised neonates [2]. Other clinical presentations of infection include bacteremia and necrotizing enterocolitis [3]. It appears that

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the frequency of *E. sakazakii* infections is low. *Enterobacter sakazakii* has been associated with life-threatening infections in premature low-birth-weight infants. Contaminated infant milk formula (IMF) has been implicated in cases of *E. sakazakii* meningitis. Sensitive and quick methods to reveal low level of pollution sporadically present in IMF preparations would positively contribute towards risk reduction across the infant formula food chain. The bacterium has been cultured from an assortment of food matrices, including meat, grain, cheese, vegetables, spices, bread and herbs [4]. Though the normal habitat of *E. sakazakii* has yet to be specified, infant milk formula (IMF) has been epidemiologically linked to cases of neonatal meningitis. As an oral pathogen reason for systemic infection, *E. sakazakii* must be in possession the instrumentarium to interfere the epithelial cell obstacle in the bowel in order to access the blood circulation and diffusion. Availability of an *in vitro* cell culture model is essential to study the primary steps of entry of *E. sakazakii* into eukaryotic host cells and to identify possibility virulence factors involved in such procedures. We used gentamicin protection examinations and confocal imaging of fluorescently tagged *E. sakazakii* cells to presenting that this bacterium actively invades human epithelial Caco-2 cells. Both f-actin and microtubule structures are needs for infestation. Disruption of cellular tight intersection increased the primary assembly of *E. sakazakii* with Caco-2 cells and significantly enhanced the ability to pervade. Showed that *E. sakazakii* is able to penetrate rat brain endothelial cells and to survive inside macrophages. The locative evidence also implies that *E. sakazakii* has to be able to translocate through the intestinal barrier and establish a systemic infection with symptoms such as bacteremia and meningitis [5]. Invasive bacteria are able to manipulate the host cell cytoskeleton. Doing this either directly by active secretion of bacterium while actin microfilaments are frequently associated with the bacterial invasion process, microtubules can be also involved in breakthrough by microbial pathogens [6].

2. Objectives and Aim of the Study

Study aimed to detection and identification *Enterobacter sakazakii* from different type of infant dry milk by new methods and confirmatory API 20 E and Vitek compact-2.

3. Material and Methods

3.1. Milk Samples

Fifty one samples have been received from public health laboratory of the Central. Samples were collected over a period of nine months between February and December of 2014.

3.2. Isolation of *Enterobacter sakazakii*

Enrichment step dilution in peptone water and transfer (1 gm, 10 gm & 100 gm into (9 ml, 90 ml & 900 ml)) triple cat in EE broth (Enrichment Enterobacter broth) subsequent isolation of pure colonies on violet red bile glucose agar. Several isolated colonies were selected and streaked onto tryptone soy agar (TSA). Typical yellow-pigmented colonies are detected after an overnight incubation for 48 to 72 h at 25˚C.

3.3. The Identification

These presumptive colonies are identified biochemically [7]. This approach provides only a generic test for Enterobacteriaceae and lacks comparison of the effectiveness of antibiotics for the first and third generation cephalosporins within the group against bacteria the necessary capability to specifically identify *E. sakazakii*. Recently, a number of eclectic have become available to assist identification. One of these is the API 20 E biochemical test and vitek confirmatory system. Sakazakii were time and enriched for 6 h at 42˚C. summarizes the findings of these experiments. This method dependably revealed between 1 and 5 CFU *E. sakazakii* in 100 g with higher inocula producing a higher recovery of *E. sakazakii*. Tests were conducted according to the instructions and approach Food and Drug Administration [7]. The limit of detection was determined to be 10 CFU/ml (equivalent to 2.5×10^3 CFU in (250 ml)).Biochemical profiles are frequently used following primary isolation (**Figure 1**), but contradictions in identification may occur in different biochemical kits for the same strain [8].

3.4. Antibiotic Susceptibility Test

Antibiotic was done for each isolate by standard Kirby Bauer method [9]. Muller Hinton agar plates were used

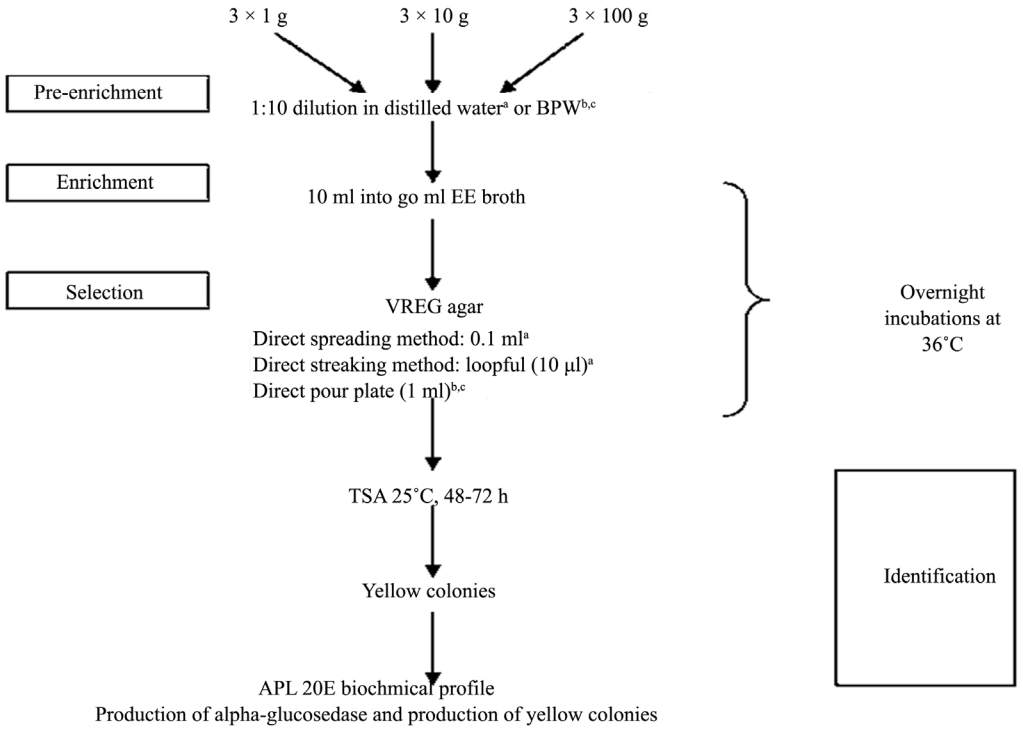

Figure 1. Quantitative *E. sakazakii* isolation procedure.

for bacterial culturing, disc of Cefotaxime, Sifutetan & Siftadizim. Were put over bacterial growth, plates were incubated for 18 hr at 37C, diameter of cleared zones were recorded for results.

3.5. Biochemical Test

Studies have demonstrated that 100% of *Enterobacter sakazakii* are positive for *α*-glucosidase while 100% of other Enterobacter species are negative for this enzyme [10]. On the basis of these observations, the chromogenic substrate 5-bromo-4-chloro-3-indolyl-*α*-D-glucopyranoside (X-*α*-glucoside) has been proposed to differentiate *Enterobacter sakazakii* from other members of the Enterobacteriaceae family [11]. The enzyme *α*-glucosidase hydrolyzes the X-*α* (**Tables 1-3**).

4. Results and Discussion

Enterobacter sakazakii was isolated from four (1.56%) out of the thirty-nine tested infant formula milk. Five strains were isolated and were identified by the FDA method 2002 and API 20 E and Vitek compact-2 (biomero) suggested that the inability to isolate *Enterobacter sakazakii* from powdered formula may have been due to its unequal allocation within the powdered formula or "its existence at such a low concentricity that it escaped disclosure by traditional methods as described by the Food and Agriculture Organization [5] [12]. This study proved that some international global milk powder contaminated with bacteria *Enterobacter sakazakii* and that the children of these bacteria have done a pathogencity represented by diarrhea and severe acute diarrhea. Bacteria were isolated from these four marks: Novolac (AD), Novolac (AR), Novolac (Allernova), Dialak and proven contamination by screening method that recommended by FDA 2002 where the isolation rate (1.56%). Isolates obtained from milk cans indicate resistance to these isolates heating during the manufacturing process and to the inefficiency of the manufacturing process [13]. The results of this study were in line with the results obtained by the researchers [14], as the isolated bacteria from baby milk powder and by (6.80%). The results of the study differed with the study [1]. The researchers have stressed the milk contamination with bacteria *Bacillus cereus* and the reason for their appearance is due to the production of toxins casuals and heat resistance. Tested isolates were resistant to antibiotics where used Cephalosporine group compared to the work of the antibiotic first generation and third. Study showed the first generation (Cephalothin, Safaberden, cefazolin, cephalexin and

Table 1. Biochemical test of *Enterobacter sakazakii* and different bacteria.

Citrate	Positive	Positive	Positive
Glucose	K/A Positive	A/A Positive	A/A Positive
Sucrose	Positive	Positive	Positive
Lactose	Positive	Positive	Positive
α-glucosidase	Positive	Negative	Negative
Urease	Positive	Negative	Negative

Table 2. The proportion of bacteria in milk samples.

Type of samples	Total of samples	Isolated	Percentage
Dialak 1, 2	51	1	1.49
Novolac AD	51	1	1.49
Novolac AR	51	1	1.49
Novolac Allernova	51	1	1.49

Table 3. Isolated bacteria from different types of children dry milk.

Type of Bacteria	Batch No.	Production time	Country of origin	Duplicates	Type of milk
	24 - 25	07.2013			
E. sakazakii	15 - 19	03.2013	Vietnam	3	Dialak 1, 2
	19 - 32	10.2013			
	19 - 27	11.2013			
	11 - 15	08.2013	USA	3	Celia 1, 2
	6 - 64	10.2013			
	15 - 20	04.2013			
	22 - 30	08.2013	Switzerland	3	Primalac
	12 - 45	12.2013			
	8500	10.2013			
	5500	07.2013	Belgium	3	Biomil 1, 2
	2000	04.2013			
	09 - 42	11.2013			
	03 - 26	07.2013	Ireland	3	Nursoy
	02 - 17	03.2013			
	41306	03.2013			
	41308	03.2013	Poland	3	Mami
	41900	11.2013			
	36420	03.2013			
	64222	06.2013	France	3	Nicalia 1, 2
	92391	09.2013			
	02 - 20	03.2013			
	06 - 44	07.2013	Ireland	3	Similac
	02 - 57	09.3013			
	20 - 57	02.2013			
E. sakazakii	16 - 32	07.2013	Germany	3	Novolaclernova
	05 - 46	10.2013			
	08 - 52	02.2013			
E. sakazakii	10 - 58	07.2013	Germany	3	Novolac AD
	03 - 36	10.2013			
	19 - 44	02.2013			
E. sakazakii	08 - 30	03.2013	Germany	3	Novolac AR
	16 - 16	07.2013			
	2A02	10.2012			
	2A27	03.2013	Ireland	3	S-26 AR
	2A34	10.2013			
	2L14	07.2012			
	2L22	03.2013	USA	3	S-26 LF
	2L28	11.2013			

Table 4. Sensitivity of test *Enterobacter sakazakii* for third generation measured in Millimeter (mm) zone of inhibition according to (NCCL).

Antibiotic	E1	E2	E3	E4
Cefotaxime	25	25	25	25
Sifutetan	22	22	22	22
Siftadizim	18	18	18	18

cephradine) did not have any result zone of inhibition. The third generation of the same batch of antibiotic which shows sensitive results through inhibition processes such as (cefotaxime (25 mm), Sifutetan (22 mm), Siftadizim (18 mm)), where the diameters of inhibition zone for Cefotaxime, Sifutetan and Siftadizim. The use of minimum inhibitory concentration Cephalosporine batch shows cefotaxime, Sifutetan and Siftadizim inhibition zone. The major cause is due to the group operates stop the work of the gyrase enzyme is present in prokaryotes and some eukaryotes work by competitive suppression of energy transduction of DNA gyrase by binding to the ATPase active site located on the GyrB subunit. Quinolones bind to these enzymes and prevent them from decatenation replicating DNA. Quinolone-resistant bacteria repeatedly harbor convert topoisomerases that resist quinolone binding 2009 Eukaryotic Cell, 11(8), 1759-1769. Gore J., Bryant Z., Stone M.D., Nollmann M., Cozzarelli N.R. 2006. Used in the study [5], this was identical to the present study in the case reported cephalosporins, such as cefepime, in collection with a second agent. Two studies in [15] and [2], supported the use of cefotaxime for childhood bacterial meningitis. In each of these studies, *Enterobacter sakazakii* meningitis was identified in at least 1 child, and the infection was treated successfully. In another study, [16] described *E. sakazakii* infection in 4 adults that occurred in 1995 and 1996 at the University of Massachusetts Medical Center. Of the 4 adults, 2 presented with pneumonia and 2 with bacteremia. The isolates were uniformly resistant to ampicillin, cefazolin, and extended-spectrum penicillins and were not uniformly susceptible to third-generation cephalosporins or to quinolones. This study proved that the four isolates were sensitive to the third generation cephalosporin group 9, **Table 4**.

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