Original Article

Clinical Profile and Antibiotic Susceptibility Patterns of *Cronobacter sakazakii* in the Northern Region of Oman

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Abstract

Background: *Cronobacter sakazakii* is an opportunistic pathogen that mostly affects neonates, infants, and elderly people with weakened immune systems. No study has reported the frequency and antibiotic susceptibility patterns of *C. sakazakii* from Oman, and thus this study was conducted to fill this gap in the literature.

Materials and Methods: This single-center retrospective study included *C. sakazakii* isolates identified from different clinical samples of patients treated at Sohar Hospital, Oman, between January 2017 and December 2023. Bacterial identification and antibiotic susceptibility testing were done using the VITEK II automated microbiological system in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines.

Results: A total of 185 *C. sakazakii* isolates were included, most commonly from patients aged >60 years (42.7%) and <1 year (11.4%). *C. sakazakii* strains had high susceptibility (>80%) to most of the tested antibiotics; however, for beta-lactam antibiotics, it ranged from 0% to 50%. Approximately 26.5% of the strains were multidrug resistant. Independent risk factors for increased frequency of multidrug-resistant strains were urinary catheterization ($P = 0.002$), surgery ($P = 0.021$), previous antibiotic therapy ($P = 0.047$), and critical care unit admission ($P = 0.048$). About one-fifth of the patients experienced life-threatening *C. sakazakii* infections such as septicemia (15%) and pneumonia (4.7%). All deaths due to septicemia occurred in the >60 years ($n = 12$) and <1 year ($n = 4$) age groups.

Conclusions: *Cronobacter sakazakii* isolates from the North Batinah region of Oman were most frequently isolated from elderly and infant patients and had high antibiotic susceptibility; however, the significant resistance against beta-lactams suggests their low effectiveness. The high number of multidrug-resistant strains coupled with the independent risk factors suggests the need for following stricter antibiotic stewardship protocols and infection control practices.

Keywords: Antimicrobial susceptibility, catheterization, *Cronobacter sakazakii*, *Enterobacter sakazakii,* infants, multidrug resistance, Oman, septicemia

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INTRODUCTION

Cronobacter sakazakii, previously known as *Enterobacter sakazakii*, is a facultative anaerobic, Gram-negative flagellated bacterium belonging to the *Enterobacteriaceae* family.[1] *C. sakazakii* is classified as a foodborne pathogen and is found in contaminated food items, such as powdered milk infant formula (PMIF), dairy products, vegetables, cereals, herbs, and in contaminated feeding items such as breast pump equipment.[2-6] *C. sakazakii* has the ability to infect all age groups;[2,7] however, premature neonates, infants with low birth weight, and elderly people with weakened immune system, including cancer patients and who are on immunosuppressive therapy, are most vulnerable to severe form of the infection that includes septicemia, pneumonia, meningitis, necrotizing enterocolitis, and osteomyelitis.[7-9]

Cronobacter infections in infants aged <1 year are frequently associated with PMIF. Infants, particularly aged <2 months, are most likely to develop meningitis, which is associated with high mortality.[6] Furthermore, findings indicate acute, long-term, and chronic consequences in survivors of *Cronobacter* infection, including brain abscesses, quadriplegia, hydrocephalus, neurodevelopmental delays, and other neurological issues.[3] The global prevalence of *C. sakazakii* varies according to geographic region and sampling methodology.[3] Between January 2002 and July 2022, the US CDC documented 76 cases of severe *Cronobacter* infections in neonates, and in 2024, invasive *Cronobacter* infections in babies was classified as a nationally notifiable illnesses, mandating health professionals to report identified cases to help determine the actual incidence of this infection.[6] Globally, *Cronobacter* meningitis or bloodstream infections result in death in >40% of the cases in newborns, and in the United States, in >20% of the cases in infants.[6]

There are significant challenges arising in the treatment of *C. sakazakii* owing to their ability to acquire antibiotic resistance through biofilm formation and gene transfer mechanisms, and the rapid emergence of extremely drug-resistant strains such as carbapenem-resistant and extended-spectrum beta-lactamase (ESBL) producers.[10-13] Prevalence of *C. sakazakii* infections and their antibiotic susceptibility patterns have a geographic variation.[7,12-16] To the best of the authors' knowledge, no study from Oman has reported the frequency of *C. sakazakii* infection and its antibiotic susceptibility patterns. The current study aimed at ascertaining the rate and clinical characteristics of *C. sakazakii* infection in patients treated at Sohar Hospital, Oman, from January 2017 to December 2023, with an emphasis on evaluating antibiotic susceptibility patterns, comorbidities, contributory variables, and the infection outcomes.

MATERIALS AND METHODS

This single-center, retrospective study was conducted at Sohar Hospital, which is the main secondary care center in the North Batinah region of Oman. *C. sakazakii* strains isolated from different clinical samples of patients treated at Sohar Hospital between January 2017 and December 2023 were included. All patients who were suspected of having *C. sakazakii* infection but had a negative bacterial culture report were excluded from the study. Patients with incomplete data were also excluded. Data pertinent to the patients, such as demographics, clinical characteristics, antibiotic susceptibility patterns, risk factors, and outcome of infection, were retrieved from the electronic health record system of the hospital.

The study received approval from the Research Ethical Review and Approval Committee of the Ministry of Health, Oman, and from the Institutional Review and Ethics Committee, College of Medicine and Health Sciences, National University of Science and Technology, Oman.

Bacterial identification and antibiotic susceptibility testing

Different clinical samples received at the microbiology laboratory at Sohar Hospital were cultured on blood agar and nutrient agar, as per the Clinical Laboratory Standards Institute (CLSI) guidelines.[17] Pathogen identification up to the species level was performed by the conventional identification method or the VITEK II automated microbiological system.[17] The antibiotic susceptibility of the isolated *C. sakazakii* was determined by the Kirby–Bauer disc diffusion method. The results of the antibiotic sensitivity were reported as sensitive, intermediate, and resistant, as per the CLSI guidelines. The tested antibiotics included ampicillin, amoxicillin, amoxicillin–clavulanic acid, amikacin, gentamicin, cefoxitin, cefuroxime, ceftriaxone, ceftazidime, cefotaxime, ciprofloxacin, imipenem, meropenem, piperacillin-tazobactam, nalidixic acid, and trimethoprim–sulfamethoxazole.

Broth microdilution and epsilometer tests were used to determine the minimum inhibitory concentrations of colistin and tigecycline. Multidrug-resistant (MDR) *C. sakazakii* were classified by non-susceptibility to at least one agent of three or more antimicrobial classes by standard susceptibility testing methods.[17] *C. sakazakii* strains that showed non-susceptibility to carbapenems by standard susceptibility testing methods (i.e., a minimum

inhibitory concentration of ≥4 μg/mL for imipenem and/or meropenem) were categorized as carbapenem-resistant strains. *C. sakazakii* were initially screened for ESBL production by testing their resistance to ceftazidime. Further confirmation of ESBL production in the screen-positive strains was done using the double-disc synergy test and the CLSI confirmatory test.[17]

Statistical analysis

The data were processed in MS Excel and then analyzed using SPSS version 26 (SPSS Inc., IBM, Chicago, IL, United States). Categorical variables are presented as frequencies and proportions, and quantitative variables as mean and standard deviation (SD). For categorical variables, association was assessed using the chi-square test or Fisher's exact test, with a significance level set at $P < 0.05$. Odds ratios were estimated with 95% confidence intervals for two-by-two tables of risk factors.

RESULTS

A total of 185 *C. sakazakii* isolates were obtained from 185 patients during the study period. Most patients were male (51.4%) and aged >60 years (42.7%), and had one or more comorbidities (69.2%). The most frequent comorbidities were cardiovascular diseases (73%), diabetes mellitus (41.6%), and renal diseases (28.6%). The most common surgical or medical interventions were antibiotic therapy within the past 2 months (40.5%), urinary catheterization (33.5%), and critical-care unit admission (23.2%). Isolates were most frequently recovered from urine (31%), pus and wound swabs (26%), and blood samples (25%) [Table 1].

In terms of antibiotic susceptibility patterns, *C. sakazakii* demonstrated high susceptibility to gentamicin (85.1%), trimethoprim–sulfamethoxazole (82.8%), ciprofloxacin (81.4%), amikacin (80.1%), imipenem (78.6%), meropenem (78.2%), and piperacillin–tazobactam (78.1%). All strains were sensitive to tigecycline; however, 6.2% of the strains showed resistance to colistin. Beta-lactam antibiotic susceptibility levels ranged from 0% (ampicillin) to 52.2% (cefotaxime) [Table 2].

Figure 1 illustrates the yearly isolation of *C. sakazakii* and the MDR strains. Among the 185 isolates, 26.5% had resistance to multiple drugs, with 13.0%, 9.2%, and 4.3% being MDR, carbapenem-resistant, and ESBL strains, respectively. The frequency of isolation and number of MDR organisms (MDROs) strains was highest during the year 2020, which was the peak period of the COVID-19 pandemic in Oman.

A significant correlation was found between the emergence of MDR strains and urinary catheterization ($P = 0.002$),

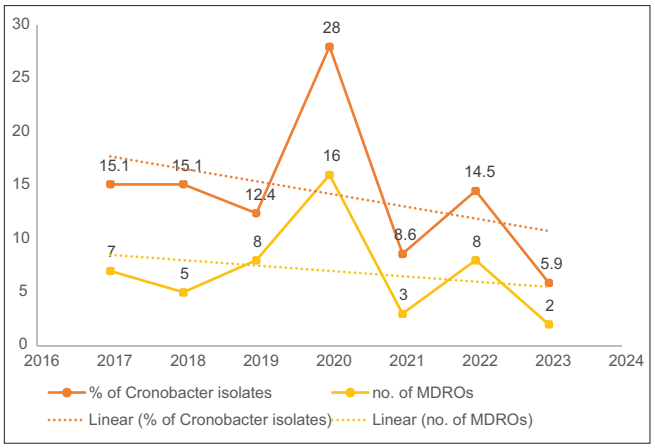

Figure 1: Yearly distribution of *Cronobacter sakazakii* isolates and multidrug-resistant organisms. MDROs – Multidrug-resistant organisms

Table 1: Demographic characteristics of the included patients (*N*=185)

Variable	*n* (%)
Gender	
Male	95 (51.4)
Female	90 (48.6)
Age (years)	
0–1	21 (11.4)
>1–30	18 (9.7)
31–60	67 (36.2)
>60	79 (42.7)
Clinical specimens	
Urine	57 (31)
Pus and wound swab	49 (26)
Blood	46 (25)
Respiratory secretions	18 (10)
Other samples	15 (8)
Patients with one or more comorbidities	127 (69.2)
Comorbidities	
Diabetes mellitus	77 (41.6)
Cardiovascular (HTN, IHD, others)	135 (73.0)
Respiratory (COPD, asthma, others)	27 (14.6)
Renal (ESRD, CKD, others)	53 (28.6)
Neurological (CVA, others)	20 (10.8)
Malignancy	8 (4.3)
Surgical or medical interventions	
Hemodialysis	41 (22.2)
Mechanical ventilation	33 (17.8)
Central venous catheterization	39 (21.1)
Urinary catheterization	62 (33.5)
Immunosuppressive therapy (chemotherapy, steroids)	15 (8.1)
Previous antibiotic therapy (within 2 months)	73 (40.5)
Critical care unit admission	43 (23.2)

HTN – Hypertension; IHD – Ischemic heart disease; COPD – Chronic obstructive pulmonary disease; ESRD – End-stage renal disease; CKD – Chronic kidney disease; CVA – Cerebrovascular accident

previous surgery ($P = 0.021$), previous antibiotic therapy ($P = 0.047$), and critical care unit admission ($P = 0.048$) [Table 3]. About one-fifth (20.5%) of the patients experienced life-threatening *C. sakazakii* infections such as septicemia ($n = 28$), pneumonia (9), and meningitis (1), and most of the cases were in infants and elderly patients. Of these, 16 patients died from bacterial septicemia and 2 from bacterial pneumonia. Infection with MDROs and

the development of septicemia significantly raised the risk of mortality in the elderly (aged >60 years; $P < 0.012$) [Table 4]. All deaths due to septicemia occurred in the elderly ($n = 12$) and infants ($n = 4$).

DISCUSSION

C. sakazakii is an emerging and opportunistic pathogen capable of causing a wide range of infections.[18] Although the incidence of *C. sakazakii* is low, its diversity, virulence, higher mortality rates, emergence of drug resistance, particularly multidrug resistance, and potential for transmission necessitate attention to prevent its spread.[19] In the current study, critical care admission, previous antibiotic therapy, and urinary catheterization were found to be independent risk factors for the increased frequency of MDRO strains.

C. sakazakii were isolated almost equally in male and female subjects. Further, most isolates (42.7%) were found in patients aged >60 years, with most having one or more comorbidities, suggesting weakened immune system. In addition, the second highest proportion of isolates were from infants. These findings are in accordance with similar studies conducted worldwide, wherein *C. sakazakii* infection have been reported to be highest in newborns with underdeveloped immune systems, likely due to being born prematurely or with low birth weight, and older individuals with some level of declining immunity due to various comorbidities.[3,18–24]

Most studies have reported *Cronobacter* infections in infants, with only a few documenting these infections in adults.[23,24] The underreporting of *Cronobacter* infections in adults could possibly be attributed to their rarity and the mild

Table 2: Antibiotic susceptibility patterns of *Cronobacter sakazakii* isolates

Antimicrobial agent	Antibiotic susceptibility	
	Susceptible *n* (%)	Resistant *n* (%)
Amikacin	75 (80.1)	18 (19.8)
Ampicillin	0	170 (100)
Amoxicillin-clavulanic acid	4 (2.3)	171 (97.7)
Cefotaxime	72 (52.2)	66 (47.8)
Cefuroxime	8 (3.4)	166 (96.6)
Ciprofloxacin	144 (81.4)	33 (18.6)
Colistin	15 (93.8)	1 (6.2)
Trimethoprim-sulfamethoxazole	140 (82.8)	29 (17.2)
Ceftriaxone	25 (31.3)	55 (68.7)
Ceftazidime	25 (31.6)	54 (68.4)
Gentamicin	149 (85.1)	26 (14.9)
Imipenem	66 (78.6)	18 (11.4)
Meropenem	68 (78.2)	19 (11.8)
Nalidixic acid	36 (63.2)	21 (36.8)
Piperacillin-tazobactam	99 (78.1)	28 (11.9)
Tigecycline	18 (100)	0

Table 3: Relationship between independent risk factors and emergence of drug-resistant *Cronobacter sakazakii*

Characteristic	Yes (*n*=49), *n* (%)	No (*n*=136), *n* (%)	*P*	OR (95% CI)
Diabetes mellitus				
Yes	23 (47)	57 (42)	0.5	1.22 (0.63–2.6)
No	26 (53)	79 (58)		
Renal				
Yes	15 (31)	35 (26)	0.8	
No	34 (69)	101 (74)		
Respiratory				
Yes	8 (18.0)	14 (10)	0.5	
No	40 (82)	122 (90)		
Neurological				
Yes	7 (14)	12 (8.8)	0.3	1.72 (0.63–4.66)
No	42 (86)	124 (91)		
Neoplasm				
Yes	3 (6.1)	6 (4.4)	0.7	1.41 (0.33–5.88)
No	46 (94)	130 (96)		
Critical care unit admission				
Yes	17 (35)	28 (21)	0.048	2.04 (0.99–4.21)
No	32 (65)	108 (79)		
Hemodialysis				
Yes	10 (20)	29 (21)	0.9	0.94 (0.42–2.12)
No	39 (80)	107 (79)		
Urinary catheterization				
Yes	26 (53)	38 (28)	0.002	2.91 (1.48–5.72)
No	23 (47)	98 (72)		
Surgery				
Yes	25 (51)	44 (32)	0.021	2.17 (1.11–4.23)
No	24 (49)	92 (68)		
Previous antibiotic therapy (within 2 months)				
Yes	26 (53)	50 (37)	0.047	1.94 (1.00–3.76)
No	23 (47)	86 (63)		

CI – Confidence interval; OR – Odds ratio

Table 4: Relationship between septicemia and multidrug-resistant organisms with mortality

Characteristics	Septicemia (*n*=28), *n* (%)	Others (*n*=157), *n* (%)	OR	*P*
Age (>60 years)	18 (64)	61 (39)	2.83 (1.22–6.54)	0.012
Death	12 (60)	8 (40)	13.25 (3.82–45.33)	<0.001
MDRO identified	11 (47.8)	12 (52.2)	6.41 (2.05–20.03)	<0.001
Age (≤1 year)	6 (28.6)	15 (71.4)	2.58 (0.91–7.36)	0.06
Death	4 (66.7)	2 (33.3)	13.00 (1.35–124.3)	0.014
MDRO identified	1 (100)	0	Not calculated	0.10

MDRO – Multidrug-resistant organism; OR – Odd ratio

nature of the infections in healthy people. Furthermore, risk factors for the transmission of infection in adults are poorly understood.[23] Routine and systematic surveillance, together with specific research, are crucial for identifying and understanding the reservoirs of *Cronobacter* infection and sources of transmission in adults.[24] In terms of the source of infection, the current study was unable to determine the same due to the retrospective study design and missing information. In general, contaminated PMIFs are frequently identified as the source of newborn infection. However, *C. sakazakii* can be also found in a variety of food sources, including cereals, herbs, fruits, meat, vegetables, rehydrated powdered milk, and ready-to-eat foods, as well as through contamination of breast pumps, enteral feeding tubes, and baby food items in dairy manufacturing facilities and utensils such as spoons and blenders used for preparing baby food in hospitals and homes.[25,26] Future studies in Oman must emphasize at determining the sources of *Cronobacter* infection to formulate effective preventive strategies.

C. sakazakii is notably associated with infections such as bloodstream infection, including septicemia, urinary tract infections, skin and soft tissue infections, meningitis, and respiratory tract infections, including pneumonia.[23,25,27] In line with this, *C. sakazakii* was most frequently isolated in the present study from urine samples, pus and wound swabs, blood, and respiratory secretions. Most of our patients were exposed to medical or surgical interventions. Use of contaminated hospital instruments and equipment, such as catheters and ventilators, is an important source of healthcare-associated infection for *Enterobacterales*, the family to which *C. sakazakii* belongs.[28,29]

C. sakazakii are susceptible to most of the commonly used antibiotics. However, excessive, and improper use of antibiotics in humans, animals, and crops has caused the recent development of MDR bacteria, which can result in treatment failure, prolonged illness, and increased risk of mortality.[4,30,31] Antimicrobial resistance patterns of *C. sakazakii* differ across geographic regions due to variations in the antibiotic prescribing policy and adherence to antibiotic stewardship.[15,16] Csorba *et al*.[32] documented that all *C. sakazakii* strains found in milk powder were sensitive to all tested antimicrobials and showed no signs of multidrug resistance. A study by Pakbin *et al*.[4] revealed that *C. sakazakii* isolates were highly resistant to amoxicillin–clavulanic acid, amoxicillin, ampicillin, cefoxitin, cefepime, erythromycin, ceftriaxone, ciprofloxacin, and chloramphenicol and highly susceptible to gentamicin, tetracycline, norfloxacin, and azithromycin. In another study, Li *et al*.,[14] found that all *C. sakazakii* strains demonstrated susceptibility to ampicillin–sulbactam, imipenem, aztreonam, and trimethoprim–sulfamethoxazole, and showed maximum resistance to cephalothin. Consistent with these results, in our study, *C. sakazakii* showed high susceptibility (80%–85%) to amikacin, gentamicin, trimethoprim-sulfamethoxazole, ciprofloxacin, imipenem, meropenem, and piperacillin-tazobactam, indicating these antibiotics as preferred choices for the initial treatment. Further, *Cronobacter* strains exhibited significant resistance (50%–100%) to beta-lactam antibiotics such as amoxicillin, amoxicillin-clavulanic acid, ceftriaxone, ceftazidime, and cefuroxime, making them unsuitable for treating these infections.

In our study, a small percentage of *C. sakazakii* strains were identified as MDROs, such as MDR, carbapenem-resistant, and ESBL producing strains, with the highest incidence in 2020, coinciding with the peak of the COVID-19 pandemic in Oman. The sharp increase in the frequency and emergence of MDR strains in 2020 is partially attributed to the widespread antibiotic prescribing pattern by physicians who were uncertain about the diagnosis of COVID-19 during the early phase of the pandemic. Bloodstream infection with MDR pathogens often leads to septicemia and related complications. Moreover, the risk of death increases with the development of septicemia, particularly in immune-suppressed patients.[33] Of note, there was a decline in the frequency of MDROs in our hospital between 2017 and 2023, which may largely be due to the recent introduction of an antibiotic stewardship program and a continuous antibiotic prescription surveillance system at the hospital.

In our study, we found a significant correlation between the increased number of deaths due to septicemia in immune-suppressed individuals including neonates, infants,

and the elderly. Previous studies have firmly established this positive correlation among other *Enterobacteriaceae* organisms.[34,35] However, no previous study has been conducted to demonstrate an association between the increased frequency of MDR *C. sakazakii* and the hospital-related independent risk factors such as critical care admission, urinary catheterization, surgery, and previous antibiotic therapy for transmission of MDRO infection and mortality in adults. Therefore, future research should emphasize on establishing a correlation between independent risk factors and the emergence and dissemination of MDR *C. sakazakii* strains and the outcome of infection.

Limitations

Our study has several limitations**.** First, because of the retrospective study design, some of the information related to the source of infection and risk factors in infants, such as prematurity and low birth weight, could not be explored. Therefore, we could not relate their role as sources and risk factors for *C. sakazakii* infection. Second, biochemical test panels and VITEK identification systems are less reliable for an accurate identification of *Cronobacter* up to the species level, and thus future studies should be conducted with matrix-assisted laser desorption ionization time-of-flight mass spectrometry assays and molecular studies, which have higher accuracy.[20-23] Third, the exact cause of death and confounding factors of mortality were not reported in a few cases. Fourth, the study did not determine the antimicrobial resistance genes in MDR, ESBL, and carbapenem-resistant strains. Fifth, most of our patients were exposed to medical or surgical interventions; however, there was a lack of information about how these interventions contributed to *Cronobacter* transmission to the patients. Finally, the study was a single-center study with a small sample size, thereby limiting generalizability and requiring larger multi-center studies to validate these findings.

CONCLUSIONS

This study found that in the North Batinah region of Oman, *Cronobacter sakazakii* isolates were most frequently isolated from elderly and infant patients and had high susceptibility (>80%) to most of the tested antibiotics but exhibited significant resistance to beta-lactam antibiotics (50%–100%). In addition, about one-fourth of the strains were multidrug resistant, and the independent risk factors for increased frequency of multidrug-resistant strains were previous antibiotic therapy, urinary catheterization, and surgery. Further, large-scale studies using higher accuracy assays and molecular studies are required to validate the findings of this study.

Ethical considerations

The study received approval from the Research Ethical Review and Approval Committee of the Ministry of Health, Oman, and from the Institutional Review and Ethics Committee, College of Medicine and Health Sciences, National University of Science and Technology, Oman (Approval no.: MOH/CSR/23/27674; date: November 26, 2023). Requirement for patient consent was waived owing to the study design. The study adhered to the principles of the Declaration of Helsinki, 2013.

Peer review

This article was peer-reviewed by three independent and anonymous reviewers.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: M.B.S., V.N.; Methodology: M.B.S., O.M.S.A., M.M.S.A., I.M.A.A., Z.M.A.H., and S.A.; Data analysis: R.A.; Writing–original draft preparation: M.B.S.; Writing – review and editing: V.N.; Supervision: M.B.S.

All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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