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ORIGINAL ARTICLE

Molecular Characterization of Multidrug-Resistant *Enterobacter spp*. Isolated from Powdered Infant Formula and children with Gastroenteritis

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Key words:
Enterobacter spp; Antimicrobial resistance; Multidrug-resistance

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ABSTRACT

Background: *Enterobacter species causes many serious and life-threatening infections. Enterobacter species may be the cause of diarrhea in children. Multidrug-resistant (MDR) Enterobacter species limits treatment options. Powdered Infant Formula (PIF) was epidemiologically linked to diseases in infants caused by Enterobacter spp.* ***Objectives:*** *Our study aimed to detect the frequency and antimicrobial resistance profile, both phenotypically and genotypically of MDR Enterobacter isolated from powdered infant formula (PIF) and stools of children suffering from gastroenteritis who needed hospitalization and determine if there is a link between the presence of Enterobacter spp. in contaminated PIF & its presence in the stool of infants.* ***Methodology:*** *Isolation of Enterobacter spp. from Powdered Infant Formula and Stool of Infants, Antimicrobial susceptibility test of Enterobacter spp. isolates to antimicrobial agents, genotypic test by polymerase chain reaction (PCR) for five genes: 16S rDNA, bla_{SHV}, bla_{TEM}, aac(6')-Ib-cr, and qnrB1 genes, and molecular identification of multidrug-resistant of Enterobacter strains which will be isolated from infant formula and stools.* ***Results:*** *Frequency of multidrug-resistant Enterobacter spp. isolates from PIF were 3 isolates positive for four resistant genes in three antibiotics classes (β-Lactam, Aminoglycoside, and Fluoroquinolones) with a percentage of 27.3%, and the genotypic frequency of multidrug-resistant Enterobacter spp. isolates from infants' stool were 2 isolates positive for four resistant genes in three antibiotics classes (β-Lactam, Aminoglycosides, and Fluoroquinolones) with a percentage of 13.3%.* ***Conclusion:*** *MDR Enterobacter was isolated from young aged (less than 24 months) children and PIF, more than one resistance gene: bla_{SHV}, bla_{TEM}, aac(6')-Ib-cr, and qnrB1 genes were detected in isolates. The presence of MDR strains is risky at a young age as it limits treatment options. Drug-resistant genes may be transmitted to a child through a carrier mother or PIF or cross-infection from the hospital.*

INTRODUCTION

Diarrhea and gastroenteritis come the second position among the top ten diseases admitted to hospitals in the world, the diarrhea-specific mortality in children younger than five years of age in Africa has been estimated at 106 per 1000[1]. The annual mortality rate associated with diarrhea is 30 deaths per 100,000 among Egyptian children under 5 years old, according to recent statistics by the World Health Organization (WHO)[2]. Incidence of severe gastroenteritis being highest in the first 2 years of life[3].

Enterobacter spp. are also natural commensals of animal and human gut Microbiota, *Enterobacter* speciesare members of the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp)*[4]. In neonatal units, *Enterobacter species* have been identified as a nosocomial pathogen, and multiple infection outbreaks have been documented causing bacteremia, endocarditis, septic arthritis, osteomyelitis, skin and soft tissue infections, lower respiratory tract, urinary tract and intra-abdominal infections[5]. *Enterobacter* has been found in powdered milk infant formula as many neonates depend on it in their feeding, PMIF are infected with opportunistic *Enterobacteriaceae* pathogens, reconstituted PMIF is nutritious, and may allow rapid growth of bacteria when the prevailing water activity, time for growth, and temperature are favorable[6].

Enterobacter produces enzymes such as β-lactamase responsible for the activity of β-lactam antibiotics, a group that includes Imipenem and Cephalosporins, repeated exposure to these drugs gives rise to drug resistance[7]**.** World Health Organization placed *Enterobacter spp.* onto the list of bacteria that should be studied to create new antibiotics, In addition to having an innate resistance to Ampicillin and broad-spectrum Cephalosporins, *Enterobacter* species have developed resistance to numerous drugs, including Carbapenems and third-generation Cephalosporins, via acquiring genetic mobile elements[8]**.**

The aim of this study is isolation of *Enterobacter spp.* from Powdered Infant Formula and Stool of Infants, Antimicrobial susceptibility test of *Enterobacter spp.* isolates to antimicrobial agents, genotypic test by polymerase chain reaction (PCR) for five genes: *16S rDNA*, bla_{SHV}, bla_{TEM}, *aac(6')-Ib-cr*, and *qnrB1* genes, and molecular identification of multidrug-resistant of *Enterobacter* strains which will be isolated from infant formula and stools.

METHODOLOGY

Design of the study:

A cross-sectional descriptive research was conducted from Jan 2023 to July 2024.

Ethical statement:

This research was conducted in keeping with the ethical principles of the World Medical Association's code of ethics (Declaration of Helsinki). The study was approved by the ethical committee of Assiut University Faculty of Medicine. (**Approval No. 200376**).

Samples Size Estimation:

Sample size was calculated using statcalc program of EPI-info version 7.2 using descriptive study design calculation, according to the previous research, the prevalence of isolate *Enterobacter* organism in stool was 12.6% as reported by[1]**.** - Confidence level 90%, degree of precision 5%, and design effect 1. The minimum required sample size will be 120 cases.

Sample collection:

120 Samples of PIF and 120 infants' stool swabs were collected from infants suffering from gastroenteritis at Gastroenterology and Hepatology Unit (included 85 samples collected from acute diarrheic infants and 35 samples from non diarrheic infants), Children Hospital, Assiut University.

Samples processing

- ***Preparation of PIF samples:*** Detection of *Enterobacter spp.* and its isolation depending on three sequential steps including pre-enrichment in buffered peptone water (BPW) broth, enrichment in *Enterobacteriaceae* Enrichment Broth (EEB), then plating on (selective and chromogenic media)[9]**.**
- ***Pre-enrichment procedure:*** One gram of each homogenized PMIF sample was dissolved and pre-enriched selectively with 9mL of pre-warmed sterilized buffered peptone water following incubation for 24 hrs at 37°C[10]**.**
- ***Enrichment procedure:*** After incubation, 10 ml of each dilution was added to 90 mL of EE (*Enterobacteriaceae* enrichment) broth medium and then incubated at 35 ± 2°C for 18 to 24 hours and plated on selective media**,** suspected colonies were then picked up and cultured for biochemical identification tests[11]**.**

***Isolation and identification of Enterobacter spp. from PIF*:** Identification of *Enterobacter spp.* from PIF was carried out using Violet Red Bile Glucose Agar[6]**.**

Isolation of Enterobacter spp. from infants' stool suffering from gastroenteritis:

- ***Preparation of stool samples:***
- ***Pre-enrichment procedure:*** Each fecal swab was dissolved in 10 mL of buffered peptone solution and then incubated for 24 hours at 37°C[12]**.**
- **Enrichment procedure:** After incubation, 10 ml of each sample was added to 90 mL of EE (*Enterobacteriaceae* enrichment) broth medium and incubated at 35 ± 2°C for 18 to 24 hours, A loopful of incubated enrichment EE broth of each sample was streaked by plating out on selective media and incubated for 18 – 24 hours at 35 ± 2°C and then plated on selective media. Suspected colonies were then picked up and cultured for further microscopic and biochemical identification[9]**.**

The following culture media were used for the isolation of *Enterobacter spp:*

1. **Culture on solid media:** Identification of *Enterobacter spp.* was carried out using MacConkey Agar[1]**,** Eosin Y and Methylene blue agar (EMB)[13]**,** Tryptic Soy Agar (TSA)[9]**,** Blood agar[14]**.**
2. **Highly selective media:** Hichrome Brilliance UTI agar was used for identification of *Enterobacter spp*[15]**.**
3. **Examination of Gram stained smear prepared from suspected colonies:** Film was stained with Gram's staining technique and examined microscopically to verify the presence of characteristic features of the organism[16]**.**
4. **Biochemical tests:** Biochemical identification of *Enterobacter spp*. was carried out using The Triple Sugar Iron test (TSI)[17]**,** Citrate Utilization test[18]**,** Urease test[15]**,** Catalase test[9]**.**

Preservation of isolated *Enterobacter spp*. samples: Purified isolates were then stored at -20°C in LB broth supplemented with 20% glycerol for further processing[9]**.**

Antimicrobial susceptibility test for *Enterobacter spp*. by Kirby-Bauer disc diffusion method:-*Enterobacter* isolates submitted to sensitivity testing using Kirby-Bauer method in accordance with CLSI recommendations as follow: interpretive categories of

susceptible, intermediate and resistant was assigned according to CSLI[19].

Molecular detection of *Enterobacter spp*:

1. **DNA extraction:** Bacterial cultures were concentrated by centrifugation (5000 rpm/10 min) to obtain heavy growth, and placed in 1.5 ml eppendorf tubes containing 300 micro liter distilled water, vortex for few seconds and then we placed the tubes in 95˚C water bath for 30 min, followed by centrifugation for 10 minutes at 5000 rpm, supernatant was transferred to new sterilized eppendorf tube and stored in ice until used[20].
2. **Detection of universal *16S Ribosomal DNA (16S rDNA):*** Universal bacterial *16S rDNA* primers were used to identify *Enterobacter spp by* PCR because *16S rRNA* hypervariable regions, displayed varying levels of sequence diversity, and all bacteria cannot be distinguished by a single hypervariable area[21].

Detection of resistant *gene*s for *Enterobacter spp*:

1. **Identification of *ESBL* genotypes (bla_{TEM}, and bla_{SHV}):** Amplifying the bla_{TEM} gene and bla_{SHV} *genes* detected and the bands were positive isolates at a fragment sized 404bp for bla_{TEM} gene and 900bp for bla_{SHV} gene[22].
2. **Identification of *aac(6')-Ib-cr* gene**: Amplifying the *aac(6')-Ib-cr* gene detected and the bands were positive isolates at a fragment sized 535bp[23].
3. **Identification of *qnr B1* gene:** Amplifying the *qnrB1*gene detected and the bands were positive isolates at a fragment sized 383bp[24].

Table 1: Nucleotide sequences of primers used for the identification of *Enterobacter spp.* and resistant genes

Class	Target gene(s)	Sequence (5′ to 3′)	Amplification Size (bp)	References
Universal primers	*16S rDNA*	F: AGAGTTTGATCCTGGCTCAG R:AAGGAGGTGATCCAGCCGCA	1500bp	[21]
ESBLs; Ambler class A	bla_{TEM}	**F:**TCCGCTCATGAGACAATAACC **R:** ACGCTCAGTGGAACGAAAAC	404bp	[22, 23]
	bla_{SHV}	**F:** CGCCGGGTTATTCTTATTTG **R:** CCACGTTTATGGCGTTACCT	900bp	
Aminoglycosides And Flouroquinolones	*aac(6')-Ib-cr*	**F:**TGACCTTGCGATGCTCTATG **R:** TTAGGCATCACTGCGTGTTC	535bp	[22, 24]
Flouroquinolones	*qnrB1*	**F:**ACCTGAGCGGCACTGAATTTA **R:**TCGCAATGTGTGAAGTTTGC	383bp	[22, 25]

Statistical analysis:

Data was analyzed using SPSS version 26. Categorical data were presented in the form of frequencies and percentages. Fisher Exact test or Chi square ($\chi 2$) test were used to compare proportions of various groups. P -value considered significant if < 0.05.

RESULTS

Genotypic identification of *Enterobacter spp*:

Molecular detection of *16S rDNA* gene by using (Universal primer for detection of *Enterobacter spp*.) as shown in figure 1.

Frequency of *Enterobacter spp.* in PIF and infants' stool samples:

Frequency of *Enterobacter spp*. isolated from PIF samples was 9.2%, while those isolated from infants' stool samples were 12.5% as shown in Table 2.

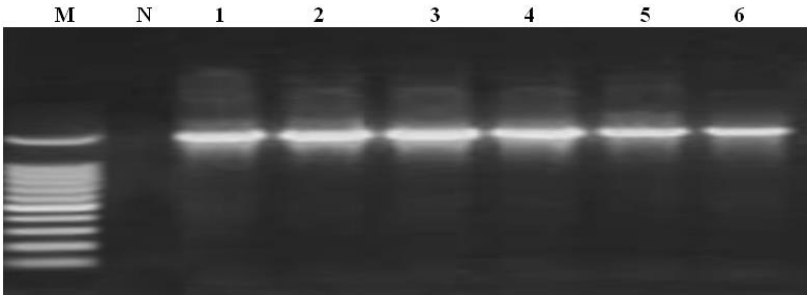

Figure 1: Agarose electrophoresis gel for *16S rDNA* gene positive *Enterobacter spp.* isolates (1500bp). Lanes (1, 2, 3, 4, 5, and 6) showed bands for the *16S rDNA* gene. Lane (N) showed negative control (Distilled Water)**.** Lane (M) showed 100-1500bp DNA Molecular Weight Marker.

Table 2: The frequency of *Enterobacter spp*. in PIF and infants' stool samples

Type of samples	*Enterobacter spp.* isolates		Negative samples		Total samples	P-value
	No.	%	No.	%	No.	
PIF samples	11	(9.2%)	109	(90.8%)	120	0.406
Infants' stool samples	15	(12.5%)	105	(87.5%)	120	

The P-value wasn't statistically significant.

Phenotypic multidrug-resistance of *Enterobacter spp.* isolated from PIF and infants' stool samples:

- **Antimicrobial susceptibility test of *Enterobacter spp.* isolates from PIF:** *Enterobacter spp.* isolates showed 100% resistance to each of Amoxacillin, Amoxacillin-Clavulanic acid, and each of Cefazolin, Cefuroxime, Cefotaxime and Ceftriaxone exhibited 81.8% resistance but Cefoperazone exhibited 72.7% resistance, Co-Trimoxazole exhibited 63.6% resistance, each of Ciprofloxacin, Ofloxacin, Norfloxacin and Lomefloxacin exhibited resistance pattern of 54.5%, Imipenem and Meropenem were 36.4% resistance, on the other hand Tetracycline, Oxytetracycline and Chloramphenicol had less resistant were 18.2%, Gentamycin and Tobramycin had the least resistance pattern of 9.1%, a category interpretation will be reported along with a Kirby-Bauer disc diffusion method, according to the interpretations defined by CLSI M100, 2023.
- **Antimicrobial susceptibility test of *Enterobacter spp.* isolates from infants' stool:** *Enterobacter spp.* isolates showed 100% resistance to each of Amoxacillin, Amoxacillin- Clavulanic acid, each of Cefazolin, Cefuroxime, Cefotaxime, and Ceftriaxone exhibited 80% resistance, Cefoperazone 73.3% resistance, Co -Trimoxazole exhibited 66.7% resistance, each of Ciprofloxacin, Ofloxacin, Norfloxacin and Lomefloxacin exhibited resistance pattern of 53.3%, Imipenem, and Meropenem were 40%, on the other hand, Tetracyclines, Oxytetracyclines, and Chloramphenicol had less resistant were 13.3%, Gentamycin, and Tobramycin had the least resistance pattern of 6.7%, a category interpretation will be reported along with a Kirby-Bauer disc diffusion method, according to the interpretations defined by CLSI M100, 2023.

Table 3: Phenotypic multidrug-resistance patterns of *Enterobacter spp*. isolates from PIF and infants' stool:

Antimicrobial classes	Frequency of (MDR) for *Enterobacter spp.* isolates from PIF samples		Frequency of (MDR) for *Enterobacter spp.* isolates from infants' stool samples	
	No.	Percentage	No.	Percentage
3 classes (β-Lactam, Sulfa drug and Flouroquinolones)	(4/11)	36.4%	(6/15)	40%
4 classes (β-Lactam, Sulfa drug, Flouroquinolones, and Tetracyclines)	(2/11)	18.2%	(2/15)	13.3%
5 classes (β-Lactam, Sulfa drug, Flouroquinolones, Tetracyclines, and Chloramphenicol)	(2/11)	18.2%	(2/15)	13.3%
6 classes (β-Lactam, Sulfa drug, Flouroquinolones, Tetracyclines, Chloramphenicol, and Aminoglycosides)	(1/11)	9.1%	(1/15)	6.7%

The relationship between phenotypic multidrug-resistance patterns of *Enterobacter spp.* isolates from infants' stool according to age:

Multidrug-resistant of *Enterobacter spp.* to three antimicrobial classes (β-Lactam, Sulfa drug, and Flouroquinolones) represented 33.3%, 33.3%, 33.3%, and 50% in infants at ages 1-3 months, >3-6 months, > 6-12 months, and >12-24 months respectively, on the other hand, multidrug-resistant of *Enterobacter spp.* to four antimicrobial classes (β-Lactam, Sulfa drug, Flouroquinolones, and Tetracyclines) and five antimicrobial classes (β-Lactam, Sulfa drug, Flouroquinolones, Tetracyclines, and Chloramphenicol) were the same percentage which represented 33.3%, zero%, zero % and 16.7 % in infants at ages 1-3 months, >3-6 months, > 6-12 months, and >12-24 months respectively, *Enterobacter spp.* (MDR) to six antimicrobial classes (β-Lactam, Sulfa drug, Flouroquinolones, Tetracyclines,Chloramphenicol, and Aminoglycosides) represented 33.3% only from 1-3 months.

The relationship between phenotypic multidrug-resistance patterns of *Enterobacter spp.* isolates from diarrheic infants' stool according to severity of diarrhea:

Multidrug-resistant *Enterobacter spp.* isolates from infants' stool according to the severity of diarrhea were found to be 36.4% resistant to 3 antimicrobial classes, in mild, moderate, and severe diarrhea with percentages of 25%, 25%, and 66.7% respectively, while 4 classes and 5 classes of antimicrobial were same resistant 9.1%, in mild, moderate, and severe diarrhea with percentage zero%, zero%, and 33.3% respectively, *Enterobacter spp.* isolates resistance to 6 antimicrobial classes were resistant only in severe diarrhea with a percentage of 33.3%.

The relationship between phenotypic multidrug-resistance patterns of *Enterobacter spp.* isolates from non-diarrheic infants' stool:

Multi-drug-resistant *Enterobacter spp.* isolates from non-diarrheic infants' stool were 50% resistant to 3 classes of antimicrobial agents (β-Lactam, Sulfa drug, and Fluoroquinolones), while 4 classes of antimicrobial agents (β-Lactam, Sulfa drug, Fluoroquinolones, and Tetracyclines) and 5 classes of antimicrobial (β-Lactam, Sulfa drug, Fluoroquinolones, Tetracyclines, and Chloramphenicol) were the same resistant 25%, and there was no multidrug-resistant *Enterobacter spp.* isolates to 6 antimicrobial classes from non-diarrheic infants' stool.

Molecular Detection of resistant genes of *Enterobacter spp*: According to molecular identification by PCR for resistant genes, the frequency of *Enterobacter spp.* isolates from PIF carrying bla_{SHV} gene and bla_{TEM} gene were the same percentage 54.5%, frequency of *Enterobacter spp.* isolates from PIF carrying *aac(6')-Ib-cr* gene was 36.4% and the frequency of *Enterobacter spp.* isolates from PIF carrying *qnrB1*gene was 45.5%, on the other hand, the frequency of *Enterobacter spp.* isolates from infants' stools carrying the bla_{SHV} gene, bla_{TEM} gene, *aac(6')-Ib-cr* gene, and *qnrB1* gene were 46.7%, 53.3%, 33.3%, and 40% respectively.

Molecular Detection of resistant bla_{SHV} gene:

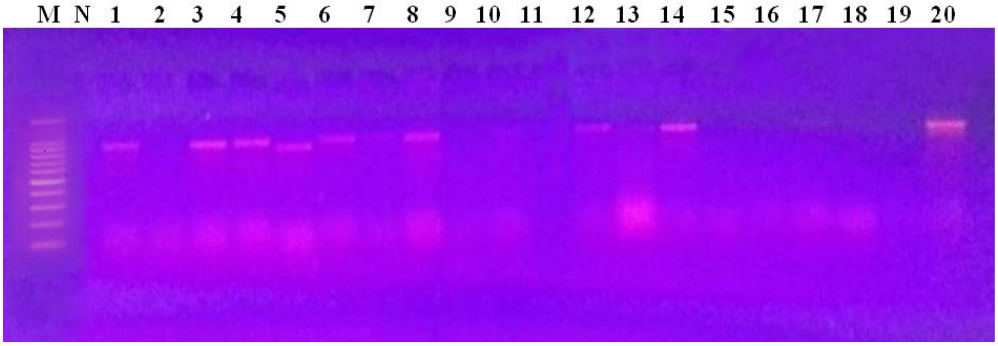

Figure 2: Agarose electrophoresis gel for the bla_{SHV} gene positive isolates (900bp). Lanes (1, 3, 4, 5, 6, 8, 12, 14 and 20) showed bands for bla_{SHV} gene 900bp. Lanes (2, 7, 9, 10, 11, 13, 15, 16, 17, 18 and 19) showed negative samples for bla_{SHV} gene. Lane (N) showed negative control (Distilled Water). Lane (M) showed 100-1500bp DNA Molecular Weight Marker.

Molecular Detection of resistant *bla$_{TEM}$* gene:

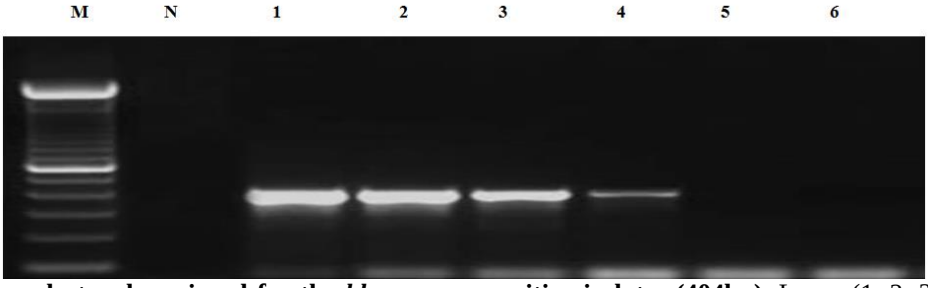

Figure 3: Agarose electrophoresis gel for the *bla$_{TEM}$* gene positive isolates (404bp). Lanes (1, 2, 3 and 4) showed bands for *bla$_{TEM}$* gene 404bp. Lanes (5 and 6) showed negative samples for *bla$_{TEM}$* gene. Lane (N) negative control (Distilled Water).Lane (M) showed 100-1500bp DNA Molecular Weight Marker.

Molecular Detection of resistant *aac(6')-Ib-cr* gene:

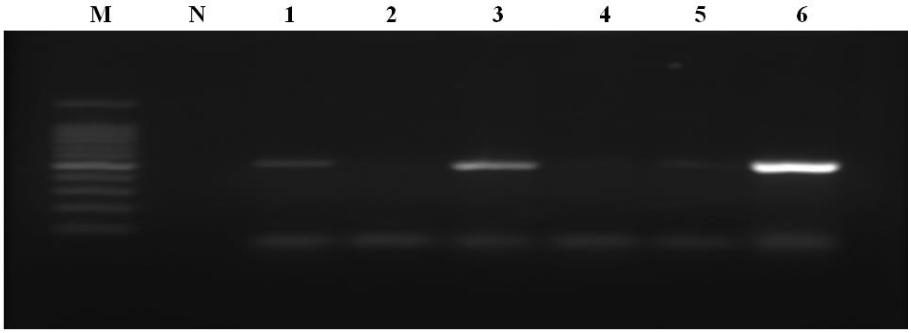

Figure 4: Agarose electrophoresis gel for the *aac(6')-Ib-cr* gene positive isolates (535bp). Lanes (1, 3, 5, and 6) showed bands for *aac(6')-Ib-cr* gene 535bp.Lanes (2 and 4) showed negative samples for *aac(6')-Ib-cr* gene. Lane (N) showed negative control (Distilled Water). Lane (M) showed a 100-1500bp DNA Molecular Weight Marker.

Molecular Detection of resistant *qnrB1* gene:

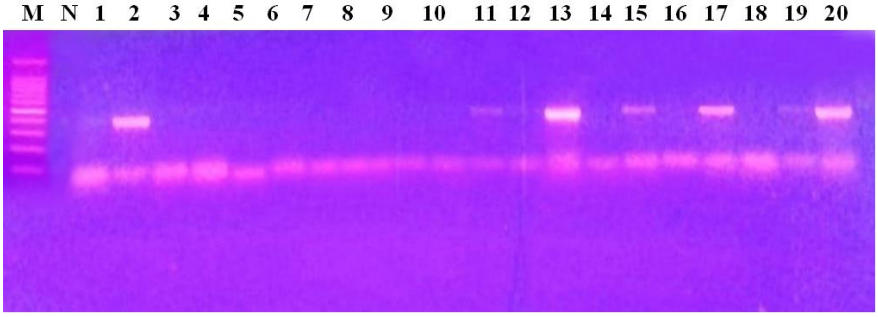

Figure 5: Agarose electrophoresis gel for the *qnrB1* gene positive isolates (383bp). Lanes (2, 11, 13, 15, 17, 19 and 20) showed bands for *qnrB1*gene 383bp. Lanes (1, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, and 18) showed negative samples for *qnrB1*gene. Lane (N) negative control (Distilled Water). Lane (M) showed 100-1500bp DNA Molecular Weight Marker.

Genotypic detection of multidrug–resistant of *Enterobacter spp.*

Genotypic detection of multidrug–resistant of *Enterobacter spp.* isolates from PIF was 27.3% which three isolates positive for four genes in three antibiotics classes (β-Lactam, Aminoglycoside, and Flouroquinolones), while genotypic frequency of multidrug- resistant of *Enterobacter spp.* isolates from infants' stools expressed a pattern of 13.3% which two isolates positive for four resistant genes in three antibiotics classes (β-Lactam, Aminoglycosides, and Flouroquinolones) as shown in table 4.

Table 4: Genotypic multidrug-resistant for *Enterobacter spp*. isolated from PIF and infants' stool:

Resistant genes	Antibiotic classes	*Enterobacter spp*. isolates from PIF samples		*Enterobacter spp.* isolates from infants' stool samples	
		No.	%	No.	%
1- bla_{TEM} gene 2- bla_{SHV} gene	1- β-Lactam	(3/11)	27.3%	(2/15)	13.3%
3- *aac(6')-Ib-cr* gene	2- Aminoglycosides				
4- *qnrB1*gene	3-Flouroquinolones				

Relationship between genotypic multidrug-resistant (MDR) for *Enterobacter spp*. isolated from infants' stool according to age:

Genotypic MDR of *Enterobacter spp.* isolates from infants' stool at ages 1-3 months, > 3-6 months, > 6-12 months, and >12-24 months were 33.3%, zero %, zero%, and 16.7% respectively.

The relationship between genotypic multidrug-resistant (MDR) for *Enterobacter spp*. isolated from infants' stool according to severity of diarrhea:

Enterobacter spp. isolates from infants' stool which expressed MDR genes according to severity of diarrhea as mild, moderate, and severe were found to be zero%, 25% and 33.3% respectively.

In this study, there was difference between phenotypic and genotypic detection for multidrug-resistant of *Enterobacter spp.* isolates as showed that phenotypic multidrug-resistant of *Enterobacter spp.* isolates from PIF for three antibiotics classes (β-Lactam, Aminoglycosides, and Flouroquinolones) was one isolate with percentage 9.1%, but genotypic multidrug-resistant of *Enterobacter spp.* isolates from PIF for the same three antibiotics classes (β-Lactam, Aminoglycoside, and Flouroquinolones) were 3 isolates with percentage 27.3%. Phenotypic multidrug-resistant of *Enterobacter spp*. isolates from infants' stool for three antibiotics classes (β-Lactam, Aminoglycosides,and Flouroquinolones) was one isolate with percentage 6.7%, but genotypic multidrug-resistant of *Enterobacter spp.* isolates from infants' stool for the same 3 antibiotics classes (β-Lactam, Aminoglycosides, and Flouroquinolones) were two isolates with percentage13.3%.

DISCUSSION

Enterobacter are commensals in the environment and are part of the normal flora of the human gastrointestinal tract. *Enterobacter* has emerged as a significant cause of nosocomial infections in recent years. This study revealed that the frequency of *Enterobacter spp.* isolates from PIF was 9.2%. Another study showed a different prevalence rates for *Enterobacter spp.* isolated from PIF which was 7.2%[11]. While another study revealed that *Enterobacter spp.* isolated from PIF samples with a percentage of 6.86%[10]. This result was different from the frequency of *Enterobacter spp.* isolated from PIF samples which ranged between 5.6% and 3.1% between the different locations where their study took place[26]. It was different from the range of prevalence of *Enterobacter spp.* isolated from PIF with a percentage of 6.7%[6]. It was very different from the range of prevalence of *Enterobacter spp.* isolated from PIF in infants1-6 months of age with a percentage of 48%[9]. Similarly, another study reported that the prevalence of *Enterobacter spp.* in infants isolated from stool samples with the same percentage of our study was 12.6%[1]. These results were different from the range of prevalence of *Enterobacter spp.* isolated from stool samples in infant age from (0-1) years was 0.5%[12]. It was very different from the prevalence of *Enterobacter spp.* isolated from stool samples from (1-6) months with a percentage of 50%[9].

According to the antimicrobial susceptibility by Kirby-Bauer disc diffusion method, interpretations defined by CLSI M100, 2023, Our study revealed that *Enterobacter spp*. isolated from PIF samples showed 100% resistance to each of Amoxacillin, Amoxacillin-Clavulanic acid, and 81.8% resistance to each of Cefazolin, Cefuroxime, Cefotaxime and Ceftriaxone, 72.7% resistance to Cefoperazone, 63.6% resistance to Co-Trimoxazole, each of Ciprofloxacin, Ofloxacin, Norfloxacin and Lomefloxacin exhibited resistance pattern of 54.5%,Imipenem and Meropenem were 36.4% resistance, on the other hand *Enterobacter spp*. isolates had less resistant to Tetracycline, Oxytetracycline, Chloramphenicol were 18.2%, while Gentamycin and Tobramycin exhibited resistance of *Enterobacter spp*. were the same percentage 9.1%. Other result showed that *Enterobacter* isolates from PIF were extremely susceptible to 100% Ciprofloxacin followed Gentamycin (75%), Cefotaxime (75%) and Meropenem (75%), while they were less sensitive to Cefoperazone (50%)[18].

According to the antimicrobial susceptibility of *Enterobacter spp*. isolated from infants' stool samples by Kirby-Bauer disc diffusion method, interpretations defined by CLSI M100, 2023, our study showed that *Enterobacter spp.* isolates showed 100% resistance to each of Amoxacillin, Amoxacillin-Clavulanic acid, each of Cefazolin, Cefuroxime, Cefotaxime and Ceftriaxone exhibited 80% resistance, Cefoperazone 73.3% resistance, Co -Trimoxazole exhibited 66.7% resistance,

each of Ciprofloxacin, Ofloxacin, Norfloxacin and Lomefloxacin exhibited resistance pattern of 53.3%, Imipenem and Meropenem were 40%, on the other hand, Tetracyclines, Oxytetracyclines and Chloramphenicol had less resistant were 13.3%, Gentamycin and Tobramycin had the least resistance pattern of 6.7%. Simillarly, another study revealed that *Enterobacter spp.* had high resistant to Cefuroxime and Ceftazidime, resistant to Cephalosporins like Ceftazidime and Cefuroxime and Penicillins like Ampicillin and Cloxacillin, and opposed to Tetracycline, Chloramphenicol, Streptomycin and Gentamycin[27]**.** In another study, *Enterobacter* displayed resistance to 84.2% of Ampicillin and had 78.9%, 63.2% and 57.9% resistant to Cloxacillin, Co-Trimoxazole and Streptomycin respectively[1]**.** *Enterobacter species* have inherent resistance to Ampicillin, Amoxicillin, first-generation Cephalosporins, and Cefoxitin. Additionally, the majority of isolates of *Enterobacter* species develop resistance to third-generation Cephalosporins, Penicillins, and Fluoroquinolones[28]**.** Another study revealed that *Enterobacter spp*. is highly resistant to Penicillins, Cephalosporins, Tetracyclines, Chloramphenicol, and Streptomycin, although most of *Enterobacter spp*. are sensitive to other Aminoglycosides, including Gentamycin, most strains are susceptible to Fluoroquinolones, Co-Trimoxazole, and Carbapenems[29]**.** Similar results showed that Aminoglycosides have a good activity to *Enterobacter spp.* isolates[4]**.** Another study revealed that *Enterobacter* isolates were high resistance rates to the Cephalosporins including Cefoxitin (82%), Cefixime (62%), Ceftazidime (46%) and Ceftriaxone (46%), resistance to Co-Trimoxazole and Ciprofloxacin were same percentage 36%, and resistance to Gentamycin and Meropenem were 30% and 22%, respectively[30]**.**

Our study revealed that phenotypic multidrug-resistant (MDR) *Enterobacter spp.* to three antimicrobial classes represented 36.4% from PIF and 40% from infants' stools and to four antimicrobial classes and five antimicrobial classes were the same percentage which represented 18.2% from PIF and13.3% from infants' stools and MDR to six antimicrobial classes represented 9.1% from PIF and 6.7% from infants' stools, our results in contrast to another study that showed all *Enterobacter spp.* isolates were multidrug-resistant with a percentage of 100%[31]**.**

According to the molecular identification of *Enterobacter spp.* by PCR. Our study revealed that the frequency of *Enterobacter spp.* isolates from PIF carrying bla_{SHV} gene and bla_{TEM} gene were the same percentage 54.5%, frequency of *Enterobacter spp.* isolates from PIF carrying *aac(6')-Ib-cr* gene was 36.4% and the frequency of *Enterobacter spp*. isolates from PIF carrying *qnr B1*gene was 45.5%.Another study showed that bla_{TEM} and bla_{SHV} resistant genes were detected in 25% of *Enterobacter spp.* isolates[32]**.** Also another study revealed that *aac(6')-Ib-cr* resistant gene was detected in 58% of *Enterobacter spp.* isolates and *qnr*-resistant genes were detected in 68% of *Enterobacter spp.* isolates[33]**.**

CONCLUSION

MDR *Enterobacter* was isolated from young aged (less than 24 months) children and PIF, and more than one resistance gene: bla_{SHV}, bla_{TEM}, *aac(6')-Ib-cr* and *qnrB1* genes were detected in isolates. In our study the most effective antibiotics for *Enterobacter spp.* isolates were Gentamycin and Tobramycin followed by Tetracycline, Oxytetracycline, and Chloramphenicol.

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