Detection Of Susceptibility of Cronobacter Sakazakii to Different Antibiotics and Biocides Among Septic Neonates

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Abstract
Background: Cronobacter is a genus within the family Enterobacteriaceae and was previously known as Enterobacter sakazakii. CS is an opportunistic pathogen. Infections caused by Cronobacter occur across all age groups and most infections although less severe are in the adult population.
Aim of the study: To identify Cronobacter Sakazakii by traditional and molecular methods in septic neonates and to detect of susceptibility of Cronobacter Sakazakii to different antibiotics and biocides.
Subjects and Methods: This was a comparative cross-sectional study and included 50 neonates: 25 full term and 25 premature. It was conducted at Pediatrics department, Zagazig University Hospital and their status fulfil the inclusion and exclusion criteria. All patients were subjected to full history taking, routine clinical examination and laboratory investigations. Collection of specimens and culture and sensitivity tests for the isolates were also done.
Results: There were insignificant difference between both groups as regard Elevated CRP or Total Leukocytic count (Cells/µl). As regard umbical artery blood gas parameters the mean PH was 7.1 (± 0.1 SD), the mean PCO2 was 45.4 (± 10 SD), the mean PO2 was 40.5 (± 12 SD), the mean Bicarbonate was 18.6 (± 4 SD), the mean Base deficit 15.3 (± 5 SD). Detected gene in Cronobacter sakazakii LMG 2789 LMG was 28141, detected Cronobacter sakazakii DSM 4485T DSM was 28141, detected Cronobacter sakazakii DSM 4485T DSM was 28141. C. sakazakii were susceptible to Colistin then Cefepime & less susceptibility to Ampicillin.
Conclusions: The resistance to different antibiotics necessitates future active surveillance to determine the incidence of laboratory-confirmed infections and contamination PIF with Cronobacter spp. This will improve our understanding of the public health effects caused by this pathogen and will eventually minimize its infections in susceptible individuals.

Keywords: Septic neonates, Cronobacter Sakazakii.

I. Introduction
Cronobacter Sakazakii (CS) is a member of the Enterobacteriaceae family that was described as a new species in 1980. Initially, it was noted to be an opportunistic pathogen responsible for neonatal sepsis and meningitis. In neonatal populations, CS outbreaks have been linked to contaminated infant formula. Most patients who survive CS meningitis suffer severe neurologic sequelae, including hydrocephalus, quadriplegia, and retardation of development (1).

Fecal carriage of CS has been described in patients as old as 18 weeks of age, illustrating the potential for mucosal adherence and long-term colonization of the human intestine by CS and supporting the need for isolation of CS-infected patients. Neonatal enterocolitis is associated with intestinal prematurity, enteral feeding, and microbial colonization. The disease affects 2–5% of all premature infants, and roughly 13% of those with birth weights < 1.5 kg (2).
Cronobacter Sakazakii is fairly resistant to osmotic, heat, and dry stresses, which may explain, in part, its presence and survival in desiccated infant powder and similarly prepared products. In fact, several studies revealed that CS was the most thermotolerant among the Enterobacteriaceae. Although the optimum temperature for CS growth is 39°C, it is reported to grow at less than 4°C, suggesting that this species would be able to replicate even during refrigeration (3).

Furthermore, CS may form biofilms and thereby resist disinfectants. Cronobacter Sakazakii is able to survive in powder for at least 12 months. The actual amount of CS contamination usually is low, ranging from 0.36 to 66 colony-forming units (CFU)/100 g. However, in dry environmental samples from infant formula factories, a 40% prevalence rate has been reported (2).

As a result of the widespread prevalence of CS in food products and the risks associated with it, reference detection methods, including selective identification techniques and enrichment procedures, have been established to quantify CS in food products. Improper storage and temperature regulation may lead to an increase in bacterial load, thus facilitating outbreaks of infection (1).

Although CS infection may arise secondary to poor storage and reheating of formula, it has not been proved definitively that hospital staff themselves are not a vector for CS. Unfortunately, hospital personnel may ignore appropriate hygiene practices, and thus may contribute to the spread of infection. Therefore, great care should be taken in adhering to strict hand washing and contact isolation of affected and susceptible infants (4).

This study aimed to identify Cronobacter Sakazakii by traditional and molecular methods in septic neonates and to detect of susceptibility of Cronobacter Sakazakii to different antibiotics and biocides.

II- Study design and Participants

From January 2021 to August 2021, a comparative cross-sectional trial that included 50 neonates: 25 full term and 25 premature and was carried out at the NICU of the pediatrics department, Zagazig University Hospital. As long as all Parents of participants signed informed consent forms and submitted them to Zagazig University's research ethics committee, the study was allowed. We followed the World Medical Association's ethical code for human experimentation, the Helsinki Declaration.

patient inclusion criteria:
- Neonates (only first 4 weeks of life).
- Both sexes.
- Neonates with proven sepsis based on clinical and laboratory evidence.
- Neonates whose feeding was powdered milk formula.
- Considering low birth weight is less than 2,500 grams while babies weighing less 1,500 at birth are considered very low birth weight (5).

Patient exclusion criteria:
- Babies more than four weeks.
- Neonates without sepsis.
- Neonates whose feeding was breast milk feeding.
- Neonates with CNS or congenital problems.

Patients were subjected to:
- Complete history taking
- Gestational age assessment
- Full clinical examination for all systems (e.g., respiratory, cardiovascular, neurological, gastrointestinal system
Routine laboratory investigations:
- Including complete blood count, ESR, CRP, ABG, etc.
- Umbilical artery blood gas parameters among neonates with umbilical artery pH < 7.00 was assessed.

- Collection of specimens:
  - For isolation of Cronobacter sakazakii from samples, the surfaces of powdered infant formula cans were sterilized with 70% ethanol and were aseptically opened in a laminar flow cabinet.
  - Samples were taken under aseptic conditions from the powdered milk.
  - Cronobacter sakazakii was recovered according to FDA protocol (6).

- Culture and sensitivity tests for the isolates:
  - Antibiotic susceptibility testing was done using Kirby-Bauer disk diffusion method on Mueller Hinton agar (Merck Co., Germany) according to CLSI guidelines (7).
  - Surviving heat resistant Cronobacter sakazakii at temperatures 54°C to 64°C in reconstituted infant powdered milk were counted.
  - Count of Cronobacter sakazakii in reconstituted infant powdered milk at refrigeration and ambient temperature storage was assessed.

Statistical Analysis:
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum). P-value ≤ 0.05 was considered statistically significant (S), p-value > 0.05 was considered statistically insignificant (NS).

III. Results
There was insignificant difference between both groups as regard gender, Type of delivery, 5 minutes Apgar, maternal age, Maternal Parity or gestational age. There was significant increase of prevalence of Cronabacter Sakazakii in preterm more than full term (table 1).

There was insignificant difference between both groups as regard Elevated CRP or Total Leukocytic count (Cells/µl) (table 2, figure 1).

The mean PH was 7.1 (± 0.1 SD), the mean PCO2 was 45.4 (± 10 SD), the mean PO2 was 40.5 (± 12 SD), the mean Bicarbonate was 18.6 (± 4 SD), the mean Base deficit 15.3 (± 5 SD) (table 3).

There was significant increase of early onset of sepsis in preterm, increase late of onset in full term (table 4).
Detected gene in Cronobacter sakazakii LMG 2789 LMG was 28141, detected Cronobacter sakazakii DSM 4485T DSM was 28141, detected Cronobacter sakazakii DSM 4485T DSM was 28141.

C. sakazakii were susceptible to Colistin then Cefepime & less susceptibility to Ampicillin (table 5, figure 2).
**Table 1:** Cronabacter Sakazakii in the studied groups in PIF:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Preterm</th>
<th>Full-Term</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cronabacter Sakazakii</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9</td>
<td>2</td>
<td>0.016</td>
</tr>
<tr>
<td>Negative</td>
<td>16</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2:** Lab. Investigations of studied groups

<table>
<thead>
<tr>
<th>Lab. Investigations</th>
<th>Preterm (N=25)</th>
<th>Full-Term (N=25)</th>
<th>Test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated CRP (&gt;1 mg/dL) (n)</td>
<td>92% (23)</td>
<td>96% (24)</td>
<td>0.35</td>
<td>0.55</td>
</tr>
<tr>
<td>Total Leukocytic count (&lt;10%L)</td>
<td></td>
<td></td>
<td>1.348</td>
<td>0.46</td>
</tr>
<tr>
<td>Mean SD Median Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute Neutrophilic count (&lt;1000 Cells/mm3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count (&lt;150 Cells/microliter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure (1):** show Total Leukocytic count (Cells/µl) regarding Mean, Median & SD.

**Table 3:** Umbilical artery blood gas parameters among neonates with umbilical artery pH <7.00:

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Median &amp; Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (units)</td>
<td>7.1 ± 0.1</td>
<td>7.15 (6.69-6.99)</td>
</tr>
<tr>
<td>PCO2 (mm Hg)</td>
<td>45.4 ± 10</td>
<td>50 (40-65)</td>
</tr>
<tr>
<td>PO2 (mm Hg)</td>
<td>40.5 ± 12</td>
<td>14 (40-90)</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>18.6 ± 4</td>
<td>19 (2-28)</td>
</tr>
<tr>
<td>Base deficit (mmol/L)</td>
<td>15.3 ± 5</td>
<td>16 (1-27)</td>
</tr>
</tbody>
</table>
Table 4: Types of sepsis in the studied groups:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Preterm</th>
<th>Full-Term</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis onset (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early Onset</td>
<td>15</td>
<td>8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Late Onset</td>
<td>10</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Meningitis (n)</td>
<td>2</td>
<td>0</td>
<td>0.05</td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Results of antimicrobial resistance tests

<table>
<thead>
<tr>
<th>Antimicrobial category</th>
<th>Antimicrobial agent</th>
<th>Resistant</th>
<th>Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>PENICILLINS</td>
<td>Ampicillin</td>
<td>70%</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>60%</td>
<td>34%</td>
</tr>
<tr>
<td>B-LACTAM COMBINATION AGENTS</td>
<td>Ampicillin</td>
<td>48%</td>
<td>16%</td>
</tr>
<tr>
<td>TETRACYCLINES</td>
<td>Tetracycline</td>
<td>29%</td>
<td>70%</td>
</tr>
<tr>
<td>MACROLIDES</td>
<td>Erythromycin</td>
<td>42%</td>
<td>56%</td>
</tr>
<tr>
<td>LINCOSAMIDES</td>
<td>Clindamycin</td>
<td>46%</td>
<td>52%</td>
</tr>
<tr>
<td>CEPHEMS</td>
<td>Cefepime</td>
<td>20%</td>
<td>78%</td>
</tr>
<tr>
<td></td>
<td>Cefoxitin</td>
<td>26%</td>
<td>64%</td>
</tr>
<tr>
<td>CARBAPENEMS</td>
<td>Doripenem</td>
<td>28%</td>
<td>62%</td>
</tr>
<tr>
<td></td>
<td>Imipenem</td>
<td>40%</td>
<td>54%</td>
</tr>
<tr>
<td></td>
<td>Meropenem</td>
<td>36%</td>
<td>48%</td>
</tr>
<tr>
<td>AMINOGLYCOSIDES</td>
<td>Gentamicin</td>
<td>46%</td>
<td>54%</td>
</tr>
<tr>
<td></td>
<td>Tobramycin</td>
<td>32%</td>
<td>48%</td>
</tr>
<tr>
<td></td>
<td>Amikacin</td>
<td>48%</td>
<td>50%</td>
</tr>
<tr>
<td>FLUOROQUINOLONES</td>
<td>Ciprofloxacin</td>
<td>48%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>Levofoxacin</td>
<td>50%</td>
<td>48%</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>34%</td>
<td>64%</td>
</tr>
<tr>
<td>PHENICOLS</td>
<td>Chloramphenicol</td>
<td>54%</td>
<td>64%</td>
</tr>
<tr>
<td>LIPOPEPTIDES</td>
<td>Colistin</td>
<td>14%</td>
<td>84%</td>
</tr>
</tbody>
</table>

Figure (2): Shows results of antimicrobial resistance tests

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IV. Discussion

Holy et al. (8) showed that the current knowledge of the virulence and epidemiology of this organism is limited. However, because neonates are frequently fed reconstituted powdered infant formula (PIF), this product has been the focus of attention for reducing infection risk to neonates because the number of exposure routes is limited. (8)

Infections with Cronobacter spp. occur across at any age, and most infections, albeit less severe, are in the adult population. However, neonates, particularly those of low birthweight, are the major identified group at risk, because the organism can cause meningitis, necrotizing enterocolitis (NEC), and sepsis in patients in neonatal intensive care units and has high mortality rate. (9)

Our study was a comparative cross-sectional study and included 50 neonates: 25 full term and 25 premature. It was aiming at comparing between the incidence of Cronobacter Sakazakii infection among preterm versus full term septic neonates PIF in septic neonates.

Regarding the demographic data in our study, the majority of babies were males, and their residence is rural areas, maternal age from 20 to 30 years. There was no difference between the two groups regarding the clinical manifestations.

In a similar recent study conducted by Elkhawaga et al (10), A total of 100 positive blood cultures from cases of neonatal sepsis admitted to the neonatal ICU, were analyzed. In addition, 1,100 food samples, including 400 powdered infant formula (PIF), 500 herbs, and 200 water samples were screened for the presence of C. sakazakii. They evaluated the antimicrobial profile and the ability of the strains to form biofilms. 100 preterm neonates of <37 weeks gestational age and aged from 0 to 28 were studied. In agreement with us, the majority of babies were males, and their residence is rural areas. (10)

This study showed that the mean PH was 7.1 (± 0.1 SD), the mean PCO2 was 45.4 (± 10 SD), the mean PO2 was 40.5 (± 12 SD), the mean Bicarbonate was 18.6 (± 4 SD), the mean Base deficit 15.3 (± 5 SD).

There was significant difference between Sepsis onset of preterm infants & post term infants. In our study, preterm were with early onset of sepsis, full term with late onset of sepsis. There was significant increase of early onset of sepsis in preterm, increase late of onset in full term. There were increase of sepsis score in preterm more than full term

Salman et al. (11) showed that among five investigate postnatal factors, only feeding method had a significant association with C. sakazakii infections. There was higher percentage of babies who received powdered infant formula (PIF) in C. sakazakii-positive group compared to C. sakazakii-negative group with a significant difference. Other factors, particularly sepsis onset and gender although showed notable differences between C. sakazakii-positive and -negative groups, these differences did not rise to significant levels. Late sepsis onset was reported in 11 children (68.75%) from C. sakazakii-positive group compared to 57(41.67%) children among C. sakazakii-negative group. On the other hand, three quarters of C. sakazakii-positive children were male compared to 61.9% among C. sakazakii-negative group

This study shows that detected gene in Cronobacter sakazakii LMG 2789 LMG was 28141, detected Cronobacter sakazakii DSM 4485T DSM was 28141, detected Cronobacter sakazakii DSM 4485T DSM was 28141.

Amer et al. (12) showed that the 3 strains (out of 9) which were identified as C. Sakazakii by biochemical tests were appeared to be E. cloacae using MALDI-TOF technique. According to MALDI-TOF technique, the difference in the incidence of isolated C. sakazakii and other Enterobacteriaceae species presented only in the examined IFMP samples (1/3) and rice pudding samples (1/2), while baby food samples give the same percentages in different species with both of them.
Our findings showed that there was a high significant difference between breast feeding and formula feeding infants regarding prevalence of C. Sakazakii infection. 30% of neonates with formula feeding showed the positivity of C. sakazakii. 10 Our tests of susceptibility revealed that most C. sakazakii were susceptible to Colistin then Cefepime & less susceptible to Ampicillin.

In the past decade, Fei et al. (13) found that different data has been reported on the prevalence of various Cronobacter isolates in PIF based on conventional and molecular methods. Very little information is available, however, on the prevalence of Cronobacter worldwide.

Elkhawaga et al. (10) showed that all isolated strains were resistant to ampicillin, amoxicillin, ampicillin/sulbactam, clindamycin, cephalothin, and cephalaxin. On the other hand, they showed 100% sensitivity for levofloxacin, tetracycline, imipenem, and chloramphenicol. The sensitivity values to other antibiotics were 94% for erythromycin, 95.2% for gentamycin, 96.4% for tobramycin, and 14.2% for cefadroxil.

Against our results, Mardaneh et al. (6) conducted a study that demonstrated that all (100%) of C. sakazakii isolates were susceptible to ciprofloxacin and levofloxacin. 88.8% of the isolates were sensitive to tetracycline and nalidixic acid.

According to Kilonzo et al. (14), as such an antibiotic resistance can easily spread within hospital wards and cause protracted outbreaks with high mortality and morbidity rates, strict infection control protocols are recommended.

In another study by Kim et al. (15), by analyzing the prevalence and genetic diversity of Enterobacter sakazakii. they reported 31.6% of isolated strains were resistant to ampicillin.

Alarming, a recent study by Parra et al. (16) reported that the incidence rates for the detection of C. sakazakii were 10 and 35% of the examined PIFs produced in Singapore and Chile, respectively.

Fei et al. (17) conducted a study that showed that C. sakazakii is the dominant species of Cronobacter spp. isolated from PIF and environmental samples and can infect infants and adults, respectively.

In China, Pan et al. (18) examined Cronobacter spp. contamination in commercial PIFs and follow-up formulas. In this study, Cronobacter spp. were detected in 49 of the 399 samples. The isolation rates from PIFs and follow-up formulas were 11.5 (19/165) and 12.8% (30/234), respectively. The isolates included 48 C. sakazakii and only one C. malonaticus.

In the study of Ahmadi et al. (19) and associates on the molecular detection of C. sakazakii in blood specimens of hospitalized neonates suspected to have sepsis in Ahvaz, this bacterium is not identified.

A microbiological analysis performed by Reich et al. (20) in a PIF processing environment revealed that the environment was correlated with Cronobacter contamination in the final products, suggesting that the processing environment may be a contamination source.

Masood et al. (21) showed that contamination of powdered infant formula (PIF) with Cronobacter after processing is also important and should not be overlooked. Infants, especially those hospitalized in NICUs and fed with PIF, are the group of population most seriously affected by Cronobacter. The Codex Committee on Food Hygiene of FAO/WHO is considering the creation of a risk pattern for C. sakazakii in PIF.

Drudy et al. (22) cleared that these high rates of positivity should lead to better control by infant formula manufacturers and healthcare authorities to avoid contamination of this dangerous bacteria. Although it was shown that C. sakazakii would not survive the pasteurization process, it is possible that contamination may occur during the addition of the dry components, such as minerals and vitamins, to the PIF before packaging or due to the poor hygienic practices during the production process.

V. Conclusion

Regarding our results of this study, we recommend underlining the importance of using ready-to-feed PIF, complying with the rules for aseptic preparation.

The resistance to different antibiotics necessitates future active surveillance to determine the incidence of laboratory-confirmed infections and contamination PIF with Cronobacter spp. This will improve our understanding of the public health effects caused by this pathogen and will eventually minimize
its infections in susceptible individuals.

References:


