

Detection of *Enterobacter sakazakii* and other *Enterobacter* sp from Dairy Cow's Milk in Boyolali and Sleman

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ABSTRACT

Enterobacter sakazakii is considered as an opportunistic pathogen that has been implicated in severe forms of necrotizing colitis and meningitis especially in neonates with a mortality rate varying from 40%-80%. The natural habitat *E.sakazakii* is not well understood and has been reported as frequently isolated from different environments including soil, rats, flies, milk powder factories, chocolate factories and households. A total of 100 samples were obtained from dairy cow's milk were studied. The presence of *E.sakazakii* and *Enterobacter* sp was detected using the Holt et al., 1994; Guillaume et al., 2005 and Turner et al., 2000 procedure on TSBA medium. *E.sakazakii* was not isolated from both district Sleman and Boyolali. However, *E.cloacae* was found in 33 of 75 isolates (44%) of samples from Sleman. Meanwhile 12.7% *E.cloacae* and 5.4% *E.gergoviae* was found of samples from Boyolali.

Key words: *Enterobacter sakazakii*, *Enterobacter* sp, dairy cow's milk

INTRODUCTION

Enterobacter sakazakii and other *Enterobacter* species have caused foodborne illness through consumption of a variety of foods. *Enterobacter sakazakii* is a Gram negative, facultative, rod-shaped bacterium. *Enterobacter sakazakii* is a Gram negative, facultative, rod-shaped, non-spore forming bacterium. The organism was called "yellow-pigmented *Enterobacter cloacae*". It belongs to the family *Enterobacteriaceae* and genus *Enterobacter* that contains a number of species including *E.agglomerans*, *E.cloacae*, *E.aerogenes* and *E.gergoviae*.

The differentiation among these species is based on biochemical reactions, and serological and molecular techniques (Hoffman and Roggenkamp, 2003; Iversen et al., 2004). *E.sakazakii*, *E. agglomerans*, and *E.cloacae* are considered the main species of this genus that are frequently isolated from clinical samples and food products (Farmer et al., 1980). *E.sakazakii* and *Enterobacter* species have been reported as frequently isolated from different environments including soil, rats, flies, milk powder factories, chocolate factories and households (Kandhai et al., 2004). *E.sakazakii* has been also isolated from a wide range of foods including ultra high-

temperature treated milk (UHT milk), cheese, meat, vegetables, grains, sorghum seeds, rice seeds, herbs, spices, fermented bread, fermented beverage, tofu, and sour tea (Iversen & Forsythe, 2004; Leclercq et al., 2002).

E. sakazakii is considered an opportunistic pathogen that has been implicated in severe forms of necrotizing colitis (Van Acker et al., 2001) and meningitis (Bar-Oz et al., 2001) especially in neonates with a mortality rate varying from 40% to 80% (Muytjens et al., 1988).

The US Food and Drug Administration (FDA, 2002) has issued an alert to health care professionals about the risk associated with *E.sakazakii* infections among neonates fed with milk-based infant formula. The alert stated that a major contribution to the avoidance of *E.sakazakii* infection in premature babies and neonates is the prevention of contamination of infant milk formula during production and bottle preparation. However, knowledge of etiological and ecological characteristic of *E.sakazakii* is sparse and its occurrence in factories that produce infant formulas and in hospital kitchens has not been studied in depth.

The natural habitat *E.sakazakii* is not well understood and has been reported as frequently isolated from different environments including

soil, rats, flies, milk powder factories, chocolate factories and households. The organism is known to tolerate extremely dry condition. In 2003-2004 announced finding that 22 % of formula milk for infants on the market in Indonesia was infected with the *E.sakazakii*. In a survey of infant formula products from 11 countries, was isolated *E.sakazakii* 13.5%.

Enterobacter cloacae and *E. aerogenes* are opportunistic pathogens. The most common infection they produce is bovine mastitis. *Enterobacter sakazakii* is known occasionally to cause meningitis and sepsis in human neonates (Carter and Wise, 2004). *Enterobacter cloacae* causes occasional bacteremia in human and *E.aerogenes* can be associated with mastitis. *Enterobacter .sakazakii* is the name given to the yellow-pigmented variant of *E. cloacae*; it is isolated from food but only rarely from human clinical specimens.

The objective of this study was to investigate the prevalence of *E.sakazakii* and *Enterobacter* sp in dairy cow's milk in Sleman and Boyolali.

MATERIALS AND METHODS

All media materials used in the study were obtained from Oxoid. Dairy cow's milk samples

A total of 100 samples were obtained by using bottles, 50 samples from dairy cow's milk from Sleman and 50 samples from dairy's cow from Boyolali. These samples were collected from udder when a time for milking.

Detection, isolation and identification of *Enterobacter* sp. The procedure for detection, isolation and identification of *E. sakazakii* and other *Enterobacter* sp by the Holt et al., 1994; Guillaume et al., 2005 and Turner et al., 2000 procedure on TSBA medium. Milk on bottles was homogenate by centrifugation. A loopful of the suspension was streak on TSBA (Enrichment culture). The plates were incubated for 18-24 h at 36 °C. Five colonies of the yellow colonies were then tested by Motility, Sitrat, MR and VP for

examine genus *Enterobacter*. *Enterobacter* sp. appeared under microscope as short-rods in shape and was Gram negative. For determine *E.sakazakii* and other *Enterobacter* sp that had tested of Sitrat (+), MR (-), VP (+) and Motil were then tested Urea, KCN and Lisin D (Holt et al., 1994).

RESULTS AND DISCUSSION

Detection by isolation *E.Sakazakii* and other *Enterobacter* from dairy cow's milk are given in Table 1. The positive *E.sakazakii*, *E.cloacae*, and *E.gergoviae* formed yellow colonies on TSBA after 18-24 h on incubation at 37 °C (Figure 1). Gram stain of *Enterobacter* sp. given in Figure 2.



Figure 1. *Enterobacter* sp in TSBA



Figure 2. Gram Stain of *Enterobacter* sp

Table 1. Percentage of *Enterobacter* sp. were Isolated from Dairy Cow's Milk in Sleman and Boyolali

Dairy cow's milk Farm location	Number of samples	<i>Enterobacter</i> sp		
		<i>E. sakazakii</i>	<i>E. cloacae</i>	<i>E. gergoviae</i>
Boyolali	50	-	19 (12.7%)	1 (0.7%)
Sleman	50	-	33 (44%)	-

E.sakazakii was not isolated from both district Sleman and Boyolali. However, *E.cloacae* was found in 33 of 75 isolates (44%) of samples from Sleman. Meanwhile 12.7% *E.cloacae* and 5.4% *E.gergoviae* was found of samples from Boyolali. This result is likely the result of Budiarsa (2007) none of *E.sakazakii* was isolated from manure, farm environment and raw milk in Yogyakarta. *Enterbacter cloacae* and *E. gergoviae* are category in B, have been associated with neonatal infections which include necrotizing enterocolitis, which is the most common important gastrointestinal illness in the new born (FAO/WHO, 2004; Iversen & Forsythe, 2004b). FAO/WHO have stated "Other Enterobacteriaceae are in category "B" because they are well-established causes of illness in infants (e.g. systemic infection, NEC and severe diarrhea) and have been found in powdered infant formula, but contaminated powdered infant formula has not been convincingly shown, either epidemiologically or microbiologically, to be the vehicle and source of infection in infants. These organisms include, *Enterobacter agglomerans*, *Hafnia alvei*, *Klebsiella pneumonia*, *Citrobacter koseri*, *C. freundii*, *Klebsiella oxytoca* and *Enterobacter cloacae* (Lehner and Stepan, 2004). Shaker et al., 2007 reported *E.sakazakii* was isolated from (2/15) infant food formula (2/8) infant milk formula and (1/8) cereal products (semolina) but none of the powder milk (full cream) cereal product (starch, ground rice, bread crumbs, oat, flour, mixed spices, fine sugar) and environmental samples. Moreover *E.cloacae* was isolated in infant formula, cereal product factory.

More research is needed to determine the sources of *E.sakazakii* for example : the raw material and particular the heat sensitive nutrient added after pasteurization, the processing environment i.e equipment and processing lines. Also studies of condition affecting biofilm formation by *E.sakazakii* in food factories.

CONCLUSIONS

The result of this study show that *E.sakazakii* was not isolated from both district Sleman and Boyolali dairy cow's milk farm. However, *E.cloacae* was found in 33 of 75 isolates (44%) of samples from Sleman, meanwhile 12.7% *E.cloacae* and 5.4% *E.gergoviae* was found of samples from Boyolali.

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