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Original article

Extended-spectrum beta-lactamase genes among Gram negative bacilli isolates from Egyptian children with diarrhea

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ABSTRACT

Background: Faecal carriage of ESBL-producing bacteria is a potential risk for transmission and infection. Antibiotic resistance in diarrheal children is considered a risk factor for these young aged ones and limit treatment options. This study aimed to investigate the frequency of ESBL-producing Gram negative bacilli (GNB) phenotypically by Vitek and genotypically by PCR. **Method:** Seventy-three diarrheal isolates from outpatient children at of age range from less than 6 months to 18 months were isolated. Bacterial strains were identified and subjected to antimicrobial susceptibility testing by using the automated Vitek 2 compact system. All isolates were screened for ESBL phenotypically by Vitek and genotypically by PCR for blaCTXM, blaTEM and blaSHV. **Results:** Seventy three different GNB identified by Vitek system. The frequency of *Escherichia coli* was 68.5%, *Acinetobacter haemolytica* was 7 /73 (9.6%), *Pseudomonas* species (sp.) 4/73 (5.5%), *Proteus mirabilis* was (2.7%), *Salmonella* sp. was (1.4%), *Enterobacter* sp. was (6.8%), *Aeromonas salmonicida* was (1.4%), and *Klebsiella* was (4.1%). ESBL carriage was significantly high among isolated strains (64.4 %). High frequency of multidrug resistance (MDR) (90.4%) was found. High resistance was detected to ampicillin (98.6%), followed by cefazolin (93.2%) and ceftazidime (82.2%), trimethoprim-sulphamethoxazol (76.7%). Resistance to carbapenems was detected as (16.4%). blaCTX-M positive was (78.1%), blaTEM positive (53.4%), blaSHV positive (31.5%). **Conclusions:** The current study reported a high rate of faecal carriage of ESBL-producing and MDR GNB and children below 2 years of age. Resistance was observed to the available antimicrobials that are used for children in treatment. This leaves few treatment options for infections caused by these bacteria.

Introduction

Gastroenteritis is a primary cause of disease and death in children under the age of five, primarily in poor countries [1]. Almost 10% of mortality was caused by diarrheal disorders, second only to pneumonia [1]. Due to the development of β-lactamase enzymes, antimicrobial resistance in Gram-negative rods is rising globally, particularly against β- lactam antibiotics [2]. The production of β-lactamases by *Enterobacteriaceae* has increased in recent years, which leads in more health-related

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infections, more deaths, and more expensive medical service [2]. Resistance to third generation cephalosporins is mainly caused by infections with AmpC β-lactamases- and extended-spectrum β -lactamases (ESBL)-producing [3].

The production of extended-spectrum β-lactamases (ESBLs) among *Enterobacteriaceae* is an emerging problem and associated with severe infections such as bloodstream infections (BSI), urinary tract infections, and meningitis [4].

Extended-Spectrum ß-Lactamase producing *Escherichia coli* (ESBL-PE) are typically multidrug-resistant and co-resistant to non-beta-lactam antimicrobial treatments. As a result, ESBL-PE infections have substantial effects, including increased morbidity and death [5].

Due to the emergence and dissemination of these antibiotic-resistant genes, patients have fewer treatment options, a longer hospital stay, and higher mortality and morbidity rates [6].

ESBL are plasmid-encoded enzymes that can hydrolyze bonds in β-lactam rings from antibiotics including penicillin, cephalosporins, and aztreonam and are inhibited by clavulanate [7]. Due to the coexistence of different modifying enzymes on the same plasmid, there is a possibility of resistance to other classes of antibiotics, such as fluoroquinolones, aminoglycosides, tetracyclines, and trimethoprim sulfamethoxazole.

In order to limit the occurrence of ESBLs, it is vital to identify them as soon as feasible and take action. Priority should be given to treating patients with potent antimicrobials, upholding hygienic standards, and making every effort to avoid invasive procedures like central venous catheterization [8].

For detection of multidrug resistance, often the conventional antimicrobial testing is not sufficient [9]. Often, microorganisms are not identified as ESBL producers and so they can spread in the hospital environment, so the identification of resistant phenotypes is crucial, especially in countries with excessive use of antibiotics and deficient infection control measures [10- 12].

The automated microbial identification and antimicrobial susceptibility testing Vitek 2 System can detect ESBLs in members of the *Enterobacteriaceae* family [13, 14]. Card wells containing 1.0 mg/L of cefepime, or 0.5 mg/L of cefotaxime or ceftazidime, are used to conduct this test. Vitek 2 can be utilized to detect ESBL production, according to studies done [15], this method is fast, sensitive and specific. The method aids in genus and species-level identification of *Enterobacteriaceae* [13], and the results of susceptibility tests performed using this method are expressed as MIC values and interpreted as susceptible, intermediate, or resistant in accordance with a CLSI (1999) [14].

Therefore, this study was carried out to investigate the frequency of ESBL-producing Gram negative bacilli (GNB), among hospitalized children suffering from diarrhea, by Vitek (phenotypic identification), and by PCR (genotypic identification).

Materials and methods

Study design/ population

All clinical isolates from March 2022 to February 2023 were included in the study, which was planned as a cross-sectional study, at Assiut University, Faculty of Medicine, Medical Microbiology & Immunology Department, the study was conducted.

Ethical consideration

Stool samples were obtained with the informed consent of the parent or legal guardian of the child. The Declaration of Helsinki was followed during the study. The Ethical Committee of the Faculty of Medicine, Assiut University, authorized the research proposal with IRB number 17300760 dated April 28, 2022.

Samples collection

It included 73 stool samples that were taken from diarrheic outpatients at children's hospitals.

A thorough history was collected, which included demographic and clinical information such name, age, sex, and a history of vomiting, diarrhea, abdominal pain, and fever.

Bacterial isolation

Stool swabs (one sample of diarrhea on sterile swab sticks) were directly plated on MacConkey's agar (Himedia) and EMB (Himedia) at 37°C overnight.

For further testing, such as DNA extraction for PCR screening, one colony from a pure culture was inoculated into BHI (Hi media, India) broth with 20% glycerol and kept at -20 °C.

Gram-stained film, culture on MacConkey's agar, and EMB were used in the diagnosis to establish whether the bacteria were lactose or non-lactose fermenters, and the Vitek 2 method provided additional confirmation [16].

Bacterial identification and antimicrobial susceptibility by Vitek 2 compact system

Using an automated Vitek 2 compact system (bioM'erieux, France), 73 isolates of diarrhea were identified and tested for antimicrobial susceptibility. The goal was to determine the minimum inhibitory concentration (MIC) of the following drugs, ampicillin, ampicillin/ sulbactam, piperacillin/ tazobactam, cefoxitin, cefepime, cefazolin, ceftazidime, ceftriaxone, meropenem, levofloxacin, ciprofloxacin, amikacin, tobramycin, gentamycin, nitrofurantoin, and sulfamethoxazole/ trimethoprim. The CLSI guidelines 2020 breakpoints were used to evaluate the findings [17].

DNA extraction

Heat treatment was used to extract bacterial DNA. Five colony form pure isolates incubated overnight on MacConkey' s agar (Difco) was suspended in 100µL of sterile distilled water. The suspension was heated at 95°C for 20 minutes centrifuged for 10 minutes at 13,000 rpm to collect 50µL of supernatant, and then kept at -20 °C for PCR (18). Positive and negative controls were used with each PCR set up. Positive controls were strains known to possess the target genes (blaSHV, blaTEM, and blaCTX-M). Sterile distilled water was used as a negative control.

Detection of ESBLs associated genes by PCR

The molecular detection of ESBLs associated genes (blaSHV, blaTEM, and blaCTX-M) was carried out using conventional PCRs in all isolates. Three primers [19, 20] were used to yield specific sequences of the genes.

PCR was performed in a 25 µL reaction mixture containing 1 µL each ofboth forward and reverse specific primer pairs, 12.5 µL of PCR master mix (Thermo Fisher Scientific, United States)), 5.5 µL of nuclease free water, and 5.0 µL of DNA template. The PCR cycling conditions were as follows: initial denaturation for 15 seconds at 95˚C, 30 cycles of denaturation at 95˚C for 10 minutes, annealing at 46˚C for 1 minute 30 seconds, elongation at 72˚C for 2 minutes and final elongation at 72˚C for 10 minutes. Bio-RadMyCycler PCR thermal cycler was used to run the PCR cycles. The post amplification products were analysed using 1.5 % agarose gel electrophoresis, stained with ethidium bromide. Gel Doc XR+ Imaging system (Bio-Rad) was used in viewing the gel after exposure to UV light.

Statistical analysis

SPSS, version 26.0, was used for all statistical analyses after the data were entered using Excel 2010. The following variables were analyzed in the distinctive features of GNB that produce ESBLs: Age, gender, vomiting, diarrhea, fever, Statistics were considered significant at a *p* value of 0.05 or lower. In order to identify the strains that produce ESBLs and express beta-lactam resistance genes, categorical data were expressed as frequency and percentage.

Results

Sociodemographic characteristics of the study

There were 73 study participants, the age of studied patients ranged from less than 6 months to 18 months. 56.2% were male and 43.8% were female. The highest frequency age group of the study participants was 1 day-6 months (28/73) (38.4%) **(Table 1).**

Bacterial identification

Vietk2 compact system was applied to the 73 isolates for bacterial identification and susceptibility. The Frequency of different isolates was illustrated in **table (2).** *Escherichia coli (E. coli*) was 50/73 (68.5%), *Acinetobacter haemolyticus* was 7 /73 (9.6%), *Pseudomonas* sp. 4/73 (5.5%), *Proteus mirabilis* was 2/73 (2.7%), *Salmonella* sp. was 1/73 (1.4%), *Enterobacter* sp. was 5/73 (6.8%), *Aeromonas* salmonicida was 1/73 (1.4%), and *Klebsiella* sp. 3/73 (4.1%).

Antimicrobial susceptibility results

The Vietk 2 compact system was applied to detect the susceptibility pattern of total seventy-three isolates as shown in **table (4)** and **figure (1).** A strain was classified as multidrug-resistant (MDR) if it was resistant to at least one agent in three or more antimicrobial classes (21). Resistance to ampicillin was n=72/73 (96.8 %), followed by cefazolin n=68/73(93.2%), ceftazidime n= 60 /73(82.2), trimethoprim-sulphamethoxazol n= 56 /73 (76.7%), ceftriaxone n= 55 /73 (75.3%), ampicillin/ sulbactam n= 53/73 (72.6%), cefoxitin n= 51/73 (69.9%), cefepime n= 44/73 (60.3%), piperacillin /tazobactam n= 26/73 (35.6%), similar resistance for gentamycin and levofloxacin n= 19/73 (26.0%). ciprofloxacin n= 14/73 (19.2%), tobramycin n= 12/73, (16.4%), to imipenem n= 12/73, (16.4%), nitrofurantoin n= 10 /73(13.7%), amikacin n=5/ 73 (6.8%). Out of 95, 54 isolates (56.8 %) were classified as MDR as shown in **table (3).**

ESBL phenotypic and genotypic prevalence

The vietk 2 compact system was used to detect ESBL phenotypes in *E. coli*, 71.4% (5/7) in *Acinetobacter* haemolyticus, 66.7% (2/3) in *Klebsiella pneumoniae*, 100.0% (5/5) in *Enterobacter* sp., and 100.0% (2/2) in *Proteus mirabilis*. The ESBL was not found in isolates of *Pseudomonas* sp., *Salmonella* sp., or *Aeromonas salmonicida*. **Table 2** shows the prevalence of blaCTXM, blaSHV, and blaTEM.

More than one gene was detected in most of the isolates. Phenotypic confirmed ESBL-PE were 14 (29.8%) which carried at least one gene encoding for ESBL production. The most frequently detected gene was blaCTX-M, (12 (85.7%). Two genes combination Were detected as blaTEM/blaCTXM as 17(73.9%), and blaSHV/blaCTXM 6 (26%). Three ESBL genes carriage was found in 6 (12.8%) **(Table 5).**

Approximately 98.2% of CTXM positive isolates were ampicillin resistant, and 94.7% were cefazolin resistant. Ampicillin resistance was found in 97.4% of TEM positive isolates, while cefazolin resistance was found in 92.3%. Ampicillin resistance was found in 95.7% of SHV positive isolates, while cefazolin resistance was found in 91.3%.

Table 1. Various characteristics of 73 patients versus ESBL.

Variable	**Total**	**ESBL positive**	**ESBL negative**	***P*-value**
	N = 73 (%)	**N=47 (%)**	**N=26 (%)**	
Age (months)				
▪ **1 day-6 months**	28 (38.4%)	18 (38.3%)	10 (38.5%)	0.968
▪ **7 months-12 months**	27 (37.0%)	17 (36.2%)	10 (38.5%)	
▪ **13 months-18 months**	18 (24.7%)	12 (25.5%)	6 (23.1%)	
Gender				
▪ **Male**	41 (56.2%)	23 (48.9%)	18 (69.2%)	0.094
▪ **Female**	32 (43.8%)	24 (51.1%)	8 (30.8%)	
Presence of vomiting	59 (80.8%)	39 (83.0%)	20 (76.9%)	0.529
Presence of abdominal pain	35 (47.9%)	24 (51.1%)	11 (42.3%)	0.473
Presence of diarrhea	69 (94.5%)	46 (97.9%)	23 (88.5%)	0.126
Fever				
▪ **Low grade**	39 (53.4%)	27 (57.4%)	12 (46.2%)	0.354
▪ **High grade**	34 (46.6%)	20 (42.6%)	14 (53.8%)	

Table 2. Percentage of ESBL and drug resistance genes of all isolates.

Organism	**Percentage and number of isolated organism**	**ESBL positive.**	**TEM positive %**	**SHV positive %**	**CTXM positive %**
E. coli	50 (68.5%)	33 (66.0%)	27 (54.0%)	15 (30.0%)	37 (74.0%)
Acinetobacter haemolyticus	7 (9.6%)	5 (71.4%)	4 (57.1%)	3 (42.9%)	6 (85.7%)
Klebsiella pneumoniae	3 (4.1%)	2 (66.7%)	2 (66.7%)	1 (33.3%)	3 (100.0%)
Pseudomonas aeruginosa	3 (4.1%)	0 (0.0%)	2 (66.7%)	2 (66.7%)	2 (66.7%)
Pseudomonas fluorescens	1 (1.4%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	1 (100.0%)
Salmonella sp.	1 (1.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)
Proteus mirabilis.	2 (2.7%)	2 (100.0%)	2 (100.0%)	1 (50.0%)	2 (100.0%)
Aeromonas salmonicida	1 (1.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Enterobacter aerogenes	2 (2.7%)	2 (100.0%)	0 (0.0%)	0 (0.0%)	2 (100.0%)
Enterobacter cloacae.	3 (4.1%)	3 (100.0%)	2 (66.7%)	0 (0.0%)	3 (100.0%)
Total	**73**	47(64.4%)	39 (53.4%)	23 (31.5%)	57 (78.1%)

Table 3. Summary of number of MDR of all isolates.

Number of resistant classes of antibiotics	**No.=73 (%)**
3 classes	20 (27.4%)
4 classes	26 (35.6%)
5 classes	10 (13.7%)
6 classes	6 (8.2%)
7 classes	4 (5.5%)
Total	66 (90.4%)

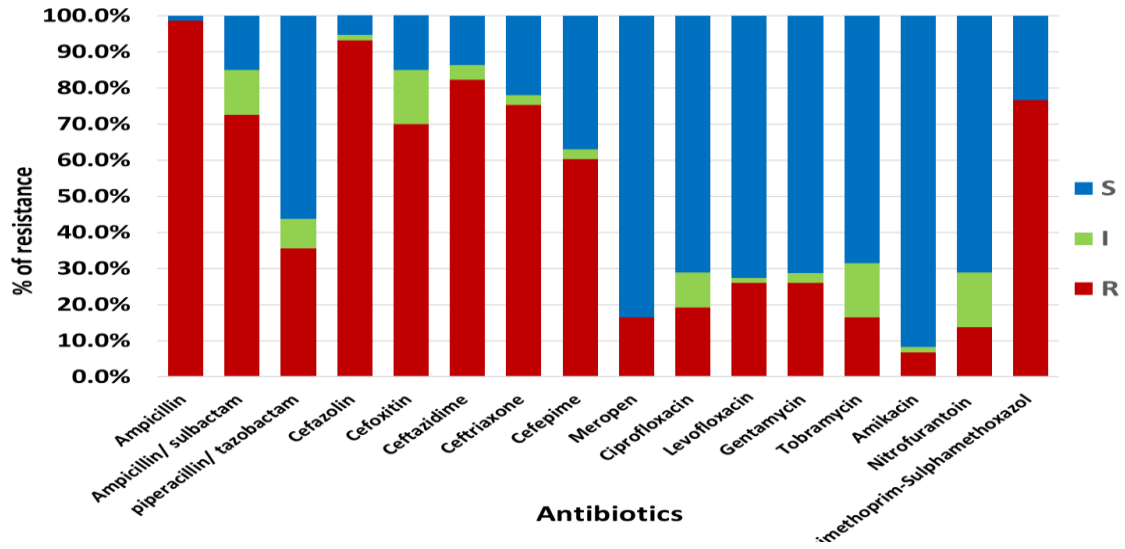
Table 4. Summary of antibiotic resistance pattern of all isolated GNB.

Antibiotic classes	*E. coli*	*Acinetobacter*	*Pseudomonas sp.*	*Proteus mirabilis*	*Salmonella*	*Klebsiella*	*Eromonas Salmonicida*	*Enterobacter*
Penicillins								
Ampicillin (20 µg)	49(98.0)	7(100.0)	4(100.0)	2(100.0)	1(100.0)	3(100.0)	1(100.0)	5(100.0)
Ampicillin/ sulbactam (30 µg)	33(66.0)	7(100.0)	2(50.0)	1(50.0)	1(100.0)	3(100.0)	1(100.0)	5(100.0)
Ureidopenicillin								
Piperacillin/ tazobactam (40µg)	19(38.0)	2(28.6)	1(25.0)	0 (0.0)	0 (0.0)	2(66.7)	0 (0.0)	2(40.0)
Cephalosporins:								
Cefazolin (30 µg)	47(94.0)	6(85.7)	4(100.0)	1(50.0)	1(100.0)	3(100.0)	1(100.0)	5(100.0)
Cefoxitin (30 µg)	34(68.0)	4(57.1)	4(100.0)	0 (0.0)	1(100.0)	2(66.7)	1(100.0)	5(100.0)
Ceftazidime(30 µg)	45(90.0)	2(28.6)	2(50.0)	2(100.0)	1(100.0)	3(100.0)	1(100.0)	5(100.0)
Ceftriaxone(30 µg)	37(74.0)	4(57.1)	3(75.0)	2(100.0)	1(100.0)	3(100.0)	0 (0.0)	5(100.0)
Cefepime (10 µg)	30(60.0)	2(28.6)	2(50.0)	2(100.0)	1(100.0)	3(100.0)	0 (0.0)	4(80.0)
Carpapenems:								
Imipenem	8(16.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2(66.7)	0 (0.0)	2(40.0)
Quinolones:								
Ciprofloxacin (5µg)	8(16.0)	3(42.9)	0 (0.0)	1(50.0)	1(100.0)	1(33.3)	0 (0.0)	0 (0.0)
Levofloxacin(5µg)	9(18.0)	5(71.4)	1(25.0)	1(50.0)	1(100.0)	1(33.3)	1(100.0)	0 (0.0)
Aminoglycosides:								
Gentamycin(10 µg)	14(28.0)	1(14.3)	0 (0.0)	0 (0.0)	1(100.0)	1(33.3)	0 (0.0)	2(40.0)
Tobramycin(10 µg)	6(12.0)	2(28.6)	1(25.0)	0 (0.0)	1(100.0)	2(66.7)	0 (0.0)	3(60.0)
Amikacin (30 µg)	3(6.0)	0 (0.0)	0 (0.0)	0 (0.0)	1(100.0)	1(33.3)	1(100.0)	5(100.0)
Nitrofurantoin								
Nitrofurantoin (50µ)	3(6.0)	3(42.9)	1(25.0)	2(100.0)	0 (0.0)	1(33.3)	0 (0.0)	2(40.0)
Trimethoprim								
Trimethoprim-Sulphamethoxazol (5 µg)	39(78.0)	4(57.1)	4(100.0)	2(100.0)	0 (0.0)	3(100.0)	1(100.0)	3(60.0)

Table 5. The distribution of ESBL genes among phenotypically ESBL positive and negative isolates.

	Gene	N (%)	Types of gene
ESBL positive (n=47)	**One gene**	14 (29.8%	TEM 1 (7.14%), SHV 1 (7.1%), CTXM 12 (85.7%)
	Two genes	23 (48.9%)	TEM+CTXM 17(73.9%), SHV+CTXM 6 (26%)
	Three genes	6 (12.8%)	TEM+CTXM+SHV
ESBL negative (n=26)	**One gene**	12 (46.2%)	TEM
	Two genes	7 (26.9%)	1 case TEM +SHV (14.2), 2 cases SHV+CTXM (28.6), 5 cases TM+CTX(71.4)
	Three genes	5 (19.2%)	TEM+CTXM+SHV

Figure 1. Summery of antibiotic sensitivity test of all isolated GNB



S=Sensitive R= Resistant I= Intermediate

Figure 2. Agarose gel electrophoresis of amplified PCR products of bla CTXM (544bp) gene of phenotypic ESBL positive isolates. Lane 1: 1200 bp DNA ladder. Lane 2: positive control. Lane 3: negative control, Lane 4-11: Amplified PCR product of bla CTXM.

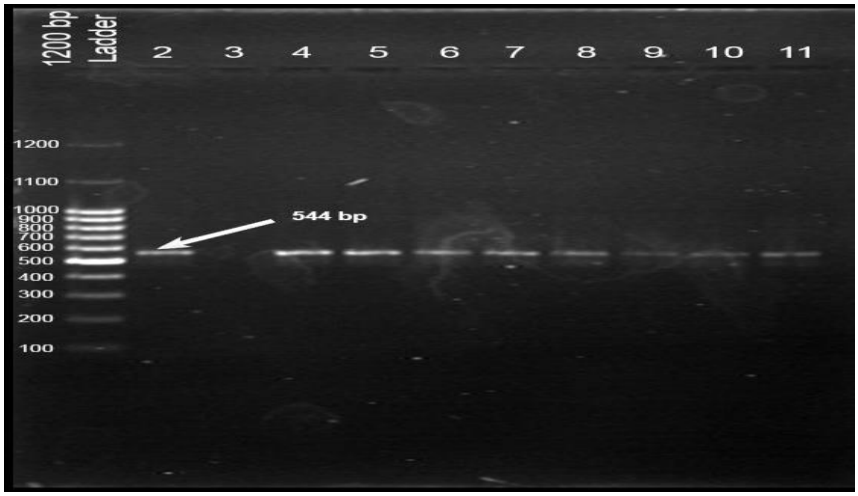


Figure 3. Agarose gel electrophoresis of amplified PCR products of blaTEMgene (800bp) of phenotypic ESBL positive isolates. Lane 1: 1200 bp DNA ladder, Lane 2: positive control., Lane 3: negative control. Lane 4-11, Amplified PCR product of blaTEM.

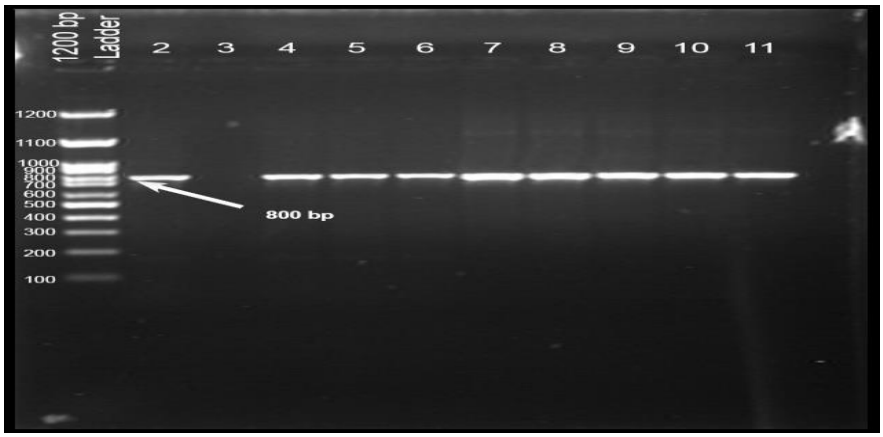
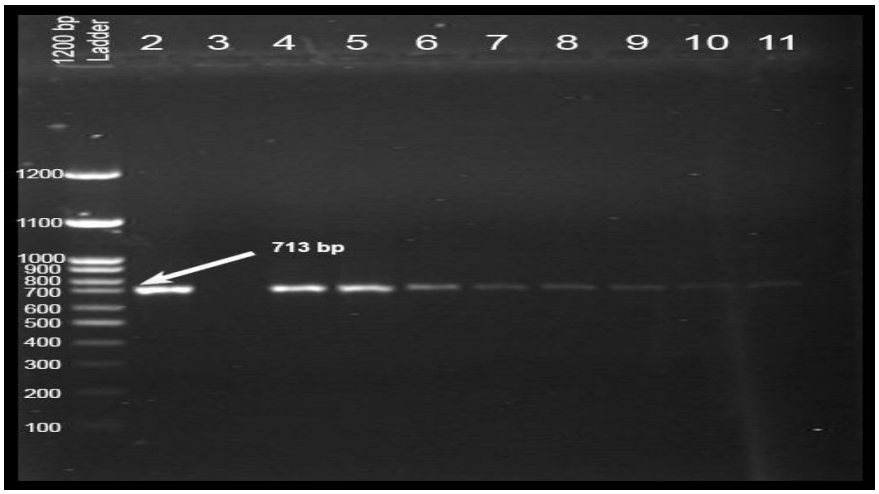


Figure 4. Agarose gel electrophoresis of amplified PCR products of blaSHVgene (713bp) of phenotypic ESBL positive isolates. Lane 1: 1200 bp DNA ladder. Lane 2: positive control. Lane 3: negative control, Lane 4-11: Amplified PCR product ofblaSHV.



Discussion

Horizontal gene transfer is thought to be a factor in the emergence of multidrug-resistant pathogens, and the gut microbiota is a source of antimicrobial resistance genes [22]. The improper use of antibiotics is largely to blame for the development of antimicrobial resistance, which accounts for the majority of morbidity and mortality from infections that could otherwise be treated. Knowing which organisms harbour mobile antimicrobial resistance genes and which organisms participate in horizontal gene transfer is crucial for preventing the spread of antimicrobial resistance [23].

In this study, the frequency of GNB that had ((MDR) pattern and ESBL producers) in kids with gastrointestinal complaints was investigated.

Seventy-three GNB isolates of diarrheal stool samples. The frequency of MDR GNB was 90.4% overall, which was more than previous study conducted in Norway (48.5%), [24] and Northwest Ethiopia (48%) [23]. However, our results are lower than those of a study conducted at the University of Mozambique (88%) [25], but it was consistent with a prior report from Egypt (65%) [26].

The excessive use of antibiotics, poor personal and environmental hygiene practices, and the frequency of multidrug-resistant bacterial strains may all contribute to Egypt's high resistance rate

[27]. Many bacteria are also resistant to commonly used antibiotics.

In this study, the frequency of the GNB group of bacteria, which produce ESBLs, was 64.4% overall in this study, exceeding reports from Tanzania 56% study [5], France (17.7%) [28], Mozambique University (20%) [25], Norway (15.8%) [24], and Northwest Ethiopia (16.3%) [23].

Other African countries, such as South Africa, have recorded a lower frequency. Babatunde et al. found 48% of hospitalized children had ESBL-PE colonization [5, 29].

Escherichia coli and *K. pneumoniae* are two common *Enterobacteriaceae* that are both pathogenic and frequently contain ESBL-encoding genes. The presence of ESBL-*producing E. coli* and *K. pneumoniae* faecal carriage, as well as a high level of multidrug resistance among ESBL-producing *E. coli* and *K. pneumoniae*, was demonstrated [30].

A meta-analysis of Eastern, Central, and Southern African countries was done [31]. likewise found that *E. coli* isolates predominated among ESBL producers.

Escherichia coli was isolated (68.5%) 50/73 in this investigation, which was consistent with previous reports from Northwest Ethiopia (62.1%) [23], Addis Ababa (70%) [32] and Mozambique University (62%) [25], but lower than a study from Norway (86%) [24].This isolated disparity may be attributed to changes in geographical location, study populations, sample size, and methodological heterogeneity, which may cause variation in prevalence.

The resistance of *E. coli* to ceftazidime and ceftriaxone was found to be (90.0%) and (74.0%), which was consistent with studies from Northwest Ethiopia and Madagascar that found 100% resistance to ceftazidime and cefotaxime, respectively [23], Addis Ababa (97%) and (98%) (32), but higher than one from Venezuela that found ceftazidime (46%) and cefotaxime (68.7%) [33].

This discrepancy could be attributed to changes in diagnostic procedure, indiscriminate antibiotic usage, illness exposure, and regional disparities among research participants.

Similar to a study done in Northwest Ethiopia (100%) [23], *K. pneumoniae* was 100% resistant to both ceftazidime and ceftriaxone, respectively. Ceftazidime (97%) and cefotaxime (98.6%) resistance was present in that.

Ampicillin resistance with a percentage of (98.6%), ampicillin/sulbactam resistance with a percentage of (72.6%), and trimethoprim resistance with a percentage of (76.7%) have all been found in Tanzania [5], Kenya [7], and Ethiopia [32].

One possible explanation is because this antibiotic is widely utilized in the community. blaCTX-M was detected in 74.0% of *E. coli*, 85.7% of *Acinetobacter*, and 100% of *Klebsiella* in our investigation. Our findings were lower than those of previous Riyadh hospital research, which discovered blaCTX-M in 90.5% (n = 19/20) of *K. pneumoniae* isolates and 60% of *E. coli* isolates [34].

Regarding *E. coli*, the percentage of blaTEM was 27/73 (54.0%), whereas in Klebsiella, it was 66.7%. Research from China, South Africa, and Iran [35], all revealed lower *E. coli* findings, with respective numbers of 17%, 17%, and 19%. Our research showed that 42.9% of *E. coli* has blaSHV. This is higher than the 10% frequency of *E. coli* found in research from Egypt [36]. However, an Indian investigation revealed a greater incidence of blaSHV expression at 44.4% [37]. Our findings outperform those of earlier Egyptian research in Zagazig, which found that diarrheagenic *E. coli* was positive for the blaTEM and blaSHV genes in 28.6% and 7.1%, respectively [38].

Our findings are in line with in a recent Kenyan study, blaCTXM (100%), blaTEM (97.6%), and blaSHV (90.4%) were detected in *Klebsiella pneumoniae* samples [39]. blaCTXM was the most often found ESBL gene among isolates from our and other environments [40]. The blaCTXM gene may predominate because these genes are expressed by conjugative epidemic plasmids, such as IncFII, which play a significant role in the successful spread of this allele; these plasmids have been frequently detected in this instance [40].

ESBL colonization was often observed in infants younger than 12 months old, according to several past studies [29]. Similar to earlier research, the greatest frequency of ESBL-PE was seen in children younger than 6 months old in the current examination. These results support the hypothesis that an unstable microbiome, which increases the susceptibility of early children to infection, is responsible for the presence of antimicrobial resistance genes in the intestines of newborns [5].

Conclusion and recommendation

Our study revealed a considerable incidence of ESBL producing GNB (64.4%) and MDR (90.4%) in different isolates in diarrhea-affected children under the age of two years. The most frequent isolation was *E. coli*. More than half of *E. coli* isolates were ESBL positive phenotypically by Vitek. BlaCTXM was the most frequently detected gene with a, followed by blaTEM gene and blaSHV gene.

These drug-resistant bacteria could be the origin of endogenous infections. So, further studies are needed for screening for carpapenemase producing GNB. Routine screening for GNB should be conducted among young aged. Infection control procedures should be improved in order to reduce cross-transmission in medical facilities. Antimicrobial susceptibility tests help in making the right treatment decisions.

Conflict of interest

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