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Occurrence, molecular characterization and antibiotic resistance of *Cronobacter* spp. isolated from wet rice and flour products in Guangdong, China

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ABSTRACT

This study explored the prevalence of *Cronobacter* spp. in wet rice and flour products from Guangdong province, China, the molecular characteristics and antimicrobial susceptibility profiles of the isolates were identified. Among 249 samples, 100 (40.16%) were positive for *Cronobacter* spp., including 77 wet rice and 23 wet flour products. Eleven serotypes were characterized among 136 isolates with *C. sakazakii* O2 (n = 32) predominating. Forty-nine MLST patterns were assigned, 15 of which were new. *C. sakazakii* ST4 (n = 17) was the dominant ST, which is previously reported to have caused three deaths; followed by *C. malonaticus* ST7 (n = 15), which is connected to adult infections. All strains presented susceptibility to ampicillin/sulbactam, imipenem, aztreonam and trimethoprim/sulfamethoxazole. The isolates showed maximum resistance to cephalothin, and the resistance and intermediate rates were 91.91% and 3.68%, each. Two strains, croM234A1 and croM283-1, displayed resistance to three antibiotics. High contamination level and predominant number of pathogenic STs of *Cronobacter* in wet rice and flour products implied a potential risk to public healthiness. This survey could provide comprehensive information for establishing more targeted control methods for *Cronobacter* spp.

1. Introduction

Cronobacter spp. are emerging food-related pathogens and previously known as *Enterobacter sakazakii* (Farmer et al., 1980). The organism comprises seven species: *C. malonaticus, C. sakazakii*, *C. universalis*, *C. turicensis*, *C. dublinensis*, *C. muytjensii* and *C. condiment* (Joseph et al., 2012a; Forsythe et al., 2014). Besides *C. condimenti*, all *Cronobacter* have been related with human infections in any age groups, predominately in more immunocompromised groups such as premature infants, neonates, young children, and elderly adults (Lehner et al., 2018; Forsythe, 2018a; Patrick et al., 2014). Notably, strains falling within *C.malonaticus*, *C. turicensis* and *C. sakazakii* may bring about uncommon but life-threatening infections, such as meningitis, necrotizing enterocolitis, and bacteremia; have high mortality rates of 40%–80% in neonates and infants (Parra et al., 2018). The International Commission for Microbiological Specifications for Foods (ICMSF) has reported that *Cronobacter* poses a serious risk to limited populations with life-threatening or significant chronic or long-lasting consequences (ICMSF, 2002).

Cronobacter spp. are ubiquitous organisms that can be found in processed foods, fresh produce, and environmental samples. Although many studies have reported the distribution and prevalence of this emerging foodborne pathogen, the vehicles of transmission and epidemiology have been poorly described. To date, only powdered infant formulas (PIF) have been confirmed to serve as vehicles for the food-related transmission of *C. sakazakii* to infants (Phair et al., 2022; Hunter and Bean, 2013); unequivocal sources of contamination in non-infant foods are still unclear (Joseph and Forsythe, 2012; Forsythe, 2018b). As the genus are not the component of the normal human and animal gut biota, the soil, water, and plant-associated are probably the major sources of *Cronobacter* spp. (Ueda, 2017; Forsythe, 2018b). To efficiently track reliable sources of non-infant population infections and propose appropriate and effective methods to control the pathogens, a better comprehension of the identification and genetic variety of *Cronobacter* spp. is required.

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As a result of their great tolerance to dry, low pH conditions and high temperature resistance (Orieskova et al., 2013; Chauhan et al., 2022), *Cronobacter* have been detected in diverse dehydrated food samples (PIF, infant foods, dairy-based preparations, flours, dried edible mushrooms, spices, herbs) (Lou et al., 2019; Li et al., 2023; Jiang et al., 2022; Cechin et al., 2022; Carvalho et al., 2020), fresh vegetables (Ling et al., 2018), meat (Zeng et al., 2020), ready-to-eat foods (Greenhalgh and Amund, 2019; Xu et al., 2015; Jang et al., 2022) and environments (water, households and processing facilities) (Vasconcellos et al., 2019; Csorba et al., 2021). Wet rice and flour products are staple foods in many parts of the world. Their rich nutritional value and high content of water provide the appropriate conditions for the growth of foodborne pathogens (Kang et al., 2021; Wang et al., 2023) which emphasized the importance to inspect the microbiological profile of wet rice and flour products for food safety. However, limited information is available on the general microbiological quality and genetic characteristics of any contaminating *Cronobacter* in these types of foods. Wet rice and flour products are usually placed in the open air for a long time before cooking, which can potentially cause secondary pollution by microorganisms, and contaminated food products may cause cross-contamination during food processing. Consequently, non-infant populations are likely to be affected by the consumption of wet rice and flour products contaminated by *Cronobacter* because these foods are the principal parts in their normal eating habits.

On the basis of the diversity of O-antigen structures, serotyping method has been manipulated to categorize Gram-negative bacteria (Forsythe, 2018a; Wang et al., 2017). O-antigen serotyping has broadly worked to figure out the diversity of genes in *Cronobacter* spp. isolates for epidemiological and surveillance purposes since 1940s (Jia et al., 2018; Wang et al., 2021). Additionally, multilocus sequence typing (MLST) allows the confirmation and serotyping of *Cronobacter* spp. which was dependent on the analysis of seven housekeeping genes (*fusA*, *gyrB*, *glnS*, *atpD*, *infB*, *gltB* and *pps*) (Joseph et al., 2012b; Forsythe, 2022). This allows both species classification and subspecies discrimination based on the PubMLST database [2] which incorporates identified sequence types (STs) covering whole *Cronobacter* spp. To date, the *Cronobacter* MLST database has recorded information on 3,725 *Cronobacter* isolates. MLST has, therefore, become the most popular tool for the molecular typing of *Cronobacter* recently. It helps researchers better understand the relationship between STs and diseases, for instance, *C. sakazakii* ST4 is significantly implicated to neonatal meningitis (Lepuschitz et al., 2019; Hariri et al., 2013), and prompts the supervision of general microbiology quality and outbreaks of this pathogen.

To the best of our knowledge, a large-scale survey of the prevalence of *Cronobacter* spp. in wet rice and flour products has never been conducted. However, such data are necessary to survey food quality and evaluate the possible risks for consumers. Our study intended to evaluate the degree of contamination of *Cronobacter* spp. in wet rice and flour products available in Chinese markets and to reveal the molecular characterization of isolates by O-antigen serotyping and MLST.

2. Materials and methods

2.1. Sampling

Between August and December in 2020, a total of 249 food samples, including 182 wet rice products and 67 wet flour products, were obtained from different retail stores and supermarkets in 5 cities geographically distributed across the Guangdong province, China (Supplementary, Table S1). The products were putted in separate sterile bags, labeled, then were carried to the laboratory by using cooler with low temperature. Finally, the microbiological experiment of each sample was operated as soon as possible.

[2] http://pubmlst.org/cronobacter.

2.2. Isolation and characterization of Cronobacter spp.

In accordance with the National Food Safety Standards of China document GB 4789.4-2010, the quantitative detection was prepared and the process was described in the former research (Ling et al., 2018). The most probable number (MPN) was calculated according to the schemes reported previously (Xu et al., 2015). By means of API 20E systems (BioMérieux, Marcy-l'Étoile, France), the presumable *Cronobacter* spp. with green or blue-green colonies in chromogenic *Enterbacter sakazakii* Agar Plate were characterized. Besides, *fusA* sequencing was manipulated to the species classification in this work.

2.3. O-Antigen serotyping and multi-locus sequence typing

Genomic DNA was extracted from the tested isolates using the HiPure Bacterial DNA Kit (Huankai, Guangzhou, China). Based on the *Cronobacter* serotyping methods reported in the earlier papers (Wang et al., 2021; Miranda et al., 2017), we identified the serotypes of *Cronobacter* spp. efficiently (Table S2). The results were characterized by agarose gel electrophoresis. MLST was applied to molecular typing of the *Cronobacter* strains (Forsythe, 2022). The amplification of seven loci were operated by using the primers and PCR conditions recommended in MLST PubMed database (Table S3). Bidirectional sequencing of the expanded products was assayed by the Beijing Genomics Institute (Shenzhen, China). The DNA sequences were then uploaded to the MLST database to allocate alleles and STs. Professor Steve Forsythe, the curator of the MLST database, designated new alleles and STs. A minimum spanning tree was created by BioNumerics 8.1.1 (Applied Maths, Sint-Martens-Latem, Belgium).

2.4. Antimicrobial susceptibility Analysis

In terms of the Kirby-Bauer method, susceptibility of *Cronobacter* spp. isolates to the antimicrobial agents were generated by diluting the antibiotics and analyzing the sensitivity of the disk displayed in Mueller-Hinton agar (Huankai) after incubating at 37 °C for 24 h. Sixteen kinds of antimicrobial agents (AMs) (Oxoid, Hampshire, United Kingdom) were detected: Ampicillin (AMP, 10 μg), Ampicillin/sulbactam (SAM, 10 μg), Ceftazidime (CAZ, 30 μg), Cefepime (FEP, 30 μg), Ceftriaxone (CRO, 30 μg), Cefazolin (KZ, 30 μg), Cephalothin (KF, 30 μg), Gentamicin (CN, 10 μg), Tobramycin (TOB, 10 μg), Ciprofloxacin (CIP, 5 μg), Nitrofurantoin (F, 300 μg), Imipenem (IPM, 20 μg), Trimethoprim/sulfameth-oxazole (SXT, 25 μg), Aztreonam (ATM, 30 μg), Chloramphenicol (C, 30 μg), Tetracycline (TE, 30 μg). We measured and scored the susceptibilities of the analyzed isolates by the instructions from the Clinical and Laboratory Standards Institute (Melvin et al., 2018).

Table 1
Incidence and contamination level of *Cronobacter* spp. in 249 samples of wet rice and flour products from Guangdong province, China.

Sample	No. of samples	No. (%) of positive samples		No. of positive samples by quantitative methods by MPN/g range		
				MPN < 10	10 ≤ MPN < 110	110 ≤ MPN
wet rice products	182	77	42.31%	24	16	37
wet flour products	67	23	34.33%	19	1	3
Total	249	100	40.16%	43	17	40

3. Results

3.1. Isolation and prevalence of Cronobacter spp. in wet rice and flour products

As can be seen from Table 1, the general degree of contamination of *Cronobacter* spp. was as high as 40.16% (100/249), with the positive samples comprising 77 and 23 wet rice and flour products, respectively. The MPN analysis revealed that the contamination level of *Cronobacter* spp. was less than 10 MPN/g in 43.00% (43/100) and between 10 and 110 MPN/g in 17.00% (17/100) of positive food samples (Table S4). Notably, 40 products exceeded 110 MPN/g, of which 37 were in the wet rice product category. Among the different cities, the prevalence of *Cronobacter* was uppermost in Guangzhou (57.65%, 49/85), followed by Zhanjiang (47.06%, 16/34), Chaozhou (36.59%, 15/41), Meizhou (27.27%%, 12/44), and Jieyang (17.78%, 8/45) (Table S5).

Table 2
Isolates, serotypes and MLST patterns of 136 *Cronobacter* strains isolated from wet rice and flour products in Guangdong province.

Isolate (Total)	Serotype	Total	Type of samples		MLST allelic type							
			wet rice products	wet flour products	*atpD*	*fusA*	*glnS*	*gltB*	*gyrB*	*infB*	*pps*	MLST pattern (no. of isolates)
C. sakazakii 71	O1	20	1	2	11	8	7	45	8	15	10	ST226 (3)
			4	0	3	8	13	15	22	20	21	ST50 (4)
			2	0	16	8	9	109	125	3	102	ST260 (2)
			3	1	1	1	1	1	1	1	1	ST1 (4)
			1	0	11	8	24	220	15	56	261	ST494 (1)
			1	0	11	8	49	58	38	38	165	ST285 (1)
			0	1	15	67	49	9	14	19	15	ST148 (1)
			1	0	44	69	80	147	96	93	123	ST327 (1)
			1	0	11	8	7	5	8	15	10	ST8 (1)
			0	1	11	15	7	109	59	56	140	ST222 (1)
			1	0	44	226[a]	3	5	5	38	59	ST892[a] (1)
	O2	32	14	1	5	1	3	3	5	5	4	ST4 (15)
			2	1	3	8	37	22	29	36	32	ST31 (3)
			2	0	15	14	15	13	22	5	16	ST13 (2)
			2	1	3	3	3	5	3	3	3	ST3 (3)
			3	0	16	1	19	19	26	5	26	ST22 (3)
			0	1	3	12	16	5	16	20	14	ST17 (1)
			2	0	20	18	16	10	3	20	27	ST23 (2)
			0	1	16	18	9	5	3	73	87	ST136 (1)
			1	0	194	8	13	368[a]	5	261	190	899[a] (1)
			1	0	69	224[a]	9	352	318[a]	297[a]	440[a]	883[a] (1)
	O3	9	3	0	11	8	7	5	8	15	10	ST8 (3)
			2	0	5	1	3	3	5	131	4	ST266 (2)
			1	1	5	1	3	3	5	5	4	ST4 (2)
			1	0	123	37	9	179	114	65	148	ST405 (1)
			1	0	3	77	302[a]	125	107	15	447[a]	ST897[a] (1)
	O4	7	3	1	3	15	28	22	5	38	19	ST40 (4)
			1	0	15	14	15	13	22	5	16	ST13 (1)
			1	0	3	77	302[a]	125	107	15	448[a]	ST898[a] (1)
			1	0	16	8	52	143	258	15	321	ST619 (1)
	O6	2	1	0	1	1	1	1	1	1	1	ST1 (1)
			1	0	15	67	49	9	14	19	15	ST148 (1)
	O7	1	1	0	11	8	7	5	8	15	10	ST8 (1)
C.malonaticus 57	O1	9	3	1	12	7	8	8	10	16	43	ST60 (4)
			3	0	57	7	64	7	28	16	449[a]	ST901[a] (3)
			1	1	51	7	6	140	135	30	50	ST307 (2)
	O2	22	9	1	10	7	6	7	9	14	9	ST7 (10)
			5	0	61	7	12	7	9	35	166	ST286 (5)
			2	0	124	13	25	7	10	22	90	ST649 (2)
			1	1	3	7	11	7	10	16	8	ST10 (2)
			1	0	10	7	6	99	9	14	9	ST201 (1)
			1	0	57	7	64	7	28	16	449[a]	ST901[a]
			1	0	10	7	107	7	17	16	445[a]	ST890[a]
	O3	7	0	1	10	13	67	7	131	124	174	ST300 (1)
			1	0	11	8	49	58	38	38	165	ST285 (1)
			0	1	10	13	67	234	131	124	174	ST551 (1)
			4	0	10	7	63	75	17	74	90	ST902[a] (4)
	O4	17	5	0	124	13	25	7	10	22	90	ST649 (5)
			5	0	10	7	6	7	9	14	9	ST7 (5)
			4	1	61	7	12	7	9	35	166	ST286 (5)
			1	0	61	7	17	7	77	16	12	ST891[a] (1)
			0	1	57	7	25	8	72	40	89	ST138 (1)
	uncertain	2	1	0	10	7	107	7	17	16	445[a]	ST890[a] (1)
			1	0	230[a]	225[a]	299[a]	364[a]	319[a]	298[a]	441[a]	ST885[a] (1)
C. dublinensis 8	O2	2	0	1	58	63	75	76	73	76	108	ST167 (1)
			0	1	70	43	69	252	221	215	303	ST584 (1)
	uncertain	6	0	1	59	20	301[a]	366[a]	82	299[a]	444[a]	ST889[a] (1)
			1	0	59	20	300[a]	366[a]	82	299[a]	444[a]	ST888[a] (1)
			0	1	65	20	152	79	320[a]	212	443[a]	ST887[a] (1)
			1	0	67	20	237	365[a]	119	41	442[a]	ST886[a] (1)
			1	1	58	20	111	367[a]	264	300[a]	446[a]	ST895[a] (2)

[a] New alleles and new STs.

Three species were categorized in 136 isolated *Cronobacter* strains from the 100 contaminated samples (Table 2) with *C. sakazakii* the highest at 71 (52.21%), followed by *C. malonaticus* (57, 41.91%), and only eight isolates identified as *C. dublinensis* (5.88%).

3.2. Serotyping and MLST Analysis

The distribution of O-antigen serotypes in 136 strains of *Cronobacter* was analyzed using a PCR-based O-antigen serotyping strategy. The results are summarized in Table 2; six *C. sakazakii* serotypes were

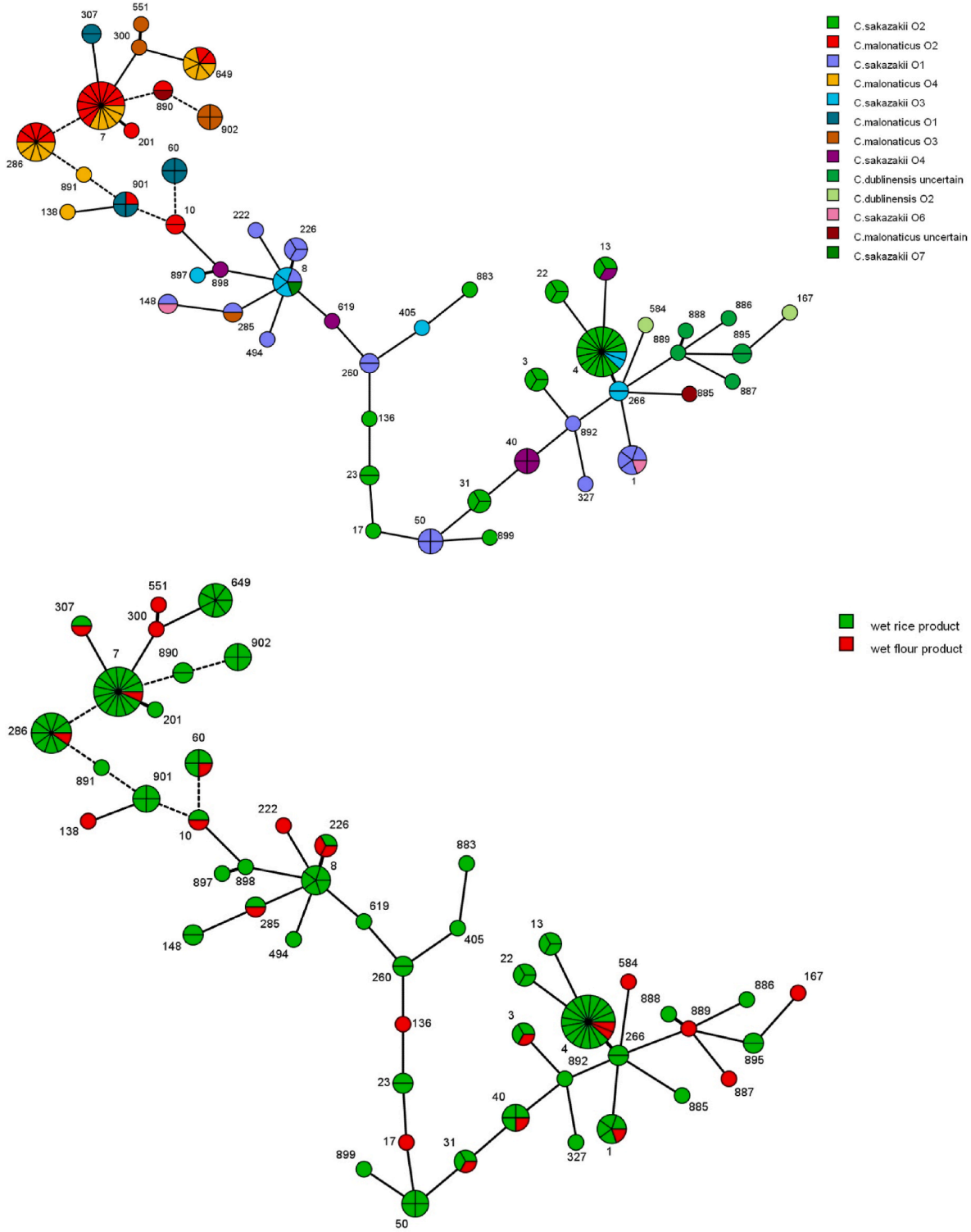

Fig. 1. Clustering relationship between *Cronobacter* spp. isolated in this study. BioNumerics 8.1.1 software was used to produce a minimum spanning tree relying on MLST results of the 136 *Cronobacter* isolates obtained from 259 samples of wet rice and flour products. Each ST was represented by one circle and the ST numbers were displayed next to the corresponding circles. The circle diameter was associated with isolate number of corresponding ST. The colors within the circles were the symbols of the relevant serotypes (A) and sources (B). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

discovered, with O2 (32 isolates) predominating and O1 (20 isolates) following closely. Fifty-seven *C. malonaticus* strains were divided into O1 (9 isolates), O2 (22 isolates), O3 (7 isolates) and O4 (17 isolates). Two *C. dublinensis* serotype O2 isolates were tested; however, six *C. dublinensis* isolates were identified as uncertain. Each of the 6 samples were recognized as containing three different serotypes and two different serotypes were detected in every of 20 samples.

According to the allelic profiles generated by the MLST analysis, 136 *Cronobacter* strains were allocated to 49 STs, of which 15 were new. Novel allele types were discovered in every housekeeping gene; the most being found in *pps,* where 10 new allele types were characterized. Of these 49 STs, 24 were associated with only one isolate and the remaining 25 STs involved 2–17 strains each. The *C. sakazakii* isolates included 5 new STs, with a total of 27 STs. ST4 was the main ST (n = 17), then, ST8 and ST50 covered 5 and 4 strains respectively. Five new STs were distinguished in *C. malonaticus* isolates, ST7 (n = 15) was the primary ST, next were ST286 (n = 10) and ST 649 (n = 7). Five new STs were assigned to *C. dublinensis* strains; the newly found ST895 was detected in two isolates, whereas the other STs of *C. dublinensis* were unique to only one strain.

On the ground of the seven housekeeping genes, a minimum spanning tree was created to illustrate evident clustering through the 136 *Cronobacter* isolates (Fig. 1). Each of the identified serotypes, except *C. sakazakii* O7, had multiple MLST patterns; with *C. sakazakii* O1 exhibiting the maximum number of MLST patterns at 11. Some STs were exclusively designated to particular serotypes, such as the isolates of ST307 and ST60 were only identified as *C. malonaticus* O1, ST226, ST260 and ST50 strains were classified into *C. sakazakii* O1 specifically, ST23, ST31, ST3 and ST22 isolates were unique to *C. sakazakii* O2. However, several STs, like ST649, ST7 and ST4 isolates, had two distinctive serotypes, the strains of ST8, especially, belonged to three serotypes: *C. sakazakii* O1, O3 and O7. Moreover, among *C. sakazakii* isolates, ST1, ST4, ST3, ST31, ST40, and ST226 were detected in wet rice and flour products concurrently. Out of *C. malonaticus* isolates, ST138, ST300, and ST551 were found only in wet flour products. Finally, ST886 and ST888 of *C. dublinensis* strains were obtained only from wet rice products.

3.3. Antimicrobial susceptibility analysis

Identified 136 *Cronobacter* isolates were exposed to sixteen antibiotics in this survey, and the susceptibility, intermediate resistance, and resistance rates were exhibited in Table 3. The strains displayed maximum resistance to cephalothin, with resistance and intermediate rates of 91.91% and 3.68% separately. Additionally, the comparatively high intermediate rates for cefazolin (30.88%) and nitrofurantoin (28.67%) were displayed in isolated strains. Notably, 14 isolates were resistant to multiple antibiotics, with *C. sakazakii* O1 accounting for the majority of these isolates (4/14), followed by *C. sakazakii* O2 (3/14) and *C. malonaticus* O1 (3/14). Two strains, croM234A1 and croM283-1, were resistant to three antibiotics: ceftriaxone, cephalothin, and cefazolin. All examined strains were susceptible to imipenem.

4. Discussion

In this study, we conducted a relatively systematic analyzation on the contamination level of *Cronobacter* spp. in 249 wet rice and flour samples with the total prevalence was 40.16%. Compared with previous studies on other food sorts performed in China, the occurrence of *Cronobacter* spp. was higher for wet rice and flour products than for dried edible mushrooms (14.80%) (Jiang et al., 2022), meat and meat products (9.18%) (Zeng et al., 2020), aquatic products (3.90%) (Li et al., 2020) and raw vegetables (30.27%) (Ling et al., 2019). In the present findings, the occurrence of *Cronobacter* spp. in wet rice products was 42.31% (77/182), however, few previous studies have focused on these species. Lou et al. reported that 3 of 21 rice noodle samples tested positive for *Cronobacter* spp., however, with limited numbers it was difficult to investigate the genetic diversity. As far as I know, the current study is the earliest research to report on the *Cronobacter* spp. prevalence of wet flour-based products. Former work has mainly concentrated on the presence of *Cronobacter* spp. in PIF (Yemis and Delaquis, 2020; Pakbin et al., 2022), aquatic products (Das et al., 2021), ready-to-eat foods (Arslan and Ertürk, 2021; Greenhalgh and Amund, 2019) as well as plant origins (Cechin et al., 2022), etc.

The prevalence of *Cronobacter* spp. was high in both types of food samples indicating a considerable risk of *Cronobacter* spp. contamination in wet rice and flour products. Therefore, it is supposed to focus on the contamination of *Cronobacter* in these foods in the future studies. The contamination level of the most of the positive wet rice samples exceeded 110 MPN/g, while majority of the wet flour samples was less than 10 MPN/g. This implies that wet rice products are more vulnerable to and seriously infected by *Cronobacter* spp., and the pollution of fresh flour products by this pathogen may happen in the process of food transportation or sales. The detected samples were purchased from stores and supermarkets in cities widely distributed in Guangdong province. There may be some variation in rice and flour samples from different locations and this should be investigated in the future effort.

Serotyping of *Cronobacter* strains in this investigation indicated that the major serotype was *C. sakazakii* O2, which is consistent with previous surveys on ready-to-eat diet in China (Xu et al., 2015) and infant

Table 3
Antimicrobial resistance rates of *Cronobacter* spp. isolates identified in the present study.

Antimicrobial group	Antibiotic	Disk code	Antimicrobial class[a] according to the WHO	No. (%) of *Cronobacter* spp. (n =136)		
				Resistant	Intermediate	Susceptible
Penicillins	Ampicillin	AMP	CI	1 (0.74%)	8 (5.88%)	127 (93.39%)
	Ampicillin/sulbactam	SAM	CI	0 (0.00%)	0 (0.0%)	136 (100%)
Cephalosporins	Ceftazidime	CAZ	CI	0 (0.00%)	4 (2.94%)	132 (97.06%)
	Cefepime	FEP	CI	0(0.00%)	11 (8.09%)	125 (91.91%)
	Ceftriaxone	CRO	CI	4 (2.94%)	3 (2.21%)	129 (94.85%)
	Cefazolin	KZ	HI	6 (4.41%)	42 (30.88%)	88 (64.71%)
	Cephalothin	KF	HI	125 (91.91%)	5 (3.68%)	6 (4.41%)
Aminoglycosides	Gentamicin	CN	CI	3 (2.21%)	10 (7.35%)	123 (90.44%)
	Tobramycin	TOB	CI	0 (0.00%)	4 (2.94%)	132 (97.06%)
Quinolones	Ciprofloxacin	CIP	CI	0 (0.00%)	1 (0.74%)	135 (99.26%)
Nitrofurans	Nitrofurantoin	F	CI	1 (0.74%)	39 (28.67%)	96 (70.59%)
Carbapenems	Imipenem	IPM	CI	0 (0.00%)	0 (0.00%)	136 (100%)
Sulfonamides	Trimethoprim/sulfameth-oxazole	SXT	HI	0 (0.00%)	0 (0.00%)	136 (100%)
Monobactams	Aztreonam	ATM	HI	0 (0.00%)	0 (0.00%)	136 (100%)
Amphenicols	Chloramphenicol	C	HI	0 (0.00%)	21 (15.44%)	115 (84.56%)
Tetracyclines	Tetracycline	TE	HI	0 (0.00%)	8 (5.88%)	128 (94.12%)

[a] CI, critically important; HI, highly important; I, important.

nourishments in Germany (Akineden et al., 2017). Our results contradict previous studies that found *C. sakazakii* serotype O1 was predominant in food and clinical cases (Y. Li et al., 2023; Jaradat et al., 2022). In addition, *C. malonaticus* O2, *C. sakazakii* O2, O4 and O1 are clinically significant and probably related to human infections (Blažková et al., 2015; Scharinger et al., 2017). Gopinathe et al. (Gopinath et al., 2018) proved that *C. sakazakii* strains (serotype O2, ST64) that are malonate positive are pathogenic. Alsonosi et al. (2015) showed that *Cronobacter* strains isolated from patients of hospital and Department of Infectious Diseases belonged to *C. sakazakii* O2 and *C. malonaticus* O2. The high percentage of these serotypes (60.00%, 81/136) in this study points to a potential and serious danger to food safety and consumer health.

In the present study, MLST revealed 49 STs among 136 *Cronobacter* isolates, of which 20 STs (ST4, ST3, ST1, ST13, ST12, ST8, ST17, ST40, ST31, ST23, ST50, ST148, ST226, ST260, and ST494 of *C. sakazakii* and ST7, ST10, ST60, ST201, and ST307 of *C. malonaticus*) had been isolated from clinical sources showed in the PubMLST database. ST4 and ST7 were the predominant STs identified in the current study. Notably, ST4 of *C. sakazakii* has been highly prevalent in newborn meningitis cases and has caused three deaths reportedly (Joseph and Forsythe et al., 2011; Hariri et al., 2013; Caubilla et al., 2007). Furthermore, ST7 of *C. malonaticus* is related with adult illnesses (Alsonosi et al., 2015). Our results suggest that greater targeted prevention and control measures against *Cronobacter* spp. in wet rice and flour products should be developed. Additionally, future studies should aim to detect more novel STs because the systematic investigation of the genetic diversity of *Cronobacter* is beneficial for tracking certain sources and allows the development of more efficient control methods for this pathogen.

Cronobacter spp. were susceptible to the majority of antibiotics in this survey. This is consistent with prior investigations regarding this organism isolated from other types of food (Lee et al., 2012; Pluta et al., 2017; Carvalho et al., 2020). Cephalothin has the highest antimicrobial resistance rate (91.91%), which is again coincided with earlier results for *Cronobacter* isolates from other food sorts (Fei et al., 2018; Zeng et al., 2020). Cephalothin is one of the most widely used antimicrobials for the treatment of bacterial infections. These results would raise concerns about the harmful effect on the unrestrained application of cephalothin in Guangdong province.

Multi-drug resistance of foodborne pathogens is directly related with public health. Here, fourteen (9.1%) strains were resistant to two or more antibiotics. Exploring the transmission mechanism of resistance genes and screening for the more effective antibiotic treatment regimen are important research properties for preventing the increasing multidrug resistance of *Cronobacter*. Furthermore, it should implement legislation to control the prudent use of antibiotics and warrant continuous monitoring on the application of antibiotics in Guangdong province, China. In previous studies, most clinical *Cronobacter* spp. isolates displayed multidrug resistance characteristics (Cui et al., 2017; Zeng et al., 2018), however, isolates from food samples usually showed low antibiotic resistance. The reason for this is unclear, hence, a complete study aiming at the molecular characterizations of antibiotic resistance in *Cronobacter* strains from different sources is required in the next research.

5. Conclusions

In conclusion, this is the first investigation to reveal a high prevalence of *Cronobacter* spp. in wet rice and flour products in Guangdong province, China. Clinically important serotypes and pathogenic STs of *Cronobacter* strains were particularly predominant in the isolates detected in this study. These findings, therefore, can provide an early alarm for potential risk to food safety and public health. Additionally, this study can supply a theoretical basis for exploring the route of transmission and genetic diversity of *Cronobacter* spp. and developing more efficient measures for the prevention of this organism.

CRediT authorship contribution statement

Qi Li: made equal contribution to this work, conceived the tests and prepared the original manuscript, operated the experiments and analyzed the results. **Chengsi Li:** made equal contribution to this work, operated the experiments and analyzed the results. **Jumei Zhang:** supervised the project. **Qingping Wu:** edited the draft, supervised the project. **Qinghua Ye**: analyzed the results. **Qihui Gu**: analyzed the results. **Shi Wu**: analyzed the results. **Youxiong Zhang**: provided the materials. **Xianhu Wei**: provided the materials. **Liang Xue**: provided the computing resources. **Moutong Chen**: provided the computing resources. **Haiyan Zeng**: provided the computing resources.

Declaration of competing interest

The authors declared that they have no conflicts of interest to this work.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at.https://doi.org/10.1016/j.crfs.2023.100554.

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