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Molecular detection of TEM-Type β lactamase producing Escherichia coli from diarrheic Egyptian children

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Abstract

Background: Diarrhea is one of the major public health problems among young children in developing countries. The objectives of this study were to characterize multiresistant *E.coli* isolated from diarrheic children and determine their prevalence and antimicrobial susceptibility pattern and the major mechanism of resistance.

Methods and Findings: A total of 214 clinical isolates were recovered and identified from stool specimens collected from Egyptian children with diarrhea. Antibacterial susceptibility pattern against antibiotics as cefotaxime, ceftazidime, imipenem and aztreonam were determined. The isolates were screened for beta-lactamase production by the Iodometric method. Multiplex PCR assay for detection of bla_{TEM-1} and bla_{TEM-2} encoding genes was carried out.

The presence of multiple drug resistance to three different antimicrobial agents was very clear in about 88 % of isolates. The results revealed that among the detected 93% Beta-lactamase producers, 90% of the examined *E.coli* isolates contain bla_{TEM-1} and/or bla_{TEM-2} genes responsible for the production of beta-lactamases. This finding proves that TEM hyper production is a frequently described mechanism by which resistance to the beta-lactam antibiotics is mediated in *E. coli* currently isolated from diarrheic Egyptian children.

Conclusions: The presence of TEM-Type β-lactamase producing *Escherichia coli* is highly prevalent. This should be considering in management of severe cases and prescribing drugs. The presence of multidrug resistant *E. coli* isolates is attributed to β-lactamases production mediated by bla_{TEM} genes.

Keywords: *Escherichia coli*, β-lactamases, TEM genes.

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Introduction

Diarrhea is a condition that has a major impact on global health. Extensive efforts to control diarrhea as one of the biggest killers of children under the school age are exerted [1]. Among those effort is the determination of causative agents and their characterization. People in developing countries suffer most from infectious forms of diarrhea. *Escherichia coli* are the most common etiologic pathogens of diarrhea in human and are common inhabitant of the human and animal gut [2]. Treatment in severe cases is typically consists of a broad-spectrum antimicrobial, although resistance to such drugs has greatly increased over the last several years. The widespread use of antibiotics could be associated with the selection of antibiotic resistance mechanisms in pathogenic and non-pathogenic isolates of *E. coli* [3, 4].

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Increasing antimicrobial resistant *E.coli* in particular among immunocompromised patients to traditional and recent agents had been documented in several areas and became an emerging problem [4-6]. Resistance of bacteria to different antimicrobials is a multifactor process [5]. Resistance to β-lactam antibiotics in Gram-negative bacteria can be due to three mechanisms; decreased accumulation of the drugs by the cell, hydrolysis of the antibiotics by β-lactamases or alteration in the penicillin binding proteins that reduce their affinity to the drugs [7]. These mechanisms may be determined by genes that reside in the chromosome, on plasmids or transposons [8].

Over 200 β-lactamases have been classified into four main groups and eight subgroups according to their functional and structural characteristics. The classical TEM and SHV enzymes are the predominant plasmid-mediated β-lactamases of gram-negative rods which have been reported [9]. The classical TEM enzyme is the predominant plasmid-mediated β-lactamases of *Enterobacteriaceae* [10]. The phenomenon of multi-resistance to commonly prescribed drugs has been repeatedly reported in *E.coli* strains of different serogroups [11]. Therefore this study was carried out to Study the spread of resistant *E. coli* recovered from diarrheic children and study the suggested major mechanism of such resistance by studying the antibiotics resistance pattern of isolated *E. coli*. Also PCR assay for detection of bla_{TEM} genes which is very helpful in determining genes responsible for resistance and in getting better and broader view on the most potent methods of management.

Materials and methods

A total of 250 stool specimens collected from Egyptian children under the age of six and suffered from diarrhea. They were collected from pediatric clinic of Egyptian public hospital.

The recovered isolates of *E.coli* were identified and confirmed using API 20E test strips (bioMérieux Vitek, Hazelwood, Mo.). *E. coli* ATCC 25922 was used as a reference strain.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed according CLSI [12]. The used antimicrobials were obtained from Oxoid (England) and included: amikacin, ampicillin, cefepime, cephalexin, cefotaxime, ceftazidime, imipenem, aztreonam, chloramphenicol, erythromycin, kanamycin and ofloxacin.

Detection of β-lactamase producing *E.coli*

The isolates were screened for β-lactamase production by Spot Iodometric Overlay Method (IOM) [13] with some modifications.

Multiplex PCR assay for Detection of TEM, encoding genes (bla_{TEM}):

PCR amplifications of the bla_{TEM} genes were carried out [14, 15]. The following primers sequences were used in these reactions; *Tem-1*-F: 5′-TTCTTGAAGACGAAAGGGC-3′. *Tem*-1-R; 5′-ACGCTCAGTGGAACGAAAAC-3′.*Tem-2*-F;5′-CTCACAAGATG AAACGGC-3′. *Tem*-2-R; 5′-ACGCGCACTC-GAACGAGAAC-3′. Total genomic DNA was extracted in order to perform the test.

Results

In the present study, among 250 stool specimens isolated from children under school age suffering from diarrhea, there were 214 positive *E. coli* specimens represent a percentage of 85.6% of total specimens.

Antimicrobial susceptibility pattern of the isolated of *E.coli* strains:

The recovered *E.coli* isolates were tested for their antimicrobial susceptibility against twelve different antimicrobials. Antimicrobials of different classes were used to estimate the incidence of resistance to each agent, **table 1.** The presence of multiple drug resistance to three different antimicrobial agents was very clear in more than 88 % of tested isolates. The most predominant resistance was recorded to Beta-lactam antibiotics.

Detection of β-lactamase production by the IOM:

A total of 214 *E.coli* isolates were tested for β-lactamase production using the IOM. A total of 199 (93 %) isolates were found β-lactamase producers.

PCR detection of bla_{TEM} genes in isolated *E.coli*:

Multiplex PCR showed that bla_{TEM} genes responsible for the production of β-lactamases were detected in 193 *E.coli* isolates (90%) of the examined *E.coli* isolates. TEM-1 encoding gene was detected in 112 isolates which represent 52.3% of total *E.coli* isolates. Both bla_{TEM-1} and bla_{TEM-2} genes were

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Table 1. Antimicrobial susceptibility pattern of clinical isolates of *E.coli*.

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	Number	%	Number	%	Number	%
Imipenem	137	64	61	28.5	16	7.5
Amikacin	123	57.5	91	42.5	0	0
Ampicillin	0	0	0	0	214	100
Cefepime	30	14	50	23.4	134	62.6
Cephalexin	0	0	14	6.5	200	93.5
Chloramphenicol	70	32.7	109	50.9	35	16.4
Erythromycin	16	7.5	4	1.8	194	90.7
Kanamycin	154	72	45	21	15	7
Cefotaxime	0	0	22	10.2	192	89.8
Ceftazidime	0	0	16	7.5	198	92.5
Ofloxacin	91	42.5	123	57.5	0	0
Aztreonam	17	8	8	3.7	189	88.3

detected in 83 (37.7%) of isolated *E.coli*. Results of this step are illustrated in **figure 1.** No isolate indicated the presence of bla_{TEM-2} gene alone.

Discussion

In our study, 93% of screened *Escherichia coli* were found β-lactamases producers. This finding was in compatible with the results of antimicrobial susceptibility test.

Results for the antimicrobial susceptibility testing of the different isolated *E. coli* strains in this study revealed higher yield of beta-lactamases producing *E. coli* than some previous studies [2, 16]. It is reasonable to attribute the emergence of higher rate of resistance to the extensive use of antibiotics without control in developing countries [5, 15].

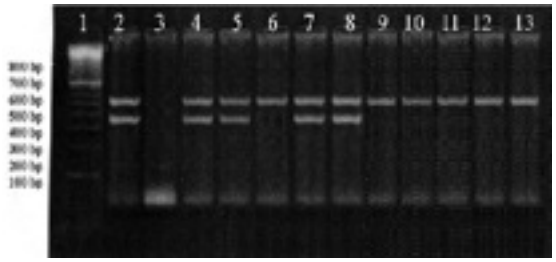

Fig.1. Gel electrophresis of DNA fragments generated by PCR amplification showing the typical pattern of bla_{TEM1-2} genes Lane1 and 2 represent 100 bp Marker and Positive control respectively, while Lane 3 represent *E.coli* strain with no bla_{TEM} genes, lanes 4,5,7,8 show *E.coli* contain $bla_{TEM-1-\&-2}$ genes. Lanes 6,9-13 represent *E.coli* strain with bla_{TEM-1} genes.

In the present study, most of isolated *E.coli* (88%) were resistant to at least three different antimicrobials. This is in agreement with another study in South India [17], where all the isolated *E.coli* from general population were multi-resistant to at least three antimicrobials.

According to the antibiograms, different resistance patterns were defined. Moreover, it should be taken into consideration that due to the declining use of old antibiotics, minimal resistance pattern were detected to old antibiotics rather than β-lactam group as amikacin, chloramphenicol and kanamycin. This can be attributed to limitation of exposure and selective pressure to old antibiotics. The same was noticed also to the relatively new antimicrobial imipenem which may be due to its availability only in injection form which limits its misuse.

Resistance of the examined *E.coli* to different Beta-lactam antibiotics revealed that several resistance mechanisms were involved as previously reported [18-21]. In the present study attention was paid especially to the most frequent reported mechanism of hyper-production beta-lactamases mediated by bla_{TEM} genes [15]. PCR detection of TEM genes in the present study revealed that about 90 % of the examined *E.coli* isolates contain bla_{TEM} genes either TEM-1 or TEM-1 and TEM-2 together which are responsible for the production of β-lactamases. Positive PCR results for the bla_{TEM-1} gene were found in 52.3 % of the studied multidrug resistant *E.coli* isolates. Both $bla_{TEM-1\&2}$ genes were found in 37.7 %

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of resistant *E.coli* isolates. Results of PCR revealed that the majority of isolated *E. coli* from diarrheic Egyptian children contained bla_{TEM} enzymes encoding genes.

The wide spread of antimicrobial resistance detected in this study reflects the overuse of antimicrobial drugs. There is no doubt that the widespread dissemination of organisms producing potent β-lactamases will severely limit the therapeutic options of physicians facing these organisms.

Molecular characterization of *E. coli* strains from stools samples differ in different studies among different geographical regions [2, 22]. Inactivation of these drugs by plasmid mediated β-lactamases was apparently the major mechanism of resistance in our study in Egypt. This indicates the importance of the characterization of diarrhea etiological agents according to the affected region in order to establish the relevant treatment policy and infection control measures.

The high rate of resistant phenotypes detected in this study strongly recommends the judicious use of antibiotics which in turn would be useful in limiting the rapidity of spread of this resistance. Finally, attention must be paid for fecal contamination, environmental sanitation, and personal hygiene as important control measures of spread of multiresistant bacterial infections.

Competing interests

The authors declare that they have no potential conflicts of interest.

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