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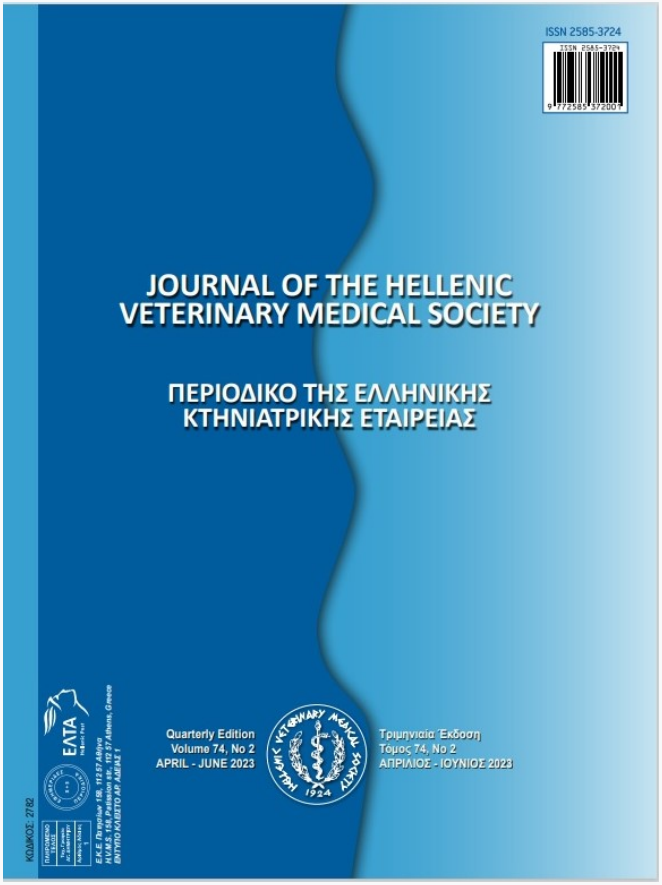

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Dried baby formula as a source of opportunistic members of *Enterobacteriaceae* for infants

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ABSTRACT: For evaluation of the prevalence of *Enterobacteriaceae* in baby formulas, we collected 60 different samples of powdered infant milk formula and milk-cereal-based infant formula from various supermarkets, shops, and pharmacies. These formulae are recommended for first-day birth and from six months of age. Six (20.0%) and thirteen (43.3%) powdered infant milk formulas and milk-cereal-based infant formulas were contaminated by different *Enterobacteriaceae*, respectively involved *Enterobacter cloacae* ssp. *cloacae, Pantoea* spp.,and *Enterobacter hormaechei*, which were identified using traditional biochemical tests and the Vitek2 compact system. Only five (16.66%) samples investigating milk-cereal-based infant formula type had coliforms count of 4 MPN/g. All species were susceptible to gentamycin, while all were resistant to cephalothin antibiotics. Eventually, 20.0% of powdered infant milk formula samples were unacceptable referred to Egyptian standards and most of the comparable international standards. All milk-cereal-based infant formula samples were agreeable with Egyptian standards and FSANZ standards, as they establish a limit of coliforms count <10 MPN/g and 20 MPN/g, respectively. In the study, we focused on the contamination of powdered milk baby formula by *Enterobacteriaceae*, which may cause significant health problems in immature and immunocompromised infants. The dried infant formula factories are more needful for good hygiene and safety systems applications. This study is one of the fewest studies conducted in Egypt to isolate *Enterobacteriaceae* members from dried foods, especially milk-cereal-based infant formula.

Keywords: *Enterobacteriaceae*, powdered infant milk formula, milk-cereal-based infant formula, Vitek2 system.

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INTRODUCTION

Nowadays, the majority of newborns and formula-fed babies are getting up their nutritional needs by consuming the efficacious breast milk replacer, dried formula milk which helps in their outgrowth and development (Kent et al., 2015). Sometimes, this powdered infant formula acts as a medium for undesirable microorganism growth.A false reconstitution of this formula may increase the risk of enteric microbial pathogens growing and the probability of dangerous enterotoxin evolvement (Forsythe, 2005). Isolation of *Enterobacteriaceae* is linked to variant infant foodborne illness in different reports. These opportunistic pathogens affect children under one year of age, which are mainly considered to be immunocompromised, and consequently commended to be absent in infant formula (Mardaneh and Dallal, 2013; Kent et al., 2015; Ibrahim et al., 2022a). The post pasteurization contamination is a significant way of entering the most *Enterobacteriaceae* members due to the intolerable heat treatment of such bacteria during the manufacturing of powdered infant formula (FAO/WHO,2004; Iversen and Forsythe, 2004). Besides, *Enterobacteriaceae* microorganisms could be isolated from the air of the dried infant formula plant environment (Jacobs et al., 2011).

The *Raoultella* spp. is a member of *Enterobacteriaceae*, which are capable of forming biofilm without being motile. This microorganism is often isolated from environments, especially in hospitals (Seng et al., 2016; Sękowska, 2017). Recently, *R. ornithinolytica* hospitals infection was significantly elevated, so improving new techniques for phenotypic identification to specialize it from *Klebsiella oxytoca* (Seng et al., 2016). The other Gram-negative opportunistic bacilli involved in *Enterobacteriaceae* is *Pantoea* spp., which is characteristic of being motile peritrichous. *Pantoea* links to human infections even in healthy babies and is at high risk in immunosuppressed neonates (Sharma et al., 2012; Mardaneh and Dallal, 2013). The emerging and opportunistic species of the *Enterobacter cloacae* complex express genomic heterogeneity and comprise six species; *E. cloacae* and *E. hormaechei* were clinically identified from samples (Paauw et al., 2008).The previously mentioned opportunistic *Enterobacteriaceae* pathogens were investigated by a few studies, such as Iversen and Forsythe, (2004); Estuningsih et al. (2006); Giammanco et al. (2011); Kim et al. (2011); Abdullah Sani et al. (2013); El-Zamkan and Mohamed (2018).

Oteo et al. (2013) conveyed the extreme reason for neonatal sickness was *E. cloacae*, which is reported to be responsible for neonatal sepsis and necrotizing enterocolitis as a widespread gastrointestinal disorder (Iversen and Forsythe, 2004; Nour et al., 2017). It has been recorded as an opportunistic pathogen for neonates and is responsible for outbreaks of various hospital-acquired infections (Davin-Regli and Pages, 2015). Several outbreaks of neonatal intensive care sepsis were recorded to cause by *Enterobacter hormaechei* (Townsend et al., 2008). *Pantoea* spp. was isolated from several dried formulas in developing or even developed countries. These species are linked with bacteriemia, osteomyelitis, septic arthritis, brain inflammation, pneumonia, and lung abscess (Mardaneh and Dallal, 2013).

The Vitek2 system is characterized by the identification and interpretation automatically of a wide range of bacteria and yeast,which is helpful in the identification of *Enterobacter* spp., depending on colorimetric reagent cards (Darbandi, 2010; Yahya and Ghosoon, 2016). It can distinguish several isolated numbers within 6 hours, giving rapid reports compared with routine methods, that are laborious and time-consuming (Yahya and Ghosoon, 2016). In the study, we aimed to examine the baby formula for *Enterobacteriaceae* due to scanty data about these bacteria in dried baby formulae in the Middle East, particularly in Egypt, and detect their antibiotic susceptibility. As *Enterobacteriaceae* frequently may contaminate the powdered infant milk formula and subsequently cause gastrointestinal disorders.

MATERIALS AND METHODS

Collection of samples

Thirty samples of imported powder infant milk formula made for infants from zero to 6 months and 30 milk-cereal-based infant formula locally produced were gathered randomly from assorted pharmacies, retail shops, and supermarkets situated in Giza and Cairo Governorate, Egypt. All samples were transported in their packages and were kept at room temperature (25°C) in a dry condition to examine in the laboratory.

Isolation of *Enterobacteriaceae* and *Cronobacter* spp. from the examined samples

The procedure was described by ISO/IDF(2006) and El-Zamkan and Mohamed(2018).Twenty-five grams of every sample was mixed with 225 ml of

buffer peptone water (Oxoid, CM0509), and these pre-enrichment broths were kept in the incubator at 37°C for 18 h. A loopful from each broth was streaked on Violet Red Bile Glucose Agar plates(Oxoid, CM0485) and incubated for 24 h at 37ºC for *Enterobacteriaceae* isolation.While other 0.1 ml of all incubated buffer peptone water were transmitted for 10 ml modified Lauryl Sulfate Tryptone broth (mLST) (Oxoid CM1133), which complemented by vancomycin supplement (10 mg/L) (Oxoid, SR0247E). These broth media was incubated at 37ºC and 44±0.5ºC/24 h. A loopful from each enriched culture was streaked on Violet Red Bile Glucose Agar,and the plates were incubated at 37 ºC for 24 h. Then five red or purple colonies circled by a halo of purple color were picked and streaked on plates of Tryptic Soya Agar (TSA) (Oxoid, CM0876), which were incubated for 72 h at 25ºC. Yellow and non-yellow colonies on TSA were picked off for further identification to *Cronobacter*-spp. and other *Enterobacteriaceae*.

Salmonella spp. isolation from the examined samples

0.1 ml from each previous homogenized sample and incubated buffer peptone was inoculated in a sterile tube of 10 ml Rappaport Vassiliadis Broth (Oxoid, CM0669), which was incubated at 42°C for 24 hr. Other 0.1 on 10 ml tetrathionate broth (Oxoid, CM0671) was incubated at 35°C for 24 h. Then streaked on Xylose Lysine Deoxycholate Agar (Oxoid, CM0469) and incubated at 37°C for 24 h. Finally, colonies were picked up and streaked on TSA (Oxoid, CM0876) for forwarding identification.The isolation of *Salmonella* spp.was done according to ISO (2017).

Determination of coliforms count and isolation of _E. coli_

Decimal dilutions of each sample were prepared, then inoculated 1 ml of appropriately prepared dilutions for samples in 3 tubes of Lauryl Sulphate Tryptose (LST) broth (Oxoid, CM0451), which were incubated at 35°C for 48 h and observed for gas production. All positive tubes were inoculated into Brilliant Green Lactose Bile Broth 2% (Oxoid, CM0031) and incubated at 35°C for 48 h. From the results of positive gas tubes, confirmed $MPNg^{-1}$ was computed, and a loopful streaked on Eosin Methylene Blue (EMB) agar plates (Oxoid, CM0069), later was incubated at 35°C for 24 h. All colonies were picked up for further examination. The determination of coliform count and isolation of *E. coli* was done according to APHA (2004).

Phenotypic identification of isolates

Following Gram's stain: all the subsequent conventional tests were carried out include,oxidase, catalase, motility, indole, methyl red, Voges–Proskauer, citrate production, Triple Sugar Iron (TSI), dulcitol & sorbitol fermentation, arginine & lysine decarboxylase, and yellow pigmentation on TSA. The identification was performed according to Whitman et al. (2015).

Identification of isolates using Vitek2compact system

The bacterial isolates were inoculated onto MacConkey agar plates (Oxoid, CM0007), and then plates were incubated overnight at 37°C. A sterile swab was used to transfer sufficient pure colonies and suspended into 3.0 ml of saline solution (0.45% to 0.50% NaCl, pH 4.5 to 7.0), subsequently mixed well. The bacterial suspension turbidity was adjusted to the equivalent of a 0.5-0.63 McFarland standard, matched with a Vitek2 instrument Densi-check. The microorganism suspension tubes and the Vitek2 ID-GN (gram-negative) card contain 64 biochemical tests were loaded into the system. The incubation of all cards was done at 35.5ºC for about 6 h with a periodical reading every fifteen min. The results were gained and printed automatically within 8 h according to BioMerieux (2013).

Detection of _Cronobacter sakazakii_ by PCR

All formula samples were reconstituted by mixing 1 gram in 9 ml of sterile peptone water, then 1 ml of each reconstituted infant formula was added to Brain Heart Infusion Broth (Oxoid, CM1135) for 18 h at 37°C. Extraction of deoxyribonucleic acid was proceeding according to (QIA ampDNA mini kit) instructions. According to Jaradat et al. (2009), the 16S rRNA gene of *C. sakazakii* was amplified using the oligonucleotide primer of the following sequences: Saka-1aFor (5'ACAGGGAGCAGCTTGCTGC3') and Saka-2bRev (5'TCCCGCATCTCTGCAGGA3') with a size of 952 base pair. PCR amplification of the ribosomal RNA gene was performed by mixing 6 µl of extracted DNA with a 19 µl of PCR mixture containing the following: 12.5 µl of Emerald Amp GT PCR mastermix (2x, premix), 4.5µl PCR grade water, and1µl from each forward primer (20 pmol) & reverse primer (20 pmol). PCR amplification was carried out as follows: initial denaturation (5 min at 94°C); annealing (35 cycles of 30 sec at 94°C, 40 sec at 52°C,

50 sec at 72°C), and elongation (10 min at 72°C). Finally, visualization of all amplified products was done on 1.5% agarose gel. After thirty minutes, the run was finished, and the gel was transferred to a UV cabinet. Then it was photographed using a gel registration system, while the data was analyzed through computer software.

Antimicrobial sensitivity testing for *Enterobacteriaceae* isolates

Enterobacteriaceae bacterial isolates were subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method (NCCLS, 2002) using Mueller-Hinton agar (Oxoid, CM0337). The antimicrobial agents tested, and their corresponding concentrations were as follows: Streptomycin (S: 10μg), Chloramphenicol (C: 30μg), Cephalothin (KF: 30μg), Gentamycin (GEN: 10μg), Neomycin (N: 10μg), Tobramycin (TOB: 10μg), Colistin sulfate (CT: 10μg), Piperacillin (PRL: 100μg) and Nalidixic acid (NA: 30μg). The evaluation of results was achieved according to CLSI (2017).

Statistical analysis

Results were analyzed statistically by Microsoft Excel 365 enterprise.

RESULTS AND DISCUSSION

The prevalence of *Enterobacteriaceae* species in the examined samples

The 1st base food used in infants formula is bovine milk, which has a significant role in supporting and improving infants' growth due to its necessary items such as butterfat, proteins, carbohydrate, minerals, and vitamins (Martin et al., 2016; Youssif et al., 2020). The dried baby formula acts a source of these significant nutrients for neonates when breast milk is unavailable. At the same time this formula may consider a potential risk factor for an infant's health (Kent et al., 2015).

Collected isolates were examined biochemically, as all were gram-negative isolates with different phenotypic reactions. As illustrated in Table 1, only 6 (20.0%) and 13 (43.3%) powdered infant formula milk and milk-cereal-based infant formula tested samples were contaminated with *Enterobacteriaceae*, respectively. The milk - cereal - based infant formula samples were more contaminated with *Enterobacteriaceae* than powdered infant milk formula, and these results were nearly similar to those of Iversen and Forsythe (2004). The high contamination of this milk-cereal-based infant formula with *Enterobacteriaceae* was due to multicomponent addition, which is agreeable with Estuningsih et al. (2006). Kent et al. (2015) evaluated the biological hazards of formula for infants and specified that supplements of nutrients are mostly non-pasteurized after heat treatment (source of recontamination).

The percentage of *Enterobacteriaceae* species in the examined samples

The data were exhibited in Table 2; represent that powdered infant milk formula samples were polluted with 10 isolates only, of which 6 *Enterobacter* s pp. {(3 *Enterobacter cloacae* ssp. *cloacae* (30.0%) and 3 *Enterobacter hormaechei* (30.0%)} and 4 *Pantoea* spp. (40.0%). The most isolated species from tested powdered infant milk formula was *Enterobacter* spp., and these were almost highly similar to the following findings: both *E. cloacae* and *Pantoea* spp. had been isolated by Iversen and Forsythe (2004) and Kim et al. (2011). While Abdullah Sani et al.(2013) and El-Zamkan and Mohamed(2018), succeeded in isolation of *E. cloacae* only. Giammanco et al. (2011) were isolated *Enterobacter cloacae* and *Enterobacter hormaechei*.

Abdullah Sani et al. (2013) informed that *Enterobacter* spp. was the most frequently isolated member of *Enterobacteriaceae* from powdered infant formula, as *Enterobacter* spp. is much present in the environment of animals and indicates fecal contamination (Sanderson et al., 2005; Nesma et al., 2020).

In milk-cereal-based infant formula, 13 samples were polluted with twenty-one *Enterobacteriaceae* isolates; that were distributed into 7 (33.3%) *Enterobacter cloacae* ssp. *cloacae*, 2 (9.5%) *Enterobacter*

Table 1. Prevalence of *Enterobacteriaceae* in the examined both type of formula (n=60)

Sample type	Positive samples		Number of isolates
	No.	%	
Powdered infant milk formula (n= 30)	6	20.0	10
Milk-cereal-based infant formula (n= 30)	13	43.3	21

n= number of examined samples. No.: Number of positive samples.

hormaechei, 11 (52.4%) *Pantoea* spp.,and 1 (4.8%) *Raoultella ornithinolytica.*The majority species that were isolated from milk-cereal-based infant formula were *Pantoea* spp. These results were nearly similar to Iversen and Forsythe (2004), who found 5 *Pantoea* spp.and 1 *Enterobacter cloacae*. Also Estuningsih et al. (2006), found 12 *Pantoea* spp. and 8 *Enterobacter cloacae*, and Abdullah Sani et al. (2013), found 1 *E. cloacae* in cereal-based infant formula.

Farmer et al. (2007) discussed that *Cronobacter* was confused with other *Enterobacteriaceae* (mainly *Enterobacter cloacae* complex and *Pantoea*). These were also approved by Jackson and Forsythe (2016). They reported confusion of biochemical test results between *Cronobacter, E. cloacae*, and *Enterobacter hormaechei* with high similarity in their biochemical identification. The results of the Vitek2 are more specialized than traditional biochemical examination, especially *Enterobacter* spp. This system was a helpful tool in a few surveys with a perfect different microorganism's identification (Yahya and Ghosoon, 2016). However, in our study, all isolates were successfully identified using traditional biochemical tests that were confirmed using the Vitek2 system as identified and confirmed by Abbas and Radhi (2016).

Table 2. Distribution and percentages of *Enterobacteriaceae* species identified from the examined samples

Powdered infant milk formula samples		**Milk-cereal-based infant formula samples**	
Sample number	**Species**	**Sample number**	**Species**
IF2	*Enterobacter cloacae* ssp. *cloacae* *Pantoea* spp.	MC6	*Pantoea* spp.
IF12	*Enterobacter hormaechei* *Pantoea* spp.	MC7	*Enterobacter cloacae* ssp. *cloacae* *Pantoea* spp.
IF15	*Enterobacter cloacae* ssp. *cloacae* *Pantoea* spp.	MC8	*Pantoea* spp.
IF18	*Enterobacter hormaechei*	MC14	*Enterobacter cloacae* ssp. *cloacae* *Pantoea* spp.
IF19	*Enterobacter cloacae* ssp. *Cloacae*	MC19	*Enterobacter cloacae* ssp. *cloacae* *Pantoea* spp.
IF26	*Enterobacter hormaechei* *Pantoea* spp.	MC20	*Enterobacter cloacae* ssp. *cloacae* *Enterobacter hormaechei* *Raoultellaornithinolytica*
		MC21	*Enterobacter cloacae* ssp. *cloacae* *Pantoea* spp.
		MC22	*Enterobacter cloacae* ssp. *cloacae* *Enterobacter hormaechei* *Pantoea* spp.
		MC23	*Pantoea* spp.
		MC24	*Pantoea* spp.
		MC27	*Pantoea* spp.
		MC29	*Pantoea* spp.
		MC30	*Enterobacter cloacae* ssp. *cloacae*

Species	Isolates of powdered infant milk formula		Isolates of Milk-cereal-based infant formula	
	No.	%	No.	%
C. sakazakii, Salmonella, and *E. coli*	0	0.0	0	0.0
Enterobacter cloacae ssp. *cloacae*	3	30.0	7	33.3
Enterobacter hormaechei	3	30.0	2	9.5
Pantoea spp.	4	40.0	11	52.4
Raoultella ornithinolytica	0	0.0	1	4.8
Total No. of isolates	10	100.0	21	100.0

IF: Infant Formula samples, MC: Milk-cereal-based infant formula samples, No.: Number of examined isolates

As shown in Table 2, we could not detect *Cronobacter sakazakii*,similar to data obtained by Abdullah Sani et al. (2013) and El-Zamkanand Mohamed (2018), neither *Salmonella* spp. nor *E. coli* could detect in all sixty examined samples. Also, Sayed (2004) and Alsharjabi et al. (2017) could not detect *E. coli* in the weaning formula. Failure detection of *E. coli* proved that; it is neither a hygienic indicator of choice for dried infant food nor the absence of other enteric pathogens (FAO/WHO, 2004; Alsharjabi et al., 2017).

Enterobacteriaceae were categorized referred to the capability in existence in the desiccated situation for long periods into four groups: some of them were isolated in this research as follows: *E. cloacae* in group 1, which could be recoverable in only six months, and group three includes *Pantoea* spp., that persevered more than two years (Barron and Forsythe, 2007).

The existence of coliforms in the examined formula samples

The thirty powdered infant milk formula samples had coliforms count of less than 3 MPN/g. However, only five samples of milk-cereal-based infant formula had a count of 4 MPN/g that agreed with Sayed;(2004). Coliforms are an indicator of unhygienic processing conditions (Abdullah Sani et al., 2013).

The antibiotic resistance of *Enterobacteriaceae* species

All *Enterobacteriaceae* species isolated from examined milk-cereal-based infant formula samples were more resistant to tested antibiotics than species from powdered infant formula samples. Results in Table 3 revealed that identified isolates were 100.0% susceptible to gentamycin and resistant to cephalothin. Chloramphenicol has inhibited the growth of most *Enterobacteriaceae* isolates except for only one *Pantoea* spp. All isolates of *Enterobacter cloacae* ssp. *cloacae* were resistant to cephalothin, neomycin, tobramycin, and colistin sulfate antibiotics. Although they were susceptible to chloramphenicol, gentamycin, and nalidixic acid.60.0% and 30.0% of isolates were resistant to streptomycin and piperacillin, respectively, and almost similar to *Enterobacter cloacae,* which were isolated by Al-Timimi (2007). Also, 40.0% of *Enterobacter hormaechei* isolates were intermediately resistant to piperacillin and nalidixic acid, and 100.0% resistant

Table 3. Degree of antibiotic resistance pattern of *Enterobacteriaceae* speciesidentified from the examined milk formula samples

Species		Degree of antibiotic resistance pattern								
		S	C	KF	GEN	N	TOB	CT	PRL	NA
Enterobacter cloacae ssp. *cloacae* (No.=10)	S	0	10 (100%)	0	10 (100%)	0	0	0	0	10 (100%)
	I	4 (40%)	0	0	0	0	0	0	7 (70%)	0
	R	6 (60%)	0	10 (100%)	0	10 (100%)	10 (100%)	10 (100%)	3 (30%)	0
Enterobacter hormaechei (No.=5)	S	0	5 (100%)	0	5 (100%)	0	0	0	0	3 (60%)
	I	0	0	0	0	0	0	0	2 (40%)	2 (40%)
	R	5 (100%)	0	5 (100%)	0	5 (100%)	5 (100%)	5 (100%)	3 (60%)	0
Pantoea spp. (No.=15)	S	0	14 (93.3%)	0	15 (100%)	0	1 (6.7%)	1 (6.7%)	0	15 (100%)
	I	3 (20%)	1 (6.7%)	0	0	1 (6.7%)	0	0	6 (40%)	0
	R	12 (80%)	0	15 (100%)	0	14 (93.3%)	14 (93.3%)	14 (93.3%)	9 (60%)	0
Raoultella ornit hinolytica (No.=1)	S	0	1 (100%)	0	1 (100%)	0	0	0	0	0
	I	1 (100%)	0	0	0	0	0	0	0	1 (100%)
	R	0	0	1 (100%)	0	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0

No.: Number of examined isolates, S: Susceptible, I: Intermediate resistant, R: Resistant, S: Streptomycin, C: Chloramphenicol, KF: Cephalothin, GEN:Gentamycin, N: Neomycin, TOB: Tobramycin, CT: Colistin sulphate, PRL: Piperacillin, NA:Nalidixic acid.

to cephalothin, streptomycin, neomycin, tobramycin, and colistin sulfate antibiotics. These results are similar to Parra-Flores et al. (2018) study;*Enterobacter hormaechei* isolates were resistant to cephalothin and susceptible to chloramphenicol and gentamycin. *Panteoa* spp. has exhibited a different antibiotic resistance among isolates, as 14/15 showed resistance to tobramycin, neomycin, and colistin sulfate but susceptible to chloramphenicol, these findings are nearly similar to Mardaneh and Dallal (2013). *R. ornithinolytica* was resistant to most antibiotics like cephalothin, neomycin, tobramycin, colistin sulfate, and piperacillin, as presented in Table 3.

Although *R.ornithinolytica* is not an extremely harmful pathogen, it may lead to infections like meningitis, osteomyelitis, cerebral abscess, pericarditis, respiratory and urinary tract infections with a predominantly lethal sequel and has the ability for developing antibiotic resistance rapidly (Seng et al., 2016; Abbas and Ahmad, 2018). The isolated species showed a different antibiotics profile and resistance to more than one antibiotic. It was considered a public health concern, as a total of 10 samples were contaminated with more than one species. The antibiotic resistance of *E. cloacae* has been the significant cause of its several nosocomial infection outbreaks and the ability to colonize and rapidly adapt to environmental stress(-Davin-Regli and Pages, 2015).

Degree of acceptability of the examined formula samples versus Egyptian and other comparable international standards

In our study, 20.0% of powdered infant milk formula samples were not complying with Egyptian standards (ES, 2006). Coliforms must be absent, while six samples would not be free from *Enterobacter* spp. as coliforms.Also, 20.0% of samples were containing *Enterobacteriaceae,* so they were unacceptable referenced to the Codex Alimentarius Commission (CAC, 2008) and European Commission (EC, 2007) which stated that samples must be0/10 g of acceptable sample for *Enterobacteriaceae.* According to FSANZ (2016), all samples had been accepted as all coliform's counts were less than 10 MPN/ g, without *Cronobacter sakazakii* (0/10 g) or *Salmonella* (0/25 g) detection (Figure 1). The vulnerability of neonates to microbial infection leads to a demand for highly restricted microbiological standards for infant formula, which conformed to national and international specifications (Forsythe, 2005; CAC, 2008).

Egyptian standard needs more attention from the authorities because it does not focus on *Cronobacter sakazakii* and *Salmonella* like other international standards as acceptable samples to be 0/10 g and 0/25 g, respectively. The pathogens of interest and significant risk factors in powdered infant milk formula were involved in *Enterobacteriaceae*, so FAO-WHO authority commended this family substitute coliforms such are less common (FAO/WHO 2004, 2006).Besides, the European Food Safety Authority (EFSA, 2007) approved that the concerning microbiological indicator key for the dried formula is *Enterobacteriaceae*.

Also, as shown in Figure 2, 100.0% of samples were agreeable with Egyptian standards (ES, 2005) and FSANZ (2016) standards as they establish a limit

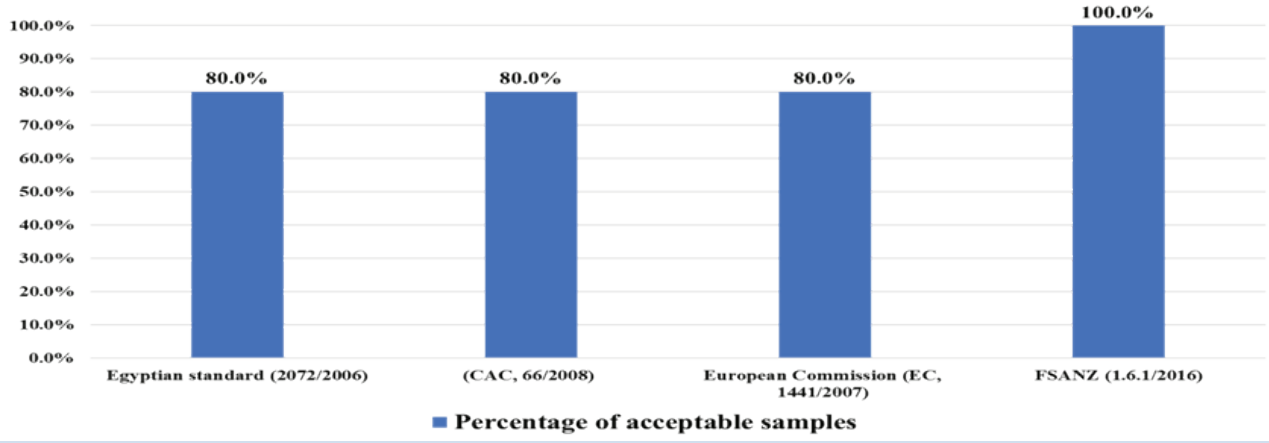

Fig. 1. Degree of acceptability of the examined powdered infant milk formula depend on various standards (n= 30)

n= number of examined samples, CAC: Codex Alimentarius Commission, FSANZ: Food Standards Australia New Zealand.

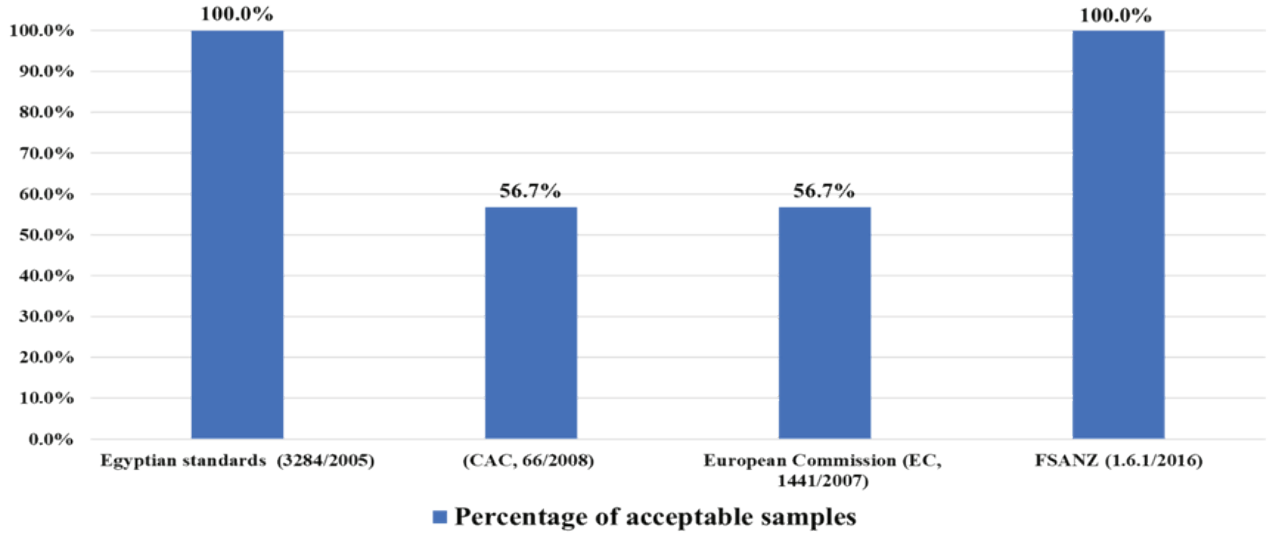

Fig. 2. Degree of acceptability of the examined milk-cereal-based infant formula depend on various standards (n= 30)

n= number of examined samples; CAC: Codex Alimentarius Commission, FSANZ: Food Standards Australia New Zealand.

of coliforms count less than 10 MPN/g and 20 MPN/g, respectively. While, about 56.7% of samples were acceptable with EC (2007) and CAC (2008) standards, which mentioned that cereal-based foods have the same food safety criterion applied for powdered follow-up formulae, as these set a limit of free *Enterobacteriaceae* in 10 g. All available standards for powdered follow-up formulae had no measure or definition for *Cronobacter* due to the low probability of causing illness in older infants > 6 months of age (CAC, 2008). Also, the Egyptian standard (ES, 2005), is not mentioned the limitation for *Salmonella* spp. presence in milk-cereal-based infant formula, dissimilar to different accessible international standards, which set a limit of 0/25 g.

A study demonstrated by Jackson et al. (2015), who conducted reassessment and analysis of isolates, implicated in an outbreak in Mexico in neonates results from consumption of rehydrated Powdered Infant Formula (PIF), these strains were *Enterobacter hormaechei* and *Enterobacter* spp. that incorrectly identified as *C. sakazakii* (Flores et al., 2011). Parra-Flores et al. (2018) declared that *Enterobacter hormaechei* and another *Enterobacter* spp. were isolated from PIF, which involved an outbreak of hemorrhagic diarrhea in Mexico. They proved the likelihood of *Enterobacter* spp. infection in infants via PIF consumption. Fei et al. (2015) recommended pathogen prevention from the beginning of manufacturing to diminishing its occurrence in the finished infant formula, as the exposure of powdered formula milk during drying and packaging to surrounding ecological conditions may cause final product pollution. World Health Organization (WHO, 2007) has recommended H_2O at more than 70ºC for reconstituted infant formula, also consumed instantaneously after preparation that should throw away even if not eaten for 2 hours. The follow-up good hygienic practices in raw milk and other raw ingredients production, handling, and transportation are recommended (Fricker-Feer, 2012). In addition, training programs for industrialists and completeness of Hazard Analysis and Critical Control Point system are needed for fulfilling the highest safe products for achieving high hygiene and sanitation levels in the production environment (Hafiz et al., 2016; Ibrahim et al., 2020; Ibrahim et al., 2021; Ibrahim et al., 2022b).

CONCLUSION

This study was demonstrating the possible entrance of *Enterobacteriaceae* members into powdered and milk-cereal-based infant formula and indicated insufficient sanitation monitoring through manufacturing steps besides equipment and staff hygiene. The identified *Enterobacteriaceae* showed resistance to more than one antibiotic, which represents an infant's health hazard. Improvements in food for an infant are now highly recommended for the prevention presence of *Enterobacteriaceae* within formula plants and the final product. Requirements of strict hygienic measures with monitoring with the supervisory authorities' support and more microbiological standard re-

strictions for this sensitive population are essential. More studies and research should be done to determine the possibility of transmitting *Enterobacter* spp., *Pantoea* spp., and *Raoultella ornithinolytica* through reconstituted infant formula and causing infection.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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