

## Antibiotic Resistance Profile of *E. coli* Strains Isolated from Clinical Specimens and Food Samples in Egypt

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**Abstract:** A total of 147 *E. coli* strains were isolated from food samples and clinical specimens. The prevalence of *E. coli* in clinical specimens was much higher than in food samples. The antibiotic resistance profile of these strains was determined against 7 classes of antimicrobial agents (26 different members). Almost 90% of *E. coli* strains were resistant to at least one of the tested antibiotics. The highest antibiotic resistance was recorded against conventional Beta-lactams. However, this resistance was significantly lower when Beta-lactams combinations and cephalosporins were tested. The highest sensitivity of the isolates was to imipenem and polymyxin-B where all isolated *E. coli* strains were sensitive to imipenem. The resistance to tetracyclines, macrolides and sulfonamides/trimethoprim were almost in the same order of magnitude of 30-37%. The resistance to quinolones and aminoglycosides were 19 and 10 % respectively. Among isolated *E. coli* 52 strains (35%) exhibited multidrug resistance (MDR) pattern to equal or more than 3 antibiotics classes with different mode of action. Interestingly, MDR *E. coli* isolated from food samples was higher than those isolated from clinical specimens.

**Key word:** *E. coli* • Antibiotics • Multi-drug resistance • Food

### INTRODUCTION

*E. coli* is an important member of the normal intestinal microflora of humans and other mammals, it has also been widely exploited as a cloning host in recombinant DNA technology. However, *E. coli* is not a laboratory workhorse or harmless intestinal inhabitant; it can also be a highly versatile and frequently deadly, pathogen [1]. Moreover, *E. coli* is well known with the high genetic flexibility to acquire and/or transfer resistance or virulence genes from or to other strains of *E. coli* as well as other organisms [2, 3].

No doubt that the growing problem of antimicrobial resistance has become a significant public health concern worldwide [4] and especially in developing countries [5] as a result of overuse and misuse of antibiotics [6, 7]. Consequently, antibiotic resistance presents a great challenge to the treatment of infections. According to the Centers for Disease Control and Prevention, healthcare-related infections led to 98,987 deaths in the USA in 2002, almost five times higher than a decade ago

(19,000 in 1992) [8]. This problem was more complicated through the emergence of multidrug resistant (MDR) strains to 3 or even more classes of antibacterial agents belonging to different chemical classes by using various mechanisms [9]. The occurrence of MDR is very common and mainly in Gram negative bacteria [10]. In this sense, MDR clinical isolates of *E. coli* pathogenic strains are commonly seen today in clinics representing a major healthcare problem with increased morbidity and mortality worldwide [11]. Moreover and a matter of fact antimicrobial resistance has been found not only in pathogenic strains but also in non-pathogenic ones [12, 13].

In this prospective, this study was conducted to screen both clinical specimens as well as environmental (food) samples for the presence of *E. coli* strains. The antibiogram of all isolated *E. coli* strains was determined against 26 different members of antibiotics representing 7 classes of antibiotics. Finally, the prevalence of MDR among these isolates was investigated.

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## MATERIALS AND METHODS

All tests were conducted under aseptic conditions and in triplicates.

### Screening, Isolation and Identification of *E. coli* Strains:

A total of 100 stool samples was collected from children with acute diarrhea in presence or absence of clinical manifestations. All samples were collected using sterile swabs and obtained from Abu-Reesh Hospital, Cairo, Egypt. Each swab was dispersed in 5 ml sterile saline solution prior to cultivation. Concurrently, a total of 1000 food samples was obtained from different food products (Ground roasted, beef lamb, turkey, chickens, salad vegetables, raw milk, cheese, koshari, etc.), available in retail stores and restaurants. Food samples were collected in sterile screw capped jars and stored at 4°C prior analysis. Initially, 1-2 grams of each food sample was transferred into sterile flasks containing 20 ml saline solution prior to cultivation. For all samples cultivation was conducted by transferring one ml of the resulted suspension into sterile Petri dishes. Then 15 ml molten sterile MacConkey agar at 45°C was added and mixed well. All plates were incubated at 37°C for 24-48 h.

Preliminary identification of the isolated bacterial strains was conducted by morphological examination using Gram stain according to Bergey's Manual of Systematic Bacteriology, [14] and biochemical regimen by API 20E (BioMérieux, France) according to the manufacturer instructions. Finally the identification was better confirmed using *E. coli* chromogenic agar medium (Oxoid, USA) [15].

### Antibiotic Susceptibility Testing of *E. coli* Isolates:

The antibiotic susceptibility testing was conducted using disc diffusion method (Kirby-Bauer method) using Mueller-Hinton agar (Difco, MI, USA) according to the guidelines of the Clinical and Laboratory Standards Institute [16]. All *E. coli* isolates were tested against 26 different members of antibiotics representing 7 different antibiotic classes. The antibiotics used were in the form of commercially available antimicrobial discs (Himedia, India). The susceptibility pattern of the tested isolates was determined according to the resulted zone of inhibition based on the standard data base [16].

**The Statistical Analysis:** All data were entered into SPSS/PC software and comparative statistics were calculated using a non parametric Mann-Whitney test to estimate the resistance rates between the *E. coli* isolates

from different sources. In all cases,  $p < 0.05$  was regarded as statistically significant depending on median.

## RESULTS

### Screening, Isolation and Identification of *E. coli* Strains:

Among the screened 100 clinical specimens 67 *E. coli* isolates were identified representing 67% (Fig. 1A). While only 80 isolates were identified as *E. coli* from those collected among the screened different 1000 food samples representing (8%) (Fig. 1B).

### Antibiotic Resistance Profile of Isolated *E. coli* Strains:

When conventional Beta-lactams represented by ampicillin was tested 85 and 95% of the *E. coli* isolates from food and clinical origins respectively showed resistance. When combinations of conventional Beta-lactams with Beta-lactamase suicidal inhibitor were tested the recorded resistance was much lower (Table 1). Only 15-20% of the isolated *E. coli* strains were resistant to cephalosporins and new generation of Beta-lactams (Table 1). Interestingly, all isolated *E. coli* strains were sensitive to imipenem.

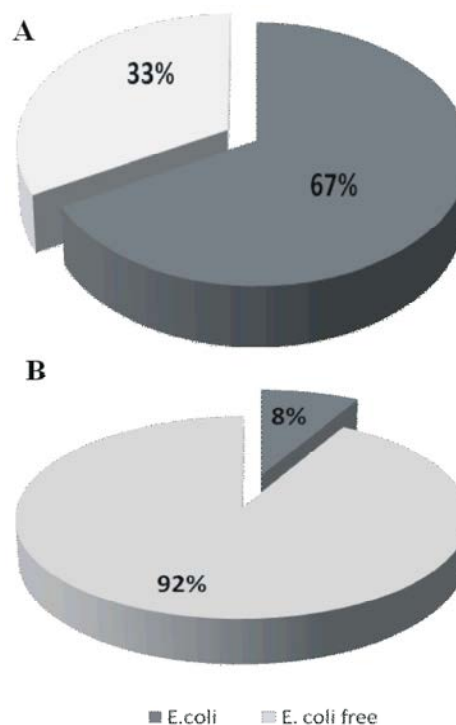


Fig. 1: The recorded prevalence (%) of the isolated *E. coli* strains compared to the *E. coli* free samples collected from 100 clinical samples (A) and 1000 food sample (B).

Table 1: Antimicrobial susceptibility pattern of *E. coli* strains isolated from clinical and food samples

Antibiotic class	Tested members (conc. $\mu\text{g ml}^{-1}$ )	Clinical Isolates (67)		Food Isolates (80)		
		Sensitive	Resistant	Sensitive	Resistant	
Beta-lactams	Conventional Beta-lactams: Ampicillin (AMP) (30)	15% (10)	85% (57)	5% (4)	95% (76)	
	Combination Beta-lactams: Amoxicillin/Clavulanic acid (AMC) (20/10); Ampicillin/Sulbactam (A/S) (10), Pipracilline/Tazobactam (PIT) (10/10)	65.6% (44)	34.4 % (23)	91.25 % (73)	8.75 % (7)	
	Cephalosporins Beta-lactams: Cefixime (CFM) (30), Cefoxitin (CX) (30), Cefotaxime (CTX) (30), Cefuroxime (CXM) (30)	79% (53)	21% (14)	78.75 % (63)	20.25 % (17)	
	Newly used Beta-lactams: Imipenem (IMP) (15), Azetronam (AT) (30)	85% (57)	15 % (10)	98.75 % (79)	1.25 % (1)	
Quinolones	Ciprofloxacin (CIP) (5), Gatifloxacin (GAT) (5), Levofloxacin (LE) (5), Lomefloxacin (LOM) (10), Ofloxacin (OF) (5), Norfloxacin (NX) (10), Sparfloxacin (SPX) (10)	82% (55)	18 % (12)	81.25 % (65)	18.75 % (15)	
	Aminoglycosides	Amikacin (AK) (30), Kanamycin (K) (30), Tobramycin (TOB) (100/10), Neomycin (N) (300 units)	91% (61)	9% (6)	90 % (72)	10% (8)
	Tetracycline	Doxycycline (DO) (5), Tetracycline (TE) (5)	63% (42)	37 % (25)	62.5% (50)	37.5% (30)
Macrolides	Azithromycin (AZM) (30)	69 % (46)	31 % (21)	66 % (53)	34 % (27)	
Polymyxins	Polymyxin-B (PB) (30)	98.5 % (66)	1.5 % (1)	97.5 % (78)	2.5 % (2)	
Sulfonamide + trimethoprim	Co-Trimethoxazole (COT) (1.25/23.75)	67% (45)	33% (22)	66 % (53)	34% (27)	

Table 2: Prevalence of MDR among clinical and food *E. coli* isolates tested against 7 classes of antibiotics

Mode of action	Antibiotic class	Isolates showed (MDR)		
		Food	Clinical	Total
Inhibition of cell wall synthesis	Beta-lactams	29 (56%)	23 (44%)	52 (100%)
Inhibition of DNA replication	Quinolones	15 (29%)	11 (21%)	26 (50%)
Inhibition of protein synthesis	Aminoglycosides	9 (17%)	3 (6%)	12 (23%)
	Tetracyclines	28 (54%)	18 (34%)	46 (88%)
	Macrolides	24 (46%)	20 (38%)	44 (84%)
Damages cytoplasmic membranes	Polymyxins	1 (2%)	1 (2%)	2 (4%)
Inhibition of folic acid metabolism (anti-folate)	Sulfonamide + trimethoprim	25 (48%)	17 (33%)	42 (81%)

The recorded resistance to tested antibiotics from tetracycline's, macrolides or sulfonamide/trimethoprim classes was almost in the same order of magnitude of 31-37 % (Table 1). A range of 9-18% of the isolated *E. coli* strains were resistant to tested members of quinolones or aminoglycosides classes (Table 1). The lowest antibiotic resistance was recorded for polymyxin-B where only 3 *E. coli* isolates showed resistance. Interestingly, statistical analysis at  $P < 0.05$  showed that there is no significant difference between the antibiotic resistance patterns of *E. coli* strains, isolated from food samples or those collected from clinical specimens when tested against all members of antibiotics on individual basis except for those members belonging to Beta-lactam class.

**Estimation of the Multi-Drug Resistance Degree among *E. coli* Strains:** A total of 133 strains representing 90% of

isolated 147 *E. coli* strains showed antibiotic resistance to at least one antibiotic (Fig. 2). However, only 52 strains (35%) showed multidrug resistance (MDR) to three or more than three different classes of antibiotics with different mode of action (Table 2 and Fig. 2). All of these MDR strains were resistant to at least one of the tested members of Beta-lactam class of antibiotics. Around 81-88 % of MDR *E. coli* were resistant to tetracycline, macrolides and sulfonamide/trimethoprim (Table 2). This was followed by quinolones then aminoglycosides, 50 and 23 % of MDR *E. coli* strains were resistant to these classes of antibiotics respectively (Table 2). The lowest contribution in the MDR was recorded for polymyxin-B (Table 2). Interestingly, except for polymyxin-B the contribution of the *E. coli* strains from food origin in MDR was significantly higher ( $P < 0.05$ ) than that of *E. coli* strains from clinical origin (Table 2 and Fig. 2).

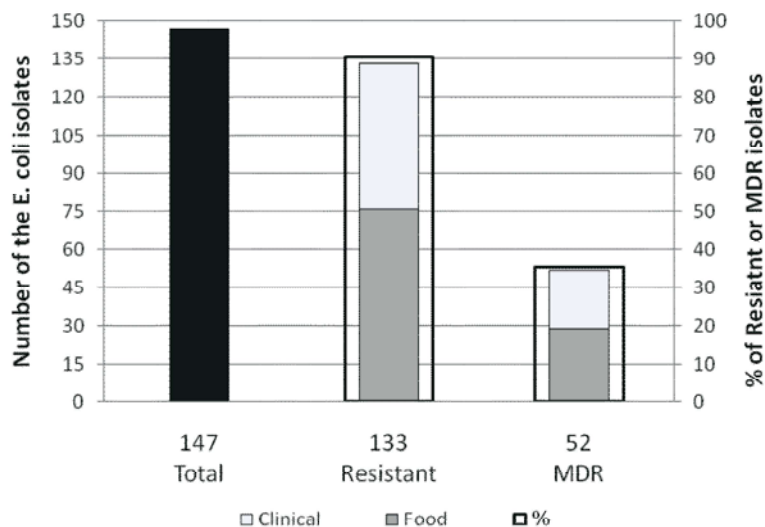


Fig. 2: The total number of isolated *E. coli* strains (black bar) from both food and clinical samples, the number of *E. coli* isolates showed resistance to at least one antibiotic and multidrug resistance to three of more of the tested antibiotics and the corresponding % (solid lines) relative to the total *E. coli* isolates.

## DISCUSSION

Indeed, members of *E. coli* are widely distributed in the environment and cause a variety of infections in hospital and community settings [17]. Diarrhea is among the most common diseases caused by *E. coli* [18]. This is in agreement with the recorded results in the present study, where members of *E. coli* were identified to be the predominant bacteria in 67 % of collected clinical specimens from children with diarrhea.

According to (CDC) [19] the main mode of transmission of *E. coli* is through contaminated food or water. Therefore, 1000 food samples were screened for presence of *E. coli*.

In the present study, 13.4% (147/1100) of the collected clinical and food samples were contaminated with *E. coli*. Rügeles *et al.* [20] reported that *E. coli* members are recovered from 9.8% of investigated food and clinical samples. Another study was conducted in Bangladesh by Nazir and K.H.M. [21], who reported a recovery of 63 *E. coli* isolates out of 120 samples from different sources (fecal, food and water). The highest recovery rate was also from fecal (clinical) samples (66.67%) and the lowest one was from food samples (10%).

In the present work, the resistance to Beta-lactam class groups gradually decreased in following order; conventional Beta-lactams > combination Beta-lactams ~

cephalosporins > newly used Beta-lactams. Indeed, combination of Beta-lactams with Beta-lactamases suicidal inhibitors is a significant tool to reduce the antibiotic resistance to Beta-lactams [22]. The recorded resistance of clinical isolates to tested cephalosporins was almost in the same order of magnitude of this reported to Beta-lactams combinations. This could be explained that these tested cephalosporins were either from the 2<sup>nd</sup> or 3<sup>rd</sup> generations, which still retain considerable potency to *E. coli* [23, 24].

Interestingly, although minor resistance was recorded to newly discovered Beta-lactams (Imipenem and azetronam), all isolates were susceptible to imipenem.

This agrees with Patricia G *et al.*, [22], who have reported that no antimicrobial resistance is observed against imipenem.

Although, quinolones are well reputed group of broad spectrum antibiotics, the recorded resistance in the present study was relatively high (18-19%). Recently, many fears are emerging concerning the increasing abuse of these broad spectrum antibiotics in many developing countries, which may lead to significant increase in the resistance rate. For instance, Bolon *et al.*, [25] have reported a consistent stepwise increase in *E. coli* resistance to ciprofloxacin. The lowest resistance was recorded to polymyxin- B, which is in agreement with HyoBi Kim *et al.*, [26] where the reported resistance of *E. coli* to polymyxin-B was the lowest.

Although 90 % of isolated 147 *E. coli* strains showed antibiotic resistance to at least one antibiotic, only 52 strains (35%) showed multidrug resistance (MDR) to  $\geq 3$  classes of antibiotics with different modes of action. This relative high prevalence could be expected in bacteria like *E. coli*, where a diversity of lifestyles is achieved through a high degree of genome plasticity, with gene losses and gains, through horizontal transfer [27, 28].

All MDR *E. coli* were resistant to at least one of the tested members of Beta-lactam class of antibiotics. This is common in Gram negative bacteria either due to the presence of the outer membrane and/or production of Beta-lactams [29]. Except for aminoglycosides the resistance to protein inhibiting antibiotics showed considerable contribution in the MDR. Although aminoglycosides, macrolides and tetracycline exert their main mechanisms of action through inhibition of the protein synthesis, different mechanisms of resistance have been reported for each of these classes [30-35]. Indeed, still members of aminoglycosides are not of frequent use compared to those belonging to either tetracyclines or macrolides classes [36].

Recently, members of quinolones are frequently prescribed in many developing countries as a treatment for enteric infections caused by Gram-negative bacteria [37], this could explain why half of the detected MDR *E. coli* were resistant to quinolones. Membrane-active agents, such as polymyxin-B, have lowest resistance potentials because they have no specific routes of entry and it is difficult to alter fundamental bacterial membrane composition by mutation [38, 39].

Interestingly, the MDR in *E. coli* strains from food origin was significantly higher than those from clinical origin. Indeed, both antibiotic resistance and virulence determinants share some basic characteristics, where both have been reported to be acquired by horizontal gene transfer from organism to another [40]. Therefore, in the present study; food samples acquired a full set of antibiotic resistance genes that enable them to colonize in antibiotic-rich environment a new ecosystem.

In conclusion, a relatively high antibiotic resistance as well as MDR were detected in the isolated *E. coli* strains. This MDR was higher in *E. coli* strains from food origin compared to those from clinical origin. Imipenem and polymyxin-B were the most effective antibiotics against all isolated *E. coli* strains.

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