Epidemiology of *Cronobacter* spp. isolates from patients admitted to the Olomouc University Hospital (Czech Republic)

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ABSTRACT

The data on the incidence of *Cronobacter* spp. was collated from hospital records for the seven-year period 2005–2011. The majority of *Cronobacter* spp. isolates (n = 91) were from throat swabs (61), followed by urine (5), tracheal aspirates (5), bronchoalveolar lavage (4), cannulae (4), and sputum (3) samples. This is the first study which profiles the carriage of *Cronobacter* spp. according to patient age, based on seven years of clinical data from 2005–2011. It reveals a high recovery (63.7% of strains, n = 91) of the organism from children, 1–14 years in age.

KEYWORDS

***Cronobacter* spp. – meningitis – nosocomial infection**

SOUHRN

Holý O., Petrželová J., Hanulík V., Chromá M., Matoušková I., Forsythe S. J.: Epidemiologie kmenů *Cronobacter* spp. izolovaných z hospitalizovaných pacientů FN Olomouc (Česká republika)
Informace o incidenci a výskytu kmenů *Cronobacter* spp. byly shromážděny ze záznamů za sedmileté období – 2005–2011. Většina izolátů *Cronobacter* spp. (n = 91) pocházela z výtěrů z krku (61), následovala moč (5), tracheální aspiráty (5), bronchoalveolární laváž (4), kanyly (4), a sputum (3). Toto je první studie, která na základě klinických dat zohledňuje věk pacienta a výskyt kmenů *Cronobacter* spp. za období sedmi let – 2005–2011. Je patrný velmi vysoký výskyt (63,7 % kmenů, n = 91) u dětí ve věku 1–14 let.

KLÍČOVÁ SLOVA

***Cronobacter* spp. – meningitida – nozokomiální infekce**

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INTRODUCTION

In recent years *Cronobacter* spp. has attracted considerable attention due to severe, though rare, neonatal infections [1–3]. Many of these cases have been attributed to contaminated reconstituted infant formula [4]. Subsequently, the FAO/WHO held three risk assessments (2004, 2006, 2008) to consider control measures for neonatal exposure to Cronobacter through formula ingestion. In addition, the FAO/WHO reported that the majority of *Cronobacter* spp. infections (ie. bacteraemia) were in the adult population [5]. This statement was based on data from the Health Protection Agency for England and Wales submitted to the FAO/WHO

following their 'call for data' [5]. However no comparable surveillance data for the bacterium has been published. This communication is the first to report an age profile for *Cronobacter* spp.

METHODS

The University Hospital Olomouc (Czech Republic) receives 45,831 patients per year in average and 776,000 outpatients per year from a catchment population size of 1,000,000. Department of Microbiology conducts microbiological examination for University Hospital Olomouc. Biological samples for bacteriological monitoring, depending on the patient's condition, are sent once or twice a week. Microbiological samples are processed by accredited methods CIA (Czech Accreditation Institute). The first sample, identified as *Cronobacter* was included.

The incidence of *Cronobacter* spp. isolation was collated from hospital records for the seven year period 2005–2011 (Table 1). The isolates had been identified using the BD Phoenix™ automated microbiological system which uses 45 phenotypic characters to identify bacterial cultures. Substrates for biochemical tests: sorbitol, methyl-beta-glucoside and esculin were positive, while adonitol was negative. The above are main biochemical tests distinguishing E. sakazakii strain from E. cloacae strain [6]. Antibiotic sensitivity of the strains was determined according to minimal inhibitory concentrations (MIC). The antibiotics tested were for Gram-negative bacteria (ATB I and II line) as given by Trios (Czech Republic). The standard microdiluting method was used [7]; the susceptibility was assessed in Mueller-Hinton broth. To establish antibiotic sensitivity, a set for determining minimal inhibitory concentrations (MIC) by means of a standard micro method in a microtiter plate was used. The majority of Cronobacter isolates (n = 91) were from throat swabs (61), followed by urine (5), tracheal aspirates (5), bronchoalveolar lavage (4), cannulae (4), and sputum (3) samples, as shown in the Table 2. These samples had been taken for general screening purposes, and not specifically according to the patient clinical presentation. Nevertheless, isolates taken from immune-deficient paediatric patients, the isolation of any microorganism particularly from sputum, bronchoalveolar lavage and indwelling cannulae could be of high clinical significance. Such additional strains had been isolated from clinical investigations (see Tables 1 and 2).

RESULTS

In the seven year period there had been a total of 61 *Cronobacter* spp. isolations from the general screening of patients, and an additional 30 iso-

Table 1. Isolation of *Cronobacter* spp. from throat swabs of patients according to age group
Tabulka 1. Izolace *Cronobacter* spp. z výtěru z krku u pacientů podle věkových skupin

	Patient age (years)								
Year	< 1	1–4	5–9	10–14	15–44	45–64	65–74	> 75	Total
2005	1	5	1	1(1)	2	0	2	0	12(1)
2006	(1)[a]	1(2)	0	5	2	0	(3)	(2)	8(8)
2007	2	9	0	2	2(1)	(1)	(1)	(1)	15(4)
2008	0	1(1)	2	2(1)	0	0	(2)	(1)	5(5)
2009	(2)	4	1	2	1	(2)	1	(1)	9(5)
2010	0	5	2(1)	0	(2)	1(1)	(2)	2	10(6)
2011	(1)	0	2	0	0	0	0	0	2(1)
Total number of Cronobacter isolates	3(4)	25(3)	8(1)	12(2)	7(3)	1(4)	3(8)	2(5)	61(30)
% of isolates/age group	7.7	30.7	9.9	15.4	11.0	5.5	12.1	7.7	100.0
Number of patients sampled[b]	808	3404	2651	1867	18223	10744	3888	3538	45123
Incidence Cronobacter isolates/1000 patients sampled	8.7	8.2	3.4	7.5	0.5	0.5	2.8	2.0	2.0

[a]Numbers in parenthesis indicate additional isolates from normally sterile sites, sampled according to the clinical presentation of the patient.
[b]Total number during period 2005–2011.
[a]Čísla v závorkách představují další izoláty z míst, která jsou normálně sterilní a z nichž byly vzorky odebrány na základě klinického stavu pacienta.
[b]Celkový počet za období 2005–2011.

Table 2. Numbers of clinical material examinations (2005–2011) and number of isolates positive for *Cronobacter* spp. for the period 2005-2011
Tabulka 2. Počty vyšetření klinického materiálu (2005–2011) a počty pozitivních izolátů *Cronobacter* spp. v období 2005–2011

Clinical source	2005	2006	2007	2008	2009	2010	2011	Total number of *Cronobacter* spp. isolations
Bronchoalveolar lavage	1505	1230 (2)[a]	1872 (1)	1375	1745	1737 (1)	1935	4
Throat swabs	1724 (12)	2715 (8)	10426 (15)	9888 (5)	10910 (9)	12222 (10)	11255 (2)	61
Urine	16900	15553 (2)	17701 (1)	17180 (1)	18973	20172 (1)	19629	5
Tracheal aspirates	1550	1460 (1)	1792 (1)	2397 (1)	2322 (1)	2261 (1)	2201	5
Cannulae	2067 (1)	1696	1983 (1)	1706 (1)	1095	939 (1)	811	4
Sputum	680	759	1602	1627	1390 (2)	1336 (1)	1385	3
Total	24426 (13)	23413 (13)	35376 (19)	34173 (8)	36435 (12)	38667 (15)	37216 (2)	82

Clinical material from which the *Cronobacter* spp. was most frequently isolated.
[a]Number of Cronobacter spp. isolates

Klinický materiál, z něhož byly kmeny *Cronobacter* spp. nejčastěji izolovány.
[a]Počet izolátů *Cronobacter* spp.

lates from normally sterile sites such as blood, which had been sampled as part of a clinical investigation. The strains were identified as *Cronobacter* spp. at the 99% confidence level. All strains were sensitive to therapeutic antibiotics. There was considerable yearly variation (2–15/y) in the number of Cronobacter isolations from screened patiens (see Table 1). There were nearly an equal number of *Cronobacter* spp. isolates from patients on the general wards (49.5%), as there were from intensive care units (45.1%), the remainder were from outpatients (4.4%) and from pathological investigations (1.1%). As shown in Table 1, *Cronobacter* spp. was isolated from all age groups. The highest incidences was in the age groups < 1 year (8.7/1000 patients) and 1–4 years (8.2/1000 patients) (see Table 1), and the majority of the strains were isolated in the age group below 14 years of age (63.7%). This corresponds with the majority of isolates being from the Department of Pediatrics (57), followed by Department of Neonatology (6), Department of Internal Medicine (5) and Department of Respiratory Medicine (5). Most of the (44%) *Cronobacter* spp. strains were from patients with immunodeficiency (i.e. acute lymphoblastic leukaemia, acute myeloblastic leukaemia, lymphoid leukaemia).

DISCUSSION

Surprisingly despite the raised awareness of *Cronobacter* spp. since 2002, there is no comparable data on its carriage by different age groups. The only age profiled data, known to the authors, is for 819 *Cronobacter* spp. bacteraemia cases reported for England and Wales between 1992 and 2007, as given by FAO/WHO [5]. In their report, the majority (91%) of bacteraemia cases were patients >15 years in age. The wide age range for the recovery of *Cronobacter* spp. is not surprising given the numerous possible routes of exposure. *Cronobacter* spp. is present in water, soil, households, flies, fresh and processed foods [8–11]. The severity of *Cronobacter* spp. infections in premature neonates is probably due to their immune-compromised status, whereas older children and adults may carry the organism as part of their normal flora. Routes of infections (ie. bacteraemia) in non-infants are uncertain, but are probably following a lowering of their immune status, and puncture wounds. Although the bacterium is isolated from many foods, no foodborne infections have not been reported to date. The data compiled here demonstrates that further research is needed to clarify the asymptomatic carriage of *Cronobacter* spp. This is of particular concern, as it is plausible that inadequate hygienic practices in the preparation of infant feed may lead to infant infection. The recent recognition of *Cronobacter* infections in the healthcare setting has shown that *C. malonaticus* is more associated with adult infections, as opposed to *C. sakazakii* which predominates infant infections [12]. Subsequently the strains collected over the 2005–2011 period from known age groups, are currently undergoing further microbiological analysis.

CONCLUSIONS

The majority of *Cronobacter* spp. isolates (n = 91) were from throat swabs (61), followed by urine (5), tracheal aspirates (5), bronchoalveolar lavage (4), cannulae (4), and sputum (3) samples. These had been taken for general screening purposes, and not specifically according to the patient clinical presentation. This is the first study which profiles the carriage of *Cronobacter* spp. according to patient age, based on seven years of clinical data from 2005–2011. It reveals a high recovery (63.7% strains, n = 91) of the organism from children, 1–14 years in age.

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