

Cronobacter Sakazakii (Enterobacter Sakazakii)

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ABSTRACT

Enterobacter sakazakii (*E. sakazakii*) is an opportunistic pathogen gram-negative, motile with peritrichous flagella, nonspore-forming, gram-negative, belonging to the family of Enterobacteriaceae. It considered being a food borne pathogen causing meningitis, septicemia and enterocolitis in neonates, cerebral infarcts with resulting in a premature infant.

In poultry industry the organism was reported to contaminate fertilized eggs and may result in weak chicks, poor chick growth and low FCR, increased mortality of embryos, lower hatchability and increased early chick mortality. Pathogenicity of *E. sakazakii* to broiler chickens in the form of clinical signs, mortality, pathological lesions and decreased FCR was reported.

Conclusion: As poultry remains a vehicle of important pathogens to human as well as the possible economic losses a strategy must be considered in the evaluation, prevention, and control of this infections.

Keywords: *E. sakazakii*, food borne pathogen, Pathogenicity, chicks, clinical signs, pathological lesions.

INTRODUCTION

Enterobacter spp. is the sixth most common cause of nosocomial infection and antibiotic resistant strains are observed with increasing frequency (Peters et al., 2000). *Enterobacter spp.* are not primary human pathogens, however *E. cloacae* have been implicated in a broad range of clinical syndromes (Liu et al., 2004).

From the public health importance; *E. sakazakii* is an opportunistic pathogen causing meningitis, septicemia and enterocolitis in neonates (Bar-OZ et al 2001), multiple cerebral infarcts with resulting multicystic encephalomalacia in a premature infant with *E. sakazakii* meningitis (Gurtler et al.,2005). Furthermore, Kothary et al (2007) stated that very little information is available regarding pathogenicity of the organism and production of virulence factors. *C. sakazakii* is a well-known special microorganism that can pass through the barrier between brain tissues and circulating blood. Therefore, it can damage human Endothelial microvascular brain cells (HBMEC) (Giri et al., 2012).

It is considered to be a food borne pathogen, which used to be known as a "yellow pigmented *Enterobacter cloacae*" until 1980, when it was

introduced as a new species (*E. Sakazakii*). Recently, a taxonomic reclassification of this pathogen to consist of 5 species within a new genus "*Cronobacter*" was proposed (Baumgartner et al., 2009).

In poultry industry the organism was reported to contaminate fertilized eggs and may result in weak chicks, poor chick growth and low FCR (Ramnoff, 1960), increased mortality of embryos, lower hatchability and increased early chick mortality (Milakovic-Novak and Prukner, 1990). The organisms were also reported on the eggshell surface, cloacal swabs, commercial eggs and fertilized eggs (Al-Bahry et al., 2010, AbdEllatif, 2013). Hai et al. (2012) isolate 11 bacteria from the affected chicks and identified as *Enterobacter*, and stated that these isolates were highly pathogenic to chickens by experimental infection and this explains the high mortalities occurred when *E. sakazakii* injected into chicks. AbdEllatif (2013) reported pathogenicity of *E. sakazakii* to broiler chickens with clinical signs, mortality, pathological lesions and decreased FCR. Poultry remains a vehicle of important pathogens such as Enterobacteriaceae (Threlfall et al., 1993, Weinstein, 1993).

ECONOMIC IMPORTANCE

In 2002, the International Commission on Microbiological Specifications for Foods (ICMSF) has ranked *C. sakazakii* as "dangerous and life threatening with substantial chronic side effects" (Iversen and Forsythe, 2003, Arroyo et al., 2011). Afterwards, it has a similar ranking with some familiar food and water-borne pathogens such as *Listeria monocytogenes*, *Clostridium botulinum* types A and B and *Cryptosporidium parvum* (Iversen and Forsythe, 2003).

INCIDENCE AND DISTRIBUTION

C. sakazakii is reported to be commonly isolated from different environments and food sources (Friedemann, 2007, Joseph et al., 2012). It has also been isolated from the surfaces of the equipments and food production environments (Ye et al., 2015).

C. sakazakii has been isolated from floor drains, air, vacuum canister, broom bristles, room heater, electrical control box, transition socks, a clean-in-place valve, floor, and condensate in a dry product processing plant (Willis and Robinson, 1988). Van Os et al. (1996) have isolated *C. sakazakii* from grass silage in the Netherlands. It has also been isolated from hospital air (Masaki et al., 2001), clinical materials (Janicka et al., 1999; Tuncer and Ozsan, 1988), rats (Gakuya et al., 2001), soil (Neelam et al., 1987), rhizosphere (Emilani et al., 2001), sediment and wetlands (Espeland and Wetzel, 2001), crude oil (Assadi and Mathur, 1991) and cutting fluids (Suliman et al., 1988).

BACTERIOLOGY OF THE ORGANISM

Scientific Classification

In 2007, the genus *Cronobacter* was created to accommodate the biogroups of *E. sakazakii*, with *C. sakazakii* as the type species. The genus was named for Cronos, the Titan of Greek myth, which devoured his children as they were born (Henry, 2018).

E. sakazakii was classified as a new genus, *Cronobacter* within the Enterobacteriaceae, initially comprising four named species in 2007. The taxonomy was expanded to five named species in 2008, and more recently (2011) to seven named species (Iversen et al., 2007, Iversen et al., 2008, Joseph and Forsythe, 2011, Joseph et al. 2012).

The initial four named species in 2007 were *C. sakazakii* (comprising two subspecies), *C.*

turicensis, *C. muytjensii* and *C. dublinensis* (comprising three subspecies) plus an unnamed species referred to as *Cronobacter* genomospecies I. The taxonomy was revised in 2008 to include a fifth named species *C. malonaticus*, which in 2007 had been regarded as a subspecies of *C. sakazakii*. In 2012, *Cronobacter* genomospecies I was formally renamed *C. universalis*, and a seventh species was described called *C. condimenti* (Iversen et al., 2007; Iversen et al. 2008; Ye et al. 2015).

Morphology

C. sakazakii is a food-borne pathogen belonging to the family of Enterobacteriaceae with characteristics such as being facultative anaerobe, nonspore-forming, gram-negative, motile with a peritrichous flagella and rapid growth on laboratory media (Farmer et al., 1980, Feeney et al., 2014).

Culture Media

This organism could grow on agar plate with two forms of colonies: glossy or matte, which depends on bacterial strain and its growth environment. The clinical isolates of the bacterium produce only slightly yellow pigmentation when cultured on nutrient agar at 37°C, but produce a non-diffusible yellow-gold pigment when incubated at room temperature. It can grow on MacConkey agar with "Blue-Green" colonies, because it can produce α -glucosidase enzyme (Fakruddin et al., 2014). This bacterium can also grow on Eosin Methylene Blue (EMB) and deoxycholate agar. It can be identified with a typical non-diffusible yellow pigment colonies on Tryptic Soy Agar (TSA) at 25°C (Iversen and Forsythe 2003, Fakruddin et al., 2014).

E. sakazakii was isolated on violet red bile glucose agar plates inoculated with rinses from shell eggs collected at various stages of processing from 3 US commercial shell egg processing plant on 3 separate visits. *E. sakazakii* constitute 28 isolates from total isolates 549 in plant x and constitute 4 isolates from total isolates 67 in plant y and 5 isolates from total isolates 220 in plants (Musgrove et al., 2008).

BIOCHEMICAL CHARACTER

Voges-Proskauer test is positive for this bacterium, while citrate assimilation, β -glucosidase (ONPG) and methyl red test reactions are negative (Nazarowec-White and Farber, 1997). The bacterium is indole positive,

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oxidase negative, catalase positive and citrate positive, MR-VP and nitrate reduction negative, and it is able to ferment glucose with the production of acid and gas, lactose rhamnose, xylose, trehalose, arabinose, cellubiose and melibiose. It can also decarboxylate arginine, hydrolyseesculin andliquefy gelatin, but it cannot ferment dulcimer and malonate (Muytjens and Van Druten, 1984, Leclerc, 2001, Mullane et. al., 2006)).

C. sakazakii is able to produce a delayed extracellular DNAase reaction against toluidine blue agar at 36°C after 7 days. It is á-glucosidase positive that can be recognized by 4- nitrophenyl-a-d- glucopyranoside after 4 h at 36°C. Researchers have found two major differences between *C. sakazakii* and other Enterobacter species; one of them is á-glucosidase activity which was shown in all *C. sakazakii* strains, but it was not found in any of the Enterobacter strains; therefore the absence of phosphoamidase enzyme was unique in *C. sakazakii* isolates (Muytjens and Van Druten, 1984).

The organism produces d-lactic acid and it is mucate negative. Most of the isolates produce esterase enzyme, this indicates another difference between *C. sakazakii* and *E. cloacae* (Postupa and Aldova, 1983) along with not fermentation of sorbitol. It can produce a novel hetero polysaccharide comprising 29-32% glucuronic acid, 23-30% dglucose, 19-24% d-galactose, 13-22% d-fucose and 0-8% d-mannose (Farmeret al., 1980, Iversen and Forsythe, 2003, Nazarowec-White and Farber, 1997, Harris and Oriel, 1989, Abdel-Galil et al., 2016). Biochemical tests for the identification of *C. sakazakii* are shown in Table 1.

Table1. Biochemical Identification Test of *C. sakazakii*

Biochemical Test	Result
Tween 80 esterase	Positive
Phosphoamidase	Positive
α-glycosidase	Positive
Yellow pigmentation on TSA	Positive
D-sorbitol fermentation	Negative

VIRULENCE FACTORS AND GENES

C. sakazakii veriolanc factors including: flagellum functions as an immune stimulus for the production of pro-inflammatory cytokines within human-derived monocytes and outer membrane proteins including OmpA, OmpX, and Inv, which are important for bacterial invasion into host cells (Chandrapala et al., 2014, Cruz-Cordova et al., 2012, Kim et al.,

2010). Lipopolysaccharides (LPS) are also essential for the invasion of *C. sakazakii* into intestinal epithelial cells via disrupting tight junctions (Chandrapala et al., 2014). Other virulence-associated genes in *C. sakazakii* include zpx encoding cell-bound zinc-containing metalloprotease, cpa encoding an outer membrane protease, mcp encoding a methyl-accepting chemotaxis protein, and bcs ABC operon responsible for cellulose biosynthesis (Kothary et al., 2007, Franco et al., 2011, Hu et al., 2015, Choi, et al.,2015).

ANTIBIOTIC SUSCEPTIBILITY

The efficiency and productivity of disinfectants are usually employed in both clinical and food environments to promote clean surfaces and to avert Cronobacter spp. (Kim et al., 2007). Both antibiotic sensitivity and bacterial resistance have been managed after a couple of deadly reported infections among the newborns (van Acker et al., 2001, Simmons et al., 1989, Arseni et al., 1987).

The tested Cronobacter isolates (53) were resistant to erythromycin, but varied in their susceptibility to tetracycline, with 35.8% of strains being susceptible to tetracycline, 60.4% of strains showing moderate resistance and 3.8% of micro-organisms being resistant to this antibiotic. All micro-organisms isolated from food samples were susceptible to b-lactams, cepheims, carbapenems, aminoglycosides, quinolones, nitrofurans compounds Chloramphenicol, diaminopyrimidine derivative, trimethoprim and co-trimoxazole (Hochel et al., 2012). Terragno et al. (2009) tested the susceptibility of 23 Cronobacter spp., isolated from powdered infant formulae, to amoxicillin / clavulanic acid, ampicillin, cefotaxime, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, tetracycline, co-trimoxazole, cefuroxime and cefixime. These authors found that all of their isolated strains were susceptible to all the antibiotics tested. Kim et al. (2008) described Cronobacter spp. strains recovered from various foods that were susceptible to tetracycline and resistant to ampicillin or cephalothin. Cronobacter spp. isolated from Mexican fruit flies *Anastrepha ludens* was also resistant to ampicillin and cephalothin, as well as to erythromycin, novobiocin and penicillin, but they were susceptible to tetracycline (Kuzina et al. 2001). In contrast, Nazarowec-White and Farber (1997) found that two of eight Cronobacter strains recovered from food samples showed resistance to chloramphenicol

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and tetracycline. Farmer et al. (1980) described the susceptibility pattern of 24 *E. sakazakii* (Cronobacter spp.); all strains were susceptible to gentamycin, kanamycin and chloramphenicol, 97% were susceptible to nalidixic acid, 92% were susceptible to streptomycin, 87% were susceptible to tetracycline and carbenicillin, 67% were susceptible to sulfadiazine and 13% were susceptible to cephalothin. All strains were resistant to penicillin. These authors noted that only 1 strain out of >100 Cronobacter strains tested were multiresistant to streptomycin, kanamycin, tetracycline and chloramphenicol.

PROBIOTICS ON INTESTINAL PATHOGENIC ENTEROBACTER

It was found several years ago that probiotic and or prebiotic play an important role in protection against intestinal pathogen, Pivnick et al. (1981) reported that the competitive exclusion concept involved introduction of intestinal bacteria from mature chickens into newly hatched chicks, implied the prevention of entry of one agent into a given environment because that space was already occupied, several years later probiotics was identified by Fuller (1989) as live cultures of microorganisms administered orally, acted beneficially on host health through inhibiting pathogens, enhancing intestinal immunity, and having a protective effect on the gut micro flora, later on Havenaar and Spanhaak (1994) found that probiotic stimulated the immunity of the chickens in two ways. Where flora from probiotic migrated throughout the gut wall and multiplied to a limited extent or antigens released by the dead organisms was absorbed and thus stimulated the immune system. Probiotic organisms can be horizontally transferred from treated birds to control non treated (Fritts et al., 2000).

Fuller (1989) defined Probiotics as live cultures of microorganisms administered orally, acted beneficially on host health through inhibiting pathogens, enhancing intestinal immunity, and having a protective effect on the gut micro flora. The mechanisms by which the indigenous intestinal bacteria inhibit pathogens included competition for colonization sites, competition for nutrient, production of toxic compounds (volatile fatty acids, low pH, and bacteriocin), or stimulation of the immune system (Patterson and Burkholder 2003).

The antimicrobial efficacy of probiotics against *E. sakazakii* (ES) was evaluated in reconstituted dried infant formula. *Enterococcus faecium* M-

74 (EF) exhibited the strongest inhibition when compared with *Lactobacillus acidophilus* 74-2 (LA) and *Pediococcus acidilacticii* (PA) (Lihono et al., 2011).

Natural Hosts

The natural habitat is not well understood; however, they have been isolated from a diverse range of environments, e.g. processing plants, domestic environments, and foods, e.g. powdered infant formula, fermented bread and cheese.

Experimental Hosts

The effect of experimental infection of layer breeder native chickens with *C. sakazakii* on layer and hatchery parameters was studied by Amer et al. (2019). The results indicate that laying chickens exposed to *C. sakazakii* infection, the infection passes to some of their eggs and affect hatchability. This result is suggestive the cycle of such infection.

Clinical Signs in Avian Species

E. sakazakii was reported to contaminate fertilized eggs and may result in weak chicks, poor chick growth and low FCR (Ramnoff, 1960), increased mortality of embryos, lower hatchability and increased early chick mortality (Milakovic-Novak and Prukner, 1990, Amer et al., 2015). Abd El-Galil et al. (1995) studied bacterial causes of lowering hatchability and early embryonic chicken deaths in balady hatcheries. Enterobacter sp. isolates from nonfertile and dead in shell embryos (7.5 and 5.5%); respectively.

Experimentally, it was reported that at the 1st dpi clinical signs were dullness, depression, sleepy and ruffled feather. These clinical signs were gradually developed to brown diarrhea, enlarged shank and coughing from 2nd to 4th dpi but in 5th dpi one chick was dead (AbdEllatif, 2013).

Gross Lesions

Septicemic picture from the 1st to the 4th dpi appeared as congested lung, air sacculitis and Spleen, hepatitis with streaks of hemorrhages on its surface, distended gall bladder, congested kidney, slight to moderate pericarditis, petechial hemorrhage on coronary fat, endocardium and on brisket muscle, Atrophied jejunum and enlarged ceci filled with yellowish to greenish or brownish materials with gases. Estimated performance parameters resulted that infected group showed in FCR (1.7) while control non infected it was 1.47 (AbdEllatif, 2013).

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Cronobacter species are opportunistic pathogens that can cause necrotizing enterocolitis, bacteremia and meningitis, predominantly in neonates. Infection in these vulnerable infants has been linked to the consumption of contaminated powdered infant formula (PIF) (Yan et al., 2012).

Histopathology

Histopathological changes in *E. Sakazakii* experimentally infected broiler chicks group at 2nd dpi liver showing focal area of necrosis infiltrated with leucocytic cells infiltrations, at 4th dpi liver showing focal necrotic area with leucocytic infiltrations. *E. Sakazakii* infected group at 6th dpi liver showing portal tract permeation leucocytic infiltrations, and at 8th dpi it was showing congestion and leucocytic permeation. Intestine of *E. Sakazakii* infected group 2 dpi, showing degenerated mucosa and submucosal gland necrosis, while infected groups with probiotic at 2nd dpi intestine showing normal mucosa with slight muscular vessels dilatation. At 4 dpi showing massive submucosal mononuclear cells infiltration, at 6 dpi showing submucosal leucocytic infiltrations. In the other hand infected group with probiotic at 6th dpi Intestine showing slight mucosal degenerative changes. At 8 dpi infected chicken intestine showing necrotic sub mucosal glands (AbdEllatif, 2013).

Clinical pathology

No available literature in this aspect.

DIAGNOSIS OF C. SAKAZAKII INFECTION

Isolation and Identification

The organism was reported on the eggshell surface, cloacal swabs, commercial eggs and fertilized eggs (Al-Bahry et al., 2010, AbdEllatif, 2013, Elmmarakeby 2014). Enterobacter was isolated from eggs and eggs shell of cracked and uncracked eggs (Edema and Atayese. 2006).

E. sakazakii was isolated from chicken carcasses without visible fecal contamination obtained from selected sites of processing line (after evisceration) and they ranged from 0.5 to 1% of the total isolates (Jimenez et al., 2003). Praxedes et al. (2012) identified Enterobacteriaceae of the broiler intestinal microbiota submitted from the 15th to the 23th day of life, while Amer et al. (2015) reported the pathogenicity of *E. Sakazakii* to 1-day old SPF chicks.

ELISA is simple, rapid and inexpensive method that requires no sophisticated equipment. It enables discrimination of *C. sakazakii* from other bacterial strains and detection of small amounts of *C. sakazakii*. Because of its speed, ease of use, reproducibility and safety, it may provide a better alternative than conventional identification methods for detecting *C. sakazakii* (Park et al., 2012).

Molecular Diagnosis

Molecular methods are used as quick and trustworthy tools to study bacterial genomic diversity and to track sources of infection (Clark et al., 1990, Nazarowec-White and Farber, 1997, Kucerova et al., 2011). For a precise characterization of Cronobacter species in PIF and its associated environments, various molecular based protocols have been improved, which contain direct target gene detection and subtype methods (Pan et al, 2014, Yan and Fanning, 2015).

Currently, molecular detection methods such as polymerase chain reaction (PCR), real-time PCR, and immunoassays are commonly employed for its identification (Bej, 2003, Krascenicová et al., 2008). Sometimes even more advanced methods such as DNA microarray-based assays have been used for the detection of *C. sakazakii* (Wang et al., 2009). In the investigation of PIF contamination, a combination of methods such as antibiograms, ribotyping, plasmid analysis, multilocus enzyme electrophoresis, and chromosomal restriction fragment analysis have been used (FAO, 2004). The gluB gene is a specific target for detecting Cronobacter and the PCR-RFLP typing assay could conveniently and rapidly differentiate *C. malonaticus*, *C. dublinensis*, *C. sakazakii* and *C. muytjensii* (Ye et al., 2015). The ropB gene was described to identify different Cronobacter species (Stoop et al. 2009, Strydom et al. 2011)

Prevention and Control

Accurate diagnosis and identification of the causative organism are of great importance for control strategy. The source of infection should be traced and determined by continuous testing and monitoring.

Biosecurity Measures

Biosecurity practices can eradicate or reduce pathogens. Biosecurity in poultry production is the application and practices against infectious biologic agents to non-infectious levels and to limit their spread in the farm level and poultry

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product handling (processing and Marketing) to be safe for human.

Inadequate biosecurity can result in epidemics of highly pathogenic or exotic disease in the industry, condemnation of flocks, infection by a low-virulent organism can reduce production, increase costs to clean, sanitize and disinfect as well as spread pathogens to human. Controlling all environmental, management and microbial factors are imperative to control bacterial infections in poultry as well as food borne infections in both poultry and man.

Application of disinfectant to destroy pathogenic microorganisms or rendering them inactive; to sanitize is to reduce microbial populations and keep them from multiplying.

Cleaning and disinfection of all equipment and utensils used in egg collection and handling to reduce egg contamination in laying hens. Sanitizing hatching eggs can decrease their prevalence, reducing a source of cross-contamination during incubation and hatching (Scott and Swetnam, 1993). Hatchery sanitation with bacteriological testing for isolation and identification of pathogenic organisms must be frequently applied. Many sanitizers, sanitizing treatments and method of application have been tested to be effective in reducing microbial numbers or prevalence in broiler hatching egg (Cox et al., 2007).

Antibiotics can be used in the treatment and control of such infection. Antibiotic must be used according to results of antibiotic sensitivity testing to obtain good results and avoid drug resistance.

Probiotics, prebiotics, and synbiotics can be used to modify the gut environment to prevent bacterial pathogens colonization, invasion, multiplication, and shedding.

CONCLUSION

As poultry remains a vehicle of important pathogens to human as well as the possible economic losses a strategy must be considered in evaluation, prevention and control of this infection.

DECLARATIONS

Ethics approval and consent to participate

Yes, (it is a review, not experiment).

Availability of data and material

All data collected in this study are included in this published article.

Authors' contributions

Mohamed M. Amer: Contributed to data collection in addition to participating in writing the manuscript. Hoda M. Mekky: Contributed to data collection in addition to participating in writing the manuscript. All authors read and approved the final manuscript.

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