Molecular Identification and Virulence Genes Detection of Cronobacter Spp Isolated from Clinical Cases in Infants Under Two Years in Mosul City, Iraq

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ABSTRACT
Cronobacter spp. are facultative-anaerobic non-spore forming Gram-negative bacteria, that are part of the Enterobacteriaceae family. They are opportunistic organisms that occasionally cause severe infections such as meningitis, necrotizing enterocolitis, and bloodstream infections in newborns and young children. There is a limited amount of information available about the diversity, pathogenicity, and virulence of Cronobacter species isolated from different sources. This study was conducted to isolate and identify Cronobacter spp from different clinical cases in children under two years in Mosul city and determine some virulence genes which are indicators for their pathogenicity. One hundred and fifty clinical specimens (diarrhea, blood and cerebrospinal fluid) were collected and cultured on Enterobacter sakazakii agar (HiCrome) and trypton soy agar. Eight Cronobacter spp were isolated and presumptively identified according to their morphological characteristics on selective media and confirmed by 16S rRNA gene sequencing and direct sequencing of ropB gene. PCR was used to amplify five potential virulence genes (cpa, hly, sip, zpx, and ompA) in order to determine their presence in our strains. The results revealed that all strains exhibited ompA and zpx, however, only four C. sakazakii strains contained cpa and hly genes. The sip gene was found in only two C. malonaticus strains. Lastly, ropB gene were detected in all Cronobacter spp isolated. Identification of Cronobacter from clinical specimens helps in understanding the sources and risks associated with these pathogens. By determining virulence genes, the study provides insights into how these bacteria cause disease which is crucial for developing targeted interventions and treatments. understanding the presence and characteristics of Cronobacter spp. in clinical cases informs diagnostic and treatment strategies, potentially leading to better patient outcomes.

1. Introduction

Cronobacter spp., previously identified as *Enterobacter sakazakii* (Farmer *et al*., 1980), are a type of non-spore froming facultative anaerobic Gram-negative bacteria. They are part of the Enterobacteriaceae family and are commonly found in the intestines. Currently, there are seven known species: *C. sakazakii*, *C. malonaticus*, *C. dublinensis*, *C. muytjensii*, *C. turicensis*, *C. universalis*, and *C. condimenti* (Stephan *et al*., 2014; Joseph and Forsythe, 2011). *Cronobacter* spp. are opportunistic organisms that occasionally cause severe infections, including meningitis, necrotizing enterocolitis, and bloodstream infections, in newborns and young children. The infections caused by these bacteria are few, although they are severe and can sometimes lead to deadly health outcomes. Meningitis, necrotizing enterocolitis, and bacteraemia are common diseases in infants and children under the age of two, but bacteremia and urosepsis are more frequently observed in adults (Holy and Forsythe, 2014; Jaradat *et al*., 2014). The mortality rate of neonatal meningitis was reported to be 41.9% (Friedemann, 2009), with death happening shortly after the onset of symptoms (Holy and Forsythe, 2014; Willis and Robinson, 1988). Typically, the individuals who survive often experience permanent consequences, such as severe neurological issues such quadriplegia and delayed cognitive development (Bowen and Braden, 2006). Infants who are less than two months old, born prematurely with low birth weight, or have a weakened immune system are at the greatest risk for infection. The clinically significant bacteria can be categorized into two groups: the first group includes *C. sakazakii* and *C. malonaticus*, while the second group consists of *C. turicensis* and *C. universalis*, which are less commonly reported. The other members of the genus are mainly environmental commensals and have less therapeutic significance. All *Cronobacter* species, with the exception of *C. condimenti*, have been found in clinical samples (Alsonosi *et al*., 2015; Holy and Forsythe, 2014; Orieskova *et al*., 2016).

Multiple PCR primers have been suggested for the identification of *Cronobacter* members by amplifying distinct sequences in both variable and conserved sections of the bacterial 16S rRNA. These primers were proposed by Lehner *et al*. (2004) and Hassan *et al*. (2007). Researchers have suggested using specific primers for the *rpoB* gene, which encodes the β region of the polymerase enzyme, to

identify *Cronobacter* species. However, these primers have not considered changes in the taxonomy of the species, leading to inaccurate positive results when used with certain *Enterobacter* species (Jackson *et al*., 2015; Jackson and Forsythe, 2016).

Virulence-related characteristics of *Cronobacter* spp. have been identified using both draft and whole genome sequencing (Grim *et al*., 2013; Kucerova *et al*., 2010). The study conducted by Cruz *et al*. (2011) found three potential virulence genes, namely siderophore interacting protein (*sip*), hemolysin (*hly*), and Cronobacter plasminogen activator (*cpa*), in isolates of *Cronobacter* spp. The strains that expressed OmpA were able to withstand destruction and proliferated within dendritic cells (Quintero-villegas *et al*., 2014).

Nevertheless, our understanding of the pathogenicity mechanisms in *Cronobacter* spp. remains limited. Several investigations have shown that these bacteria have the capability to generate enterotoxins, attach to, and infiltrate several types of epithelial cells (Pagotto *et al*., 2003; Mange *et al*., 2006). In addition, *Cronobacter* spp. demonstrate the expression of a crucial outer membrane protein known as OmpA, which plays a vital role in the invasion process (Townsend *et al*., 2007; Singamsetty *et al*., 2008; Mittal *et al*., 2009). Furthermore, *Cronobacter* spp. participate in the process of creating a biofilm on the plastic surfaces of enteral feeding tubes, which could potentially serve as a means of infection transmission for these bacteria (Hurrell *et al*., 2009a; Hurrell *et al*., 2009b). *Cronobacter* spp. is considered a newly recognized pathogen, making it important to regularly monitor its occurrence in both dietary and environmental sources (Iversen *et al*., 2006). Hence, it is crucial to ascertain the potential sources of contamination, as well as to investigate the genes implicated in the pathogenesis of these bacteria.

It is important to note that the health services of the most advanced countries, including Iraq, have not yet conducted systematic monitoring of these species. Therefore, to have a better understanding of the distribution and pathogenic mechanisms of *Cronobacter* spp., we determined the presence of several virulence genes among *Cronobacter* spp. isolated from different clinical sources in infants under two years old from Iraq/ Mosul.

2. Materials And Methods

Sample collection and sampling

A total of 150 specimens, consisting of 60 blood specimens from patients with bacteremia, 60 stool specimens from patients with diarrhea, and 30 CSF specimens from patients with meningitis, were collected from Ibn Al-Atheer and Al Khansa Hospitals in Mosul. The collection period was from September, 2023 to December, 2023. The specimens were collected prior to the initiation of antibiotic treatment and promptly sent to the microbiology laboratory at the College of Science, University of Mosul, in a refrigerated container within a time frame of 1-2 hours.

Both blood samples and CSF samples were inoculated into Brain Heart Infusion broth and placed in an incubator at a temperature of 37°C for a duration of 24 hours. Subsequently, they were transferred onto the MacConkey agar medium. The feces samples were directly placed on MacConkey agar and incubated at 37 °C for 24 hours. Afterward, all colonies were transferred to the Trypton soy agar medium and incubated at 25 °C for 72 hours. Ultimately, the samples were placed on the HiCrome *Enterobacter sakazakii* agar and kept in an incubator at a temperature of 37 °C for a duration of 24 hours, as described by Kim *et al*. (2008).

Molecular identification

DNA Extraction

Genomic DNA was extracted from *Cronobacter* spp. using a Genomic DNA isolation kit (Geneaid, Taiwan) according to the manufacturer's instructions. Concentration and purity of DNA samples were estimated by Nanodrop 2000 (Thermo Scientific, USA). Then DNA was visualized on 0.8% agarose gel after electrophoresis at 70 V. for 45 min (Younis and Faisal, 2024).

RopB gene amplification and direct sequencing

The amplification of the *ropB* gene was performed by preparing reaction mixtures with a total volume of 20µl. These mixes contained the primers RopB-F: 5-AACCAGTTCCGCGTTCCTGG-3 and RopB-R 5-CCTGAACAACACGCTCGGA-3 at a concentration of 10µM each. The GoTaq Green Master Mix from Promega was used for this purpose and PCR conditions were mentioned elsewhere (Mollet *et al.*, 1997). Following the Polymerase Chain Reaction (PCR), the resulting products were isolated using a 1% agarose gel containg the safe dye, RedSafe and visualized under UV light. The appropriate-sized products were excised from gel and purified using the PCR clean-up kit supplied by Promega (Ibrahim and Faisal, 2024). Subsequently, the products were sequenced at Psomagene sequencing company (Maryland/USA).

Amplification of 16S rRNA specific for *Cronobacter* spp.

The 1495bp fragment from 16S rRNA gene was amplified using traditional polymerase chain reaction (PCR) according to the method described by Khaleel *et al*. (2023) by using the universal primers 27F and 1522R. The amplified products were sequenced and the identity of the isolates was determined by comparing them with other *Cronobacter* spp. isolates on the GeneBank (Abdulrazzaq and Faisal, 2022).

Amplification of putative virulence genes by PCR

Five genes known to be involved in the virulence of *Cronobacter* spp. were identified in the isolates understudy. *OmpA*, *Cpa*, *zpx*, *hly*, and *sip* are virulence factors demonstrated by different authors. The genes were amplified by PCR using primers shown in Table 1 and the conditions used by each author.

Table 1: Primer sequence, amplification conditions and product size for putative virulence genes.

Primer name	Primer sequence 5′-3′	Product size (bp)	Reference
hly:F	CTAGGGTAACGGACTGTCACAGAT	880	Cruz *et al*., 2011
hly:R	CTAGGAAGAAGCGTAAGCGTCTGA		
sip:F	CTAGGCAAAAGAATCGACAAAGGG	953	Cruz *et al*., 2011
sip:R	CTAGGTTGTTGTCTTTATCCGTTC		
cpa:F	CTAGGGCGATGATAGCTGCCTCCG	1015	Cruz *et al*., 2011
cpa:R	CTAGGGGGAACAGCCACGAGGAAA		
ompA:F	GGATTTAACCGTGAACTTTTCC	469	Mohan Nair and Venkitanarayanan.,2006
ompA:R	CGCCAGCGATGTTAGAAGA		
Zpx:F	GAAAGCGTATAAGCGCGATTC	94	Kothary *et al*., 2009
Zpx:R	GTTCCAGAAGGCGTTCTGGT		

3. Result and Discussion

After culturing the clinical samples on selective culture media, colonies were examined for their morphological appearance and color, the results showed that eight samples gave colonies that exhibited a blue-greenish color on *Enterobater sakazaki* agar, at the same time these colonies appeared yellow on tryptone soy agar. Initial identification of these colonies indicate that the isolated bacteria belonged to *Cronobacter* spp. The identification of all isolated *Cronobacter* species was confirmed using the *ropB* gene and 16S rRNA sequencing and the results showed that the isolates belonged to *Cronobacter* spp. The DNA from the eight *Cronobacter* isolates under study were submitted to NCBI under the accession numbers OR825874- OR825875-PP126443- PP126444 and PP126445.

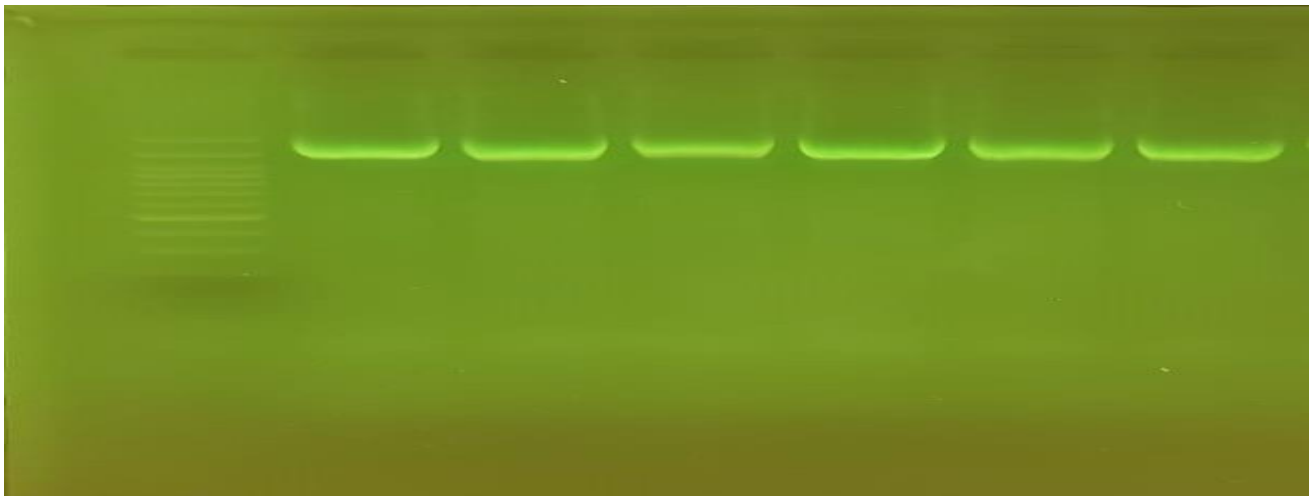


Figure (1): Ampilification of the 1495bp fragment of the 16S rRNA gene from a selection of *Cronobacter* isolates. Ladder used is 100bp DNA ladder.

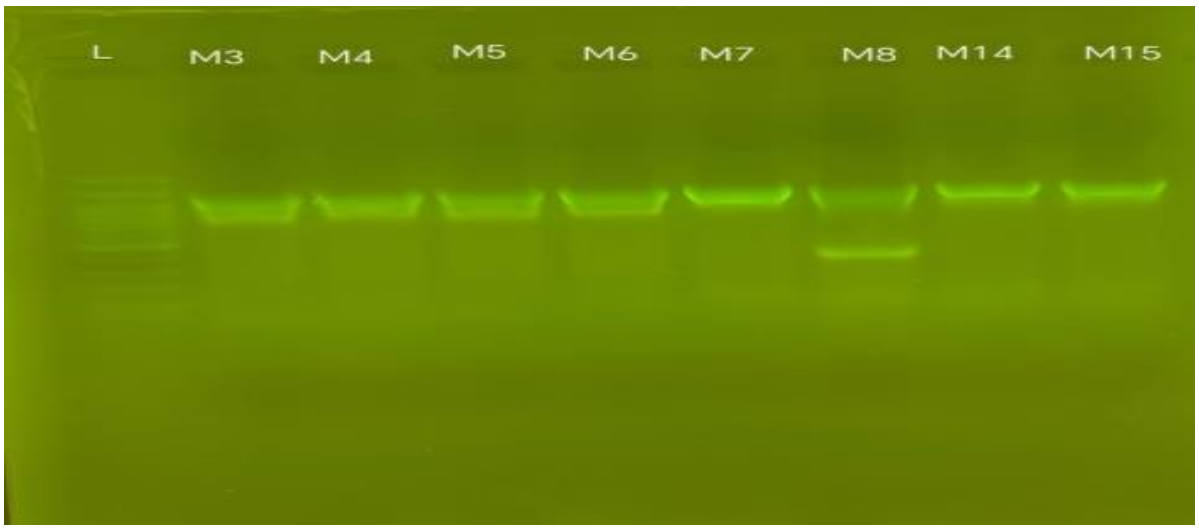


Figure (2): Agarose gel electrophoresis of *ropB* gene from different *Cronobacter* spp for genus specific gene identification. L:100 bp DNA ladder, M3, M4, M8 and M7: *C. sakazakii*, M5: *C. muytjensii*, M14 and M15: *C .malonaticus*, and M6: *C. pulveris*.

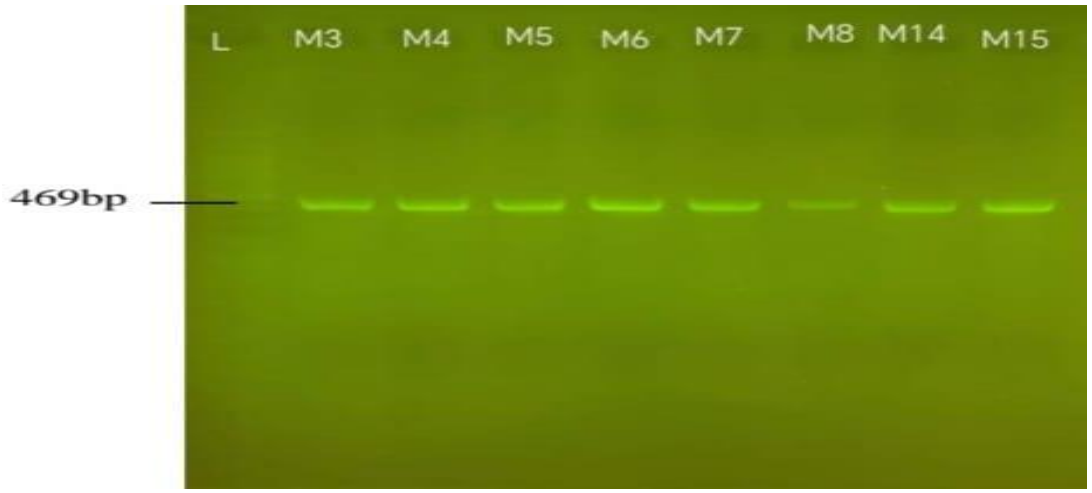
The virulence genes were analyzed in our eight local isolates by PCR technique and the results showed the presence of variability between strains in containing the studied genes (Table 2). The *ompA* and *zpx* genes were found in all eight strains that belonged to three species, while the *sip* gene was exhibited in only two strains of *C. malonaticus*, the *cpa* and *hly* genes was found only in four strains belonging to *C. sakazakii* figures (3, 4, 5, 6, 7)

Table (2): Distribution of five virulence genes in different species of *Cronobacter* isolated from clinical cases.

Species type	**Source of strain**	**Virulence gene**				
		sip	***ompA***	***ZPX***	***hly***	***Cpa***
Cronobacter sakazakii	**stool**	_	+	+	+	+
Cronobacter sakazakii	**stool**	_	+	+	+	+
Cronobacter sakazakii	**blood**	_	+	+	+	+
Cronobacter sakazakii	**blood**	_	+	+	+	+
Cronobacter malonticus	**stool**	+	+	+	_	_

Cronobacter malonticus	**stool**	+	+	+	_	_
Cronobacter muytjensii	**stool**	_	+	+	_	_
Cronobacter pulveris	**stool**	_	+	+	_	_

Figure(3): Agarose gel electrophoresis of *ompA* virulence associated gene amplification in different *Cronobacter* spp with amplicon 469 size (L:100 bp ladder ,(M3, M4, M8 and M7: *C. sakazakii*, (M5: *C. muytjensii*), (M14 and M15: *C. malonaticus*), (M6: *C. pulveris*)



Figure(4): Agarose gel electrophoresis of hly virulence associated gene amplification in different *Cronobacter* spp with amplicon 880 size (L:100 bp ladder ,(M3, M4, M8 and M7: *C. sakazakii*, (M5:*C. muytjensii*), (M14&M15: *C. malonaticus*), (M6: *C. pulveris*)

Figure (5): Agarose gel electrophoresis of *cpa* virulence associated gene amplification in different *Cronobacter* spp with amplicon 1015 size (L:100 bp ladder, M3, M4, M5 (L:100 bp ladder ,(M3, M4, M8 andM7*: C. sakazakii*, (M5: *C. muytjensii*), (M14&M15: *C. malonaticus*), (M6: *C. pulveris*)

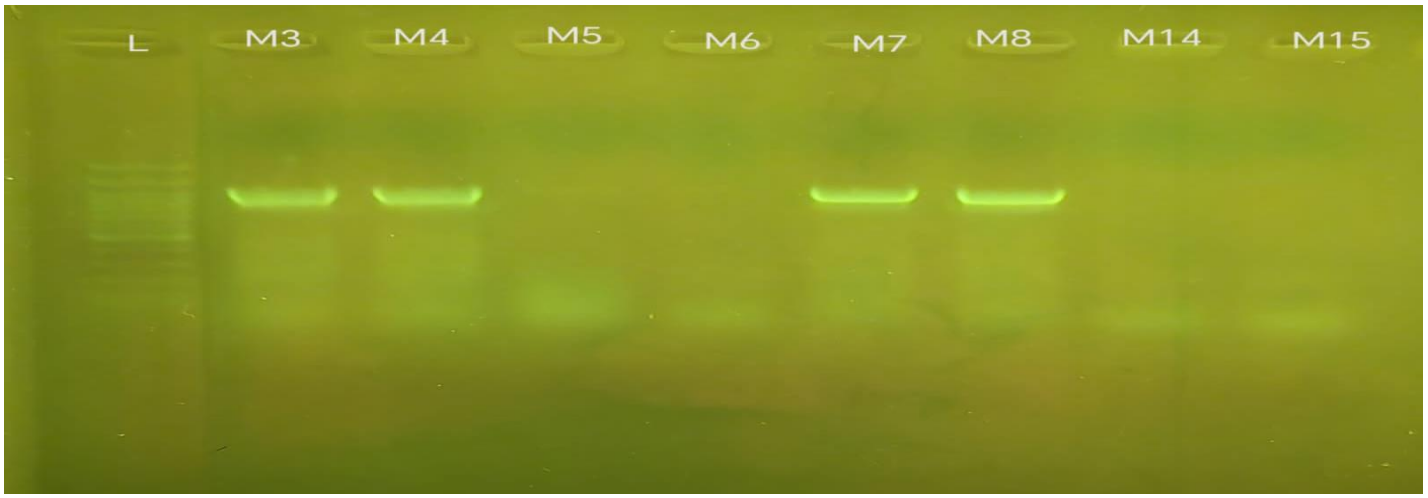


Figure (6) : Agarose gel electrophoresis of *sip* virulence associated gene amplified from different *Cronobacter* spp isolates with amplicon size of 934bp. L:100 bp DNA ladder, M3, M4, M8 and M7: *C. sakazakii*, M5: *C. muytjensii*, M14 and M15: *C. malonaticus* and M6: *C. pulveris*.

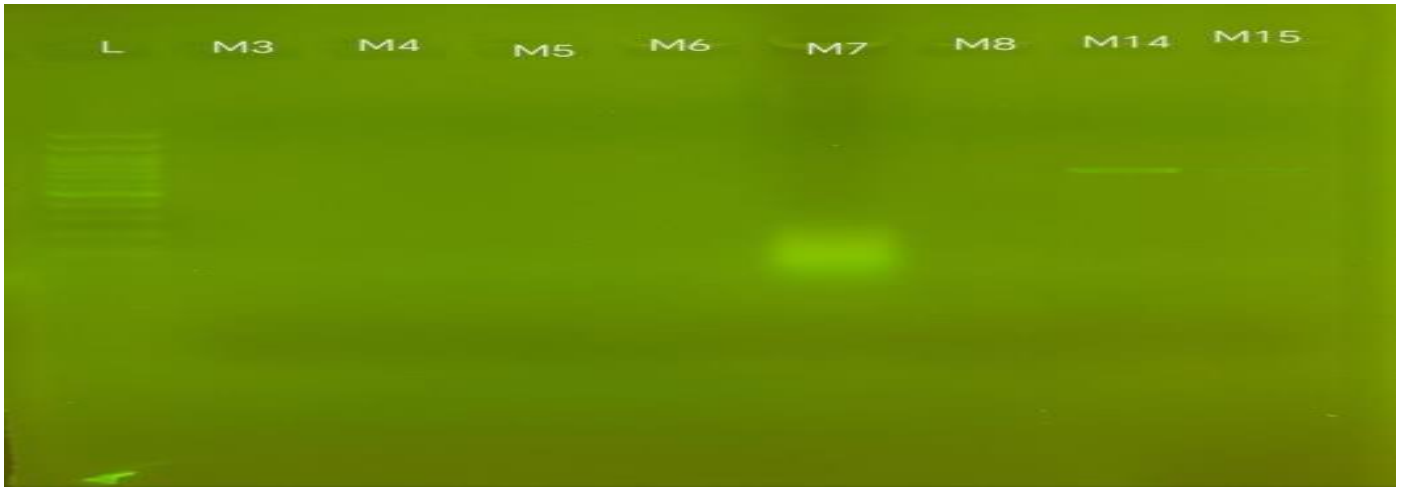
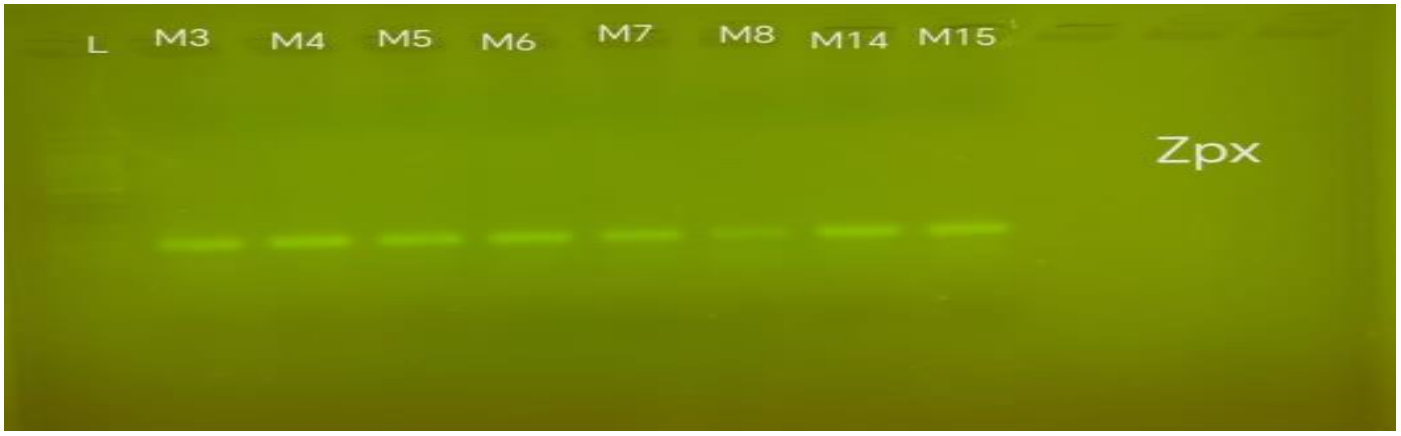


Figure (7): Agarose gel electrophoresis of *zpx* virulence associated gene amplificated from different *Cronobacter* spp isolates with amplicon size of 94bp. L:100 bp DNA ladder, M3, M4, M8 and M7: *C. sakazakii*, M5:*C. muytjensii*, M14 and M15:*C. malonaticus*, M6:*C. pulveris*.



Our study is considered the first local study in Iraq concerned with the detection of virulence genes in *Cronobacter* spp isolated from clinical cases in infants, from our results we concluded that isolated *Cronobacter* spp exhibited different putative virulence genes which confirmed their pathogenicity and ability to cause diarrhea and bacteremia in infants under two years.

The pathogenicity mechanisms of *C. sakazakii* are intricate and remain incompletely understood due

to the diverse range of virulence factors exhibited by this bacteria and its global distribution (Kucerova *et al*., 2010). Multiple investigations have been undertaken to elucidate the virulence factors and associated risk of this pathogen. However, there have been fewer studies undertaken to investigate the virulence characteristics of *C. malonaticus* (Alsonosi *et al.*,2015). *Cronobacter* species demonstrate many virulence factors during the invasion and adherence to intestinal cells, including the synthesis of toxins and hemolysins, as well as the ability to withstand killing by human serum. Nevertheless, neonatal fatalities have solely been linked to *C. sakazakii*, *C. malonaticus*, *C. muytjensii*, and *C. turicensis* (Kucerova *et al*., 2010).

The pathogenicity of *Cronobacter* is poorly understood due to limited research and characterization of its virulence components. This work aimed to characterize strains of *C. sakazakii*, *C. malonaticus*, *C. muytjensii*, and *C. pulveris* by detecting specific virulence genes. All eight strains in our study possessed the *ompA* gene. The outer membrane proteins OmpA and OmpX facilitate the invasion of *C. sakazakii* and *C. malonaticus* into cultured human enterocyte-like Caco-2 cells (Towensend *et al*., 2007). The OmpA protein is a putative virulence factor that helps *Cronobacter* spp. cells to breach the blood-brain barrier, leading to the development of meningitis. The sequencing of the *ompA* gene has been widely employed for the identification of the *Cronobacter* genus (Mohan and Venkitanarayanan, 2006). None of the strains of *C. malonaticus*, *C. muytjensii*, and *C. pulveris* contained the *cpa* gene, however it was found in all examined strains of *C. sakazakii*. This observation may suggest a characteristic feature of *C. sakazakii*. These results are consistent with the findings described in reference (Cruz *et al*., 2011). The Cpa protein is potentially a significant virulence factor that plays a role in both serum resistance and the dissemination and invasion of *C. sakazakii* into the host's body (Franco *et al*., 2011). Another noteworthy feature is the ubiquitous presence of the *hly* gene in all *C. sakazakii* strains, which is absent in our other strains. The gene in question codes for type III hemolysin, a membrane protein that has been identified in several pathogens with hemolytic activity, such as *C. sakazakii* (BAA894). This particular strain was obtained from the NICU outbreak in Tennessee in 2001 (Baida and Kuzmin, 1996; Chen *et al*., 2004; Himelright *et al*., 2002).

The *sip* was harbored in only *C. malonaticus* strains and absent in all four *C. sakazakii*, *C. muytjensii* and *C. pulveris* strains. This gene codes for an interacting protein with the siderophore and is able to transfer electrons from reduced ferrodoxin to FAD and then convert NADP + to NADPH (Cruz *et al*., 2011; Faisal and Rasol, 2022).

Identifying virulence factors was crucial in comprehending the pathogenic process and its interaction with the host. The study conducted by Holy *et al*. (2019) revealed the existence of certain genes, namely *ompA* and *cpa*, which have been linked to bacterial pathogenicity. Additionally, the investigation identified probable virulence genes, including *hly* and *sip*. However, further investigations are required to ascertain whether these proteins may be classified as virulence factors. So far, the use of whole genome sequencing on disease-causing strains has enabled us to accurately describe their features, determining whether these properties are unique or shared among different bacteria (Grad and Lipsitch, 2014). This concise explanation provides a deeper comprehension of the characteristics associated with how this particular bacteria enhances its chances of survival by utilizing various mechanisms to infect the host and induce a more severe and perhaps lethal illness response.

Cronobacter spp. have been isolated from various environmental and food sources, as well as from diverse clinical cases. Neonatal mortality have only been linked to *C. sakazakii* and *C. turicensis*. However, all *Cronobacter* spp. have the potential to cause disease in newborns, particularly due to their sensitive physiological condition (Kucerova *et al*. 2010). However, there is limited knowledge regarding the virulence genes present in *Cronobacter* spp that have been isolated from clinical cases of diarrhea and bacteremia in infants under the age of two in Mosul city, Iraq. By employing a method that examines the genetic and observable traits of the bacteria, we successfully identified distinct virulence genes in *C. sakazakii*, *C. malonaticus*, *C. pulveris*, and *C. muytjensii*. Hence, our findings aid in the additional delineation of these species and enhance comprehension of their distribution and pathogenicity.

Mohan and Venkitanarayanan (2007) provided a description of other proteins linked to invasion. The OmpA protein has the capability to attach to fibronectin, which helps in the penetration of brain endothelial cells. In their study, Mittal *et al*. (2009) showed that the expression of this protein in *E. sakazakii* 51329 (*C. muytjensii*) had an impact on the occurrence of meningitis in newborn rats. The OmpA-positive bacteria effectively traversed the intestinal barrier and proliferated within the blood-brain barrier. On the other hand, strains that do not produce this protein were unable to attach to epithelial cells. In a recent study, Kim *et al*. (2010) showed that the expression of OmpA and OmpX in *C. sakazakii* is necessary for both apical and basolateral adhesion to and invasion of mammalian cells.

A research comparing the genome of the *C. sakazakii* BAA-894 strain from the Tennessee NICU outbreak with the pathogenomics of other bacteria revealed the presence of three shared potential virulence genes (*sip*, *hly*, and *cpa*). The presence of these genes was discovered by PCR in isolates obtained from human sources, and in a limited number of isolates from nonhuman sources (Miethke and Marahiel, 2007). The presumed gene encoding type III hemolysin (*hly*) was exclusively detected in *C. sakazakii*, *C. malonaticus*, and *C. muytjensii* isolated from human specimens. The type III hemolysin, which is present in multiple diseases, is an essential outer membrane protein that exhibits hemolytic activity (Baida and Kuzmin 1996; Chen *et al*. 2004). Additionally, a hypothetical gene for the plasminogen activator (*cpa*) was mostly identified in two species, *C. muytjensii* and *C. sakazakii*, which originated from human origins. The source cited is Cruz *et al*. (2011). The plasminogen activators, which are serine proteases, indicate that this gene may have a role in exhibiting the greatest invasiveness of *Cronobacter* spp. Additional research will be necessary to determine the significance of these genes in causing disease and colonizing the host (Cruz *et al*., 2011). Nevertheless, our investigation consistently observed virulence-associated traits in four species of *Cronobacter* (*C. sakazakii*, *C. malonaticus*, *C. muytjensii*, and *C. pulveris*). Remarkably, *C. sakazakii* was discovered to possess the three presumed virulence genes at a higher frequency and demonstrated the highest level of invasiveness efficiency. Our findings indicate that *Cronobacter* spp. have the potential to be successful opportunistic infections due to their ability to invade and the presence of some putative virulence genes (*hly* and *cpa*).

4. Conclusion

Cronobacter spp. are known to cause severe infections in vulnerable populations like newborns and young children. The identification of these bacteria in clinical specimens helps in understanding the sources and risks associated with these pathogens. By determining virulence genes, the study provides insights into how these bacteria cause disease. This is crucial for developing targeted interventions and treatments. Knowing that *Cronobacter* spp. can be isolated from clinical specimens in a specific region (Mosul city) informs local health authorities about potential outbreaks and the need for vigilance and improved hygiene practices. The study addresses the limited information available about *Cronobacter* spp., contributing to the broader scientific knowledge about these bacteria, which can inform future research and policy-making.

Reference

[1] Abdulrazzaq, R., & Faisal, R. (2022). Efficiency of hichrome Enterococcus faecium agar in the isolation of Enterococcus spp. and other associated bacterial genera from water. Journal of Life and Bio Sciences Research, 3(01), 01-06.

[2] Alsonosi, A. Hariri S., M. Kajsik, M. Orieskova, V. Hanulik, M. Roderova, J. Petrzelova, H. Kollarova, H. Drahovska, S. Forsythe, O. Holy, The speciation and genotyping of Cronobacter isolates from hospitalised patients, Euro J Clin Microbiol 34 (2015) 1979–1988.

[3] Baida G.E., N.P. Kuzmin, Mechanism of action of hemolysin III from Bacillus cereus, Biochim. Biophys. Acta 1284 (1996) 122–124.

[4] Bowen A.B., C.R. Braden, Invasive Enterobacter sakazakii disease in infants, Emerg. Infect. Dis. 12 (8) (2006) 1185–1189.

[5] Chase H.R., G.R. Gopinath, A.K. Eshwar, A. Stoller, C. Fricker-Feer, J. Gangiredla, I.R. Patel, H.N. Cinar, H. Jeong, C.

Lee, F. Negrete, S. Finkelstein, R. Stephan, B.D. Tall, A. Lehner, Comparative genomic characterization of the highly persistent and potentially virulent Cronobacter sakazakii ST83, CC65 strain H322 and other ST83 strains, Front. Microbiol. 8 (2017) 136.

[6] Chen Y.C., Chang M.C., Y.C. Chuang, C.L. Jeang, Characterization and virulence of hemolysin III from Vibrio vulnificus, Curr. Microbiol. 49 (2004) 175–179.

[7] Cruz A ., Xicohtencatl J., B. Gonzalez, M. Bobadilla, C. Eslava, I. Rosas, Virulence traits in Cronobacter species isolated from different sources, Can. J. Microbiol. 57 (2011) 735–744.

[8] Faisal, R. M., & Rasol, A. H. (2022). Physiological role of isocitrate lyase in dibenzo-p-dioxin and dibenzofuran metabolism by Sphingomonas wittichii RW1. Journal of Genetic Engineering and Biotechnology, 20(1), 52.

[9] Farmer, J.J., Asbury, M. A., Hickman, F.W. & Brenner, D.J. (1980). Enterobacter sakazakii: a new species of "Enterobacteriaceae" isolated from clinical specimens. International Journal of Systematic Bacteriology. 30:569–84

[10] Friedemann .M. Epidemiology of invasive neonatal Cronobacter (Enterobacter sakazakii) infections, Eur. J. Clin. Microbiol. Infect. Dis. 28 (11) (2009) 1297–1304.

[11] Grad, Y. H., & Lipsitch, M. (2014). Epidemiologic data and pathogen genome sequences: a powerful synergy for public health. Genome biology, 15, 1-14.

[12] Grim C.J., M.L. Kotewicz, K.A. Power, G. Gopinath, A.A. Franco, K.G. Jarvis, Q.Q. Yan, S.A. Jackson, V. Sathyamoorthy, L. Hu, F. Pagotto, C. Iversen, A. Lehner, R. Stephan, S. Fanning, B.D. Tall, Pan-genome analysis of the emerging foodborne pathogen Cronobacter spp. suggests a species-level bidirectional divergence driven by niche adaptation, BMC Genomics 14 (2013) 366.

[13] Hamby S.E., Joseph S., Forsythe S.J., Chuzhanova N., In silico identification of pathogenic strains of Cronobacter from biochemical data reveals association of inositol fermentation with pathogenicity, BMC Microbiol. 11 (2011) 204.

[14] Hassan, A., Akineden, A., Kress, C., Estuningsih, S., Schneider, E., and Usleber, E. (2007). Characterization of the gene encoding the 16S rRNA of Enterobacter sakazakii and development of a species specific PCR method. Int. J. Food Microbiol.116,214–220.doi:10.1016/j.ijfoodmicro.2006.12.011

[15] Holy .O and Forsythe. S, Cronobacter spp. as emerging causes of healthcare-associated infection, J. Hosp. Infect. 86 (2014) 169–177.

[16] Hunter C.J., Singamsetty V.K., N.K. Chokshi, P. Boyle, V. Camerini, A.V. Grishin, J.S. Upperman, H.R. Ford, N.V. Prasadarao, Enterobacter sakazakii enhances epithelial cell injury by inducing apoptosis in a rat model of necrotizing enterocolitis, J. Infect. Dis. 198 (2008) 586–593.

[17] Hurrell, E., Kucerova, E., Loughlin, M., Caubilla-Barron, J., and Forsythe, S.J. 2009a. Biofilm formation on enteral feeding tubes by Cronobacter sakazakii, Salmonella serovars and other Enterobacteriaceae. Int. J. Food Microbiol. 136(2): 227–231. doi:10.1016/j.ijfoodmicro.2009.08.007. PMID:19720416.

[18] Hurrell, E., Kucerova, E., Loughlin, M., Caubilla-Barron, J., Hilton, A., Armstrong, R., et al. 2009b. Neonatal enteral feeding tubes as loci for colonization by members of the Enterobacteriaceae. BMC Infect. Dis. 9(1): 146. doi:10.1186/1471-2334-9-146. PMID: 19723318.

[19] Ibrahim, M. A., & Faisal, R. M. (2024). Molecular characterization of antibiotic resistance and virulence genes on plasmids of Proteus mirabilis isolated from urine samples of Hospitals in Mosul City, Iraq. Journal of Applied and Natural Science, 16(2), 830-841.

[20] Iversen, C., Waddington, M., Farmer, J.J., 3rd, and Forsythe, S.J. 2006. The biochemical differentiation of Enterobacter sakazakii genotypes. BMC Microbiol. 6(1): 94. doi:10.1186/1471-2180-694. PMID:17067387.

[21] Jackson, E. E., Parra-Flores, J., Fernandez Escartin, E., and Forsythe, S. J. (2015). Reevaluation of a suspected Cronobacter sakazakii outbreak in Mexico.J.Food Prot.2015,1191–1196.doi:10.4315/0362-028X.JFP-14-563

[22] Jackson, E., and Forsythe, S. J. (2016). Comparative study of Cronobacter identification according to phenotyping methods. BMC Microbiol. 16:146. doi: 10.1186/s12866-016-0768-6

[23] Jaradat Z.W., Al Mousa W., A. Elbetieha, A. Al Nabulsi, B.D. Tall, Cronobacter spp.– opportunistic food-bornepathogens.Areviewoftheirvirulenceandenvironmentaladaptive traits, J. Med. Microbiol. 63 (8) (2014) 1023–1037.

[24] Joseph. S and Forsythe S.J., Predominance of Cronobacter sakazakii sequence type 4 in neonatal infections, Emerg. Infect. Dis. 17 (2011) 1713–1715.

[25] Khaleel, A. M., Faisal, R. M., & Altaii, H. A. (2023). The efficiency of molecular methods compared to traditional methods in identifying bacteria from blood and cerebrospinal fluid samples. Malaysian Journal of Microbiology, 19(2).

[26] Kim K., Kim K.P., J. Choi, J.A. Lim, J. Lee, S. Hwang, S. Ryu, Outer membrane proteins a (OmpA) and X (OmpX) are

essential for basolateral invasion of Cronobacter sakazakii, Appl. Environ. Microbiol. 76 (15) (2010) 5188–5198.

[27] Kothary, M., McCardell, B., Frazar, C., Deer, D. and Tall, B. (2007). Characterization of the zinc containing met alloprotease encoded by zpx and development of a species-specific detection method for Enterobacter sakazakii. Applied and Environmental Microbiology, 73(13), pp.4142-4151.

[28] Kothary, M., McCardell, B., Frazar, C., Deer, D. and Tall, B. (2009). Characterization of the zinc containing met alloprotease encoded by zpx and development of a species-specific detection method for Enterobacter sakazakii. Applied and Environmental Microbiology, 73(13), pp.4142-4151.

[29] Kucerova E, Clifton S.W., X.Q. Xia, F. Long, S. Porwollik, L. Fulton, C. Fronick P. Minx, K. Kyung, W. Warren, R. Fulton, D. Feng, A. Wollam, N. Shah, V. Bhonagiri, W.E. Nash, K. Hallsworth-Pepin, R.K. Wilson, M. McClelland, S.J. Forsythe, Genome sequence of Cronobacter sakazakii BAA-894 and comparative genomic hybridization analysis with other Cronobacter species, PLoS One 5 (2010) e9556.

[30] Lehner, A., Tasara, T., and Stephan, R. (2004). 16S rRNA gene based analysis of EnterobactersakazakiistrainsfromdifferentsourcesanddevelopmentofaPCR assayforidentification.BMCMicrobiol.4:43.doi:10.1186/1471-2180-4-43

[31] M. Aldubyan,I.Almami, F.Benslimane, A. Alsonosi, S. Forsythe,Comparative outer membrane protein analysis of high and low-invasive strains of Cronobacter malonaticus, Front. Microbiol. 8 (2017) 2268.

[32] Mange J.P., R. Stephan, L. Borel, P. Wild, K.S. Kim, A. Pospischil, A. Lenher, Adhesive properties of Enterobacter sakazakii to human epithelial and brain microvascular endothelial cells, BMC Microbiol. 6 (2006) 58–68.

[33] Miethke. M and Marahiel M.A., Siderophore-based iron acquisition and pathogen control, Microbiol. Mol. Biol. Rev. 71 (3) (2007) 413–451.

[34] Mittal, R., Wang, Y., Hunter, C.J., Gonzalez-Gomez, I., and Prasadarao, N.V. 2009. Brain damage in newborn rat model of meningitis by Enterobacter sakazakii: a role for outer membrane protein A. Lab. Invest. 89(3): 263–277. doi:10.1038/labinvest. 2008.164. PMID:19139724.

[35] Mohan Nair M.K.and Venkitanarayanan K.S., Cloning and sequencing of the ompA gene of Enterobacter sakazakii and development of an ompA-targeted PCR for rapid detection of Enterobacter sakazakii in infant formula, Appl. Environ. Microbiol. 72 (2007) 2539–2546.

[36] MohanNair,M.K.;Venkitanarayanan,K.S.Cloning and sequencing of the ompAgene of Enterobacter sakazakii and development of an ompA-targeted PCR for rapid detection of Enterobacter sakazakii in infant formula. Appl. Environ. Microbiol. 2006,72, 2539–2546.

[37] Orieskova M., Kajsik M., Szemes T., Holy O., Forsythe S., J. Turna, H. Drahovska, Contribution of the thermotolerance genomic island to increased thermal tolerance in Cronobacter strains, Antonie Leeuwenhoek 109 (3) (2016) 405–414.

[38] Pagotto, F.J., Nazarowec-White, M., Bidawid, S., and Farber, J.M. 2003. Enterobacter sakazakii: infectivity and enterotoxin production in vitro and in vivo. J. Food Prot. 66(3): 370–375. PMID: 12636287.

[39] Quintero-Villegas M., Wittke A.and Hutkins R., Adherence inhibition of Cronobacter sakazakii tointestinalepithelial cellsbylactoferrin, CurrentMicrobiol 69(4)(2014) 574–579. (60) V. Singamsetty, Y. Wang, H. Shimada, N. Prasadarao, Outer membrane protein A expression in Enterobacter sakazakii is required to induce microtubule condensation in human brain microvascular endothelial cells for invasion, Microb. Pathog. 45 (3) (2008) 181–191.

[40] Singamsetty, V.K., Wang, Y., Shimada, H., and Prasadarao, N.V. 2008. Outer membrane protein A expression in Enterobacter sakazakii is required to induce microtubule condensation in human brain microvascular endothelial cells for invasion. Microb. Pathog. 45(3): 181–191. doi:10.1016/j.micpath.2008.05.006. PMID: 18606523.

[41] Stephan, R., Lehner, A., Tischler, P. and Rattei, T. (2010). Complete genome sequence of Cronobacter turicensis LMG 23827, a food-borne pathogen causing deaths in neonates. Journal of Bacteriology, 193(1), pp.309-310.

[42] Townsend ,S., E. Hurrell, S. Forsythe, Virulence studies of Enterobacter sakazakii isolates associated with a neonatal intensive care unit outbreak, BMC Microbiol. 8 (2008) 64.

[43] Townsend,S.M.,Hurrell,E.,Gonzalez-Gomez,I.,Lowe,J.,Frye,J.G., Forsythe, S., and Badger, J.L. 2007. Enterobacter sakazakii invades brain capillary endothelial cells, persists in human macrophages influencing cytokine secretion and induces severe brain pathology in the neonatal rat. Microbiology, 153(10): 3538–3547. doi:10.1099/mic.0.2007/009316-0. PMID:17906151.

[44] Willis J. Robinson J., Enterobacter sakazakii meningitis in neonates: the pediatric infectious disease journal, Pediatr. Infect. Dis. J. 7 (1988) 196–199.

[45] Younis R. M. and Faisal R. M. (2024). Effect of antibiotics on the expression of pyocyanin synthetic genes in

Pseudomonas aeruginosaisolated from different clinical sources of a few hospitals in Mosul, Iraq. Journal of Applied and Natural Science, 16(2), 812 -819. https://doi.org/10.31018/jans.v16i2.5590