Article

Co-Existence of Certain ESBLs, MBLs and Plasmid Mediated Quinolone Resistance Genes among MDR E. coli Isolated from Different Clinical Specimens in Egypt

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Abstract: The emergence of multi-drug resistant (MDR) strains and even pan drug resistant (PDR) strains is alarming. In this study, we studied the resistance pattern of E. coli pathogens recovered from patients with different infections in different hospitals in Minia, Egypt and the co-existence of different resistance determinants. E. coli was the most prevalent among patients suffering from urinary tract infections (62%), while they were the least isolated from eye infections (10%). High prevalence of MDR isolates was found (73%) associated with high ESBLs and MBLs production (89.4% and 64.8%, respectively). blaTEM (80%) and blaNDM (43%) were the most frequent ESBL and MBL, respectively. None of the isolates harbored blakpc and blaoxa48 carbapenemase like genes. Also, the fluoroquinolone modifying enzyme gene aac(6’)-Ib-cr was detected in 25.2% of the isolates. More than one gene was found in 81% of the isolates. Azithromycin was one of the most effective antibiotics against MDR E. coli pathogens. The high MAR index of the isolates and the high prevalence of resistance genes, indicates an important public health concern and high-risk communities where antibiotics are abused.

Keywords: MDR E. coli; ESBLs; MBLs; MAR index

1. Introduction

Escherichia coli, belongs to the family Enterobacteriaceae, is the most common human gastrointestinal commensal as well as important etiological agent of many hospital and community-acquired infections. Pathogenic strains are capable of causing a wide variety of diseases including diarrhea, dysentery, overwhelming sepsis, and the hemolytic-uremic syndrome and neonatal meningitis. E. coli can be sorted into intestinal or extraintestinal according to the site of infection [1].

Antibiotics have been the most successful form of chemotherapy developed in the 20th century, saving human lives every day [2]. The evolution of pathogens resistant to antibiotics limits their clinical use, making such infections difficult to control. The antimicrobial resistance (AMR) can be of chromosomal or mobile genetic elements origin [3]. The most common resistance mechanism is the production of the β-lactamase hydrolytic enzymes, which specifically have an inactivated β-lactam ring so that they cannot inhibit the bacterial transpeptidases [4].

β-lactamases are classified into four classes. Serine classes (A, C and D) have serine residue at the hydrolysis active sites. Metallic- β-lactamases (MBLs) (class B) in which the hydrolytic action is promoted by one or two zinc ions at the active site [5]. Class A enzymes include blatem which is the first identified plasmid-encoded β-lactamase; blashv which has similar activity to blatem; blactx-m (cefotaximase) and blakpc which confers carbapenem...
resistance [6]. Class A Extended-spectrum β-lactamase (ESBL) producing strains (\(\text{bla}_{\text{TEM}}\), \(\text{bla}_{\text{SHV}}\) and \(\text{bla}_{\text{CTX-M}}\) types) are of the most clinically significant pathogens which can resist all β-lactam drugs including monobactams [5,6]. The most clinically significant class B enzymes are \(\text{bla}_{\text{VIM}}\), \(\text{bla}_{\text{IMP}}\) and \(\text{bla}_{\text{NDM}}\). MBLs are a group of carbapenemases that resist most β-lactam drugs except the monobactams. Monobactams (e.g., aztreonam) are intrinsically stable to MBLs, but their susceptibility to other serine β-lactamases which are often co-expressed with the MBL limit their usage against MBL expressing strains [7]. Another group that able to hydrolyze carbapenems in addition to other β-lactams are class D β-lactamases (e.g., \(\text{bla}_{\text{OXA-48}}\) like enzymes) [8].

Another example for enzymatic inactivation of antibiotics is the enzymatic modification at different -OH or -NH\(_2\) groups of aminoglycosides. As a result of the induced steric and/or electrostatic interactions, the modified antibiotic is unable to bind to the target RNA. They can be nucleotidyltransferases (ANTs), phosphotransferases (APHs), or acetyltransferases (AACs) [4]. In addition, the enzyme variant \(\text{aac(6')}\)-Ib-cr has two amino acid changes that allow the enzyme to inactivate quinolones as well [9,10].

Being plasmid-encoded, hydrolyzing enzymes are likely to be transmissible and widespread. As a single plasmid may encode more than one enzyme, a strain may express many different enzymes, as each one deactivates a different antibiotic [5]. As a result, MDR, or even PDR strains, arising and returning to the pre-antibiotic era has become a nightmare for medical professionals.

The present study aimed to report the resistance pattern of \(E.\ coli\) pathogens, detect the co-existence of different resistance determinants and their correlations to the resistance of \(E.\ coli\) pathogens of different infection origins, which would help in identifying local effective therapeutic options and infection control.

2. Results

2.1. Prevalence of \(E.\ coli\) Among Samples

In the present study, 200 (47%) \(E.\ coli\) pathogens were isolated from 425 patients suffering from different infections attending three hospitals in EL-Minia, Egypt. The highest prevalence was among urinary tract infections (62%) while it was lowest among eye infections (10%) (Table 1). Among the three hospitals, \(E.\ coli\) isolates were most prevalent in Minia University Hospital samples (51.37%), followed by Minia General Hospital (40.8%) (Table 2).

Table 1. Prevalence of \(E.\ coli\) among different clinical samples.

| Infection                        | No. of Samples | No. of \(E.\ coli\) Isolates | \(E.\ coli\) (%) * |
|----------------------------------|----------------|------------------------------|-------------------|
| Wound infections (burns, diabetic foot, surgery wound, cuts) | 150            | 66                           | 44%               |
| Urinary tract infection          | 100            | 62                           | 62%               |
| Gastro-enteritis                 | 50             | 24                           | 48%               |
| Blood                            | 75             | 39                           | 52%               |
| Chest infection                  | 20             | 4                            | 20%               |
| Ear infection                    | 20             | 4                            | 20%               |
| Eye                              | 10             | 1                            | 10%               |
| Total                            | 425            | 200                          | 47%               |

* Percent of \(E.\ coli\) were correlated to the number of samples of each infection.
Table 2. Distribution of *E. coli* among samples collected from different hospitals.

| Hospitals                  | No. of Samples | *E. coli* No. | % *      |
|----------------------------|----------------|---------------|----------|
| Minia University Hospitals | 290            | 149           | 51.37    |
| Minia Chest Hospital       | 20             | 4             | 20       |
| Minia General Hospital     | 115            | 47            | 40.8     |
| Total                      | 425            | 200           |          |

* Percent of *E. coli* were correlated to the number of collected samples from each hospital.

2.2. Antibiotic Resistance of the *E. coli* Pathogens

The antibiotic susceptibility was tested in 27 antibiotics that cover most of the available antibiotics in the Egyptian market. Table S1 (Supplementary Data) indicates the different used antibiotics and their different targets. The test revealed that 73% of *E. coli* were MDR. The pathogens were approximately totally resistant to Amoxycillin/clavulanic (97.5%), cephalothin (97%) and cefadroxil (93%). Also, high resistance levels were observed for Ceftazidime (78%) and Aztreonam (65.5%). Imipenem was the most effective antibiotic (20%), followed by Azithromycin (26%) (Figure 1). Our supplementary spread sheet indicates the resistance patterns of the isolates. One hundred MDR isolates were subjected for further investigation.

![Figure 1. Antibiotic resistance of the total *E. coli* isolates.](image)

2.3. Serotyping of the Intestinal *E. coli*

Out of the selected 100 isolates, 20 were isolated from stool. Since *E. coli* normally inhabit the intestine, stool isolates were serotyped to confirm its pathogenicity. Out of 20 intestinal *E. coli* isolates 15 (75%) isolates were diarrheagenic *E. coli* (DEC). Three isolates (20%) were identified as Enterohaemorrhagic *E. coli* (EHEC) O157:H7. Different O serotypes were observed as O115, O158, O55, O126, O125 and O86a. The identified pathotypes are...
listed in Table S3 (Supplementary Data). Untyped five isolates were excluded from the further testing so that 95 isolates were further tested phenotypically and genotypically.

2.4. Multiple Antibiotic Resistance Index MDR E. coli Pathogens

The multiple antibiotic resistance index (MARI) ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics the organism is exposed to, have been calculated for 95 MDR E. coli. It was found that 98.9% of isolates have showed MAR index higher than 0.2, indicating high risk communities where antibiotics are abused. However, there was a statistically significant difference in MARI mean among E. coli isolates of different sources (p value < 0.05). Eye and blood isolates showed highest MARI mean of 0.82 and 0.74, respectively. On the other hand, stool samples had the lowest MARIs (Figure 2).

![Figure 2. MAR index mean values (x̅), p value calculated by One-way ANOVA test.](image)

2.5. Phenotypic Characteristics

ESBL production phenotypically tested by combined disk test (CDT). It was found that 89.4% (85/95) of the tested strains were ESBL producers. Carbenemase production was tested by Modified Hodge test (MHT), then carbenemase producers were tested for MBL using combined-disk synergy test. MBL producers accounted for 50.50% of the isolates (64.8% of carbenemase producers). Positive significant association was found between ESBL and MBL phenotypes (p = 0.001) as all MBL producers were ESBL producers. Statistically significant difference in distribution of ESBL and MBL producers between different infection groups was observed (p < 0.001). Regardless of the eye infection, MBL producers were mostly frequent in UTIs (71%) while no intestinal E. coli was reported as MBL producers (Table 3).

![Image of Table 3. Distribution of ESBLs and MBLs producing isolates among clinical specimens.](image)

| Type of Infection     | β-Lactam Resistant Isolates | ESBLs | MBL |
|-----------------------|-----------------------------|-------|-----|
|                       | No. | % * | No. | % * |
| Wound infections      | 25  | 100%| 12  | 48% |
| Urinary tract infection| 28  | 100%| 20  | 71.4% |
| Gastro-enteritis      | 15  | 33.3%| 0  | 0% |
| Blood                 | 20  | 100%| 12  | 46.67% |
| Respiratory infection | 4   | 100%| 2   | 50% |
| Ear infection         | 2   | 100%| 1   | 50% |
| Eye                   | 1   | 100%| 1   | 100% |
| Total                 | 95  | 89.4% **| 48 | 50.5% ** |

* percent was correlated to the total number of samples of each infection type; ** percent was correlated to total number of samples. Significant if p ≤ 0.05.
2.6. Antimicrobial Resistance of ESBLs and MBLs Producers

Resistance patterns of ESBL-producers revealed that ESBL producers were highly resistant to β-lactam antibiotics such as amoxicillin/clavulanic (99%), cefadroxil (95%), ceftazidime (85%) and meropenem (69%). MBL producers showed higher resistance rates to same antibiotics (100%, 97.9%, 93.75% and 93.75%, respectively). Azithromycin and chloramphenicol were the most effective drugs against ESBLs and MBLs producers. Table S4 (Supplementary Data) indicates the antibiogram of ESBLs and MBLs producers.

2.7. Prevalence of Resistance Genotypes Among the Tested Isolates

There was a statistically significant difference in the distribution of the different genotypes between different infections. The most prevalent gene was the \( \text{bla}_{\text{TEM}} \) (80%) followed by \( \text{bla}_{\text{SHV}} \), \( \text{bla}_{\text{CTX-M}} \) and \( \text{bla}_{\text{NDM}} \) (54.7%, 42% and 44.2%, respectively). All isolates were negative for \( \text{bla}_{\text{KPC}} \) or \( \text{bla}_{\text{oxa-48}} \) genes. Also, the \( \text{aac-(6')-Ib-cr} \) gene was observed in 26.3% of the tested pathogens (Table 4).

Table 4. Distribution of detected MBLs and ESBLs genotypes.

| Type of Infection | β-Lactam Resistant Isolates N | \( \text{bla}_{\text{NDM}} \) N (%) * | \( \text{bla}_{\text{TEM}} \) N (%) * | \( \text{bla}_{\text{CTX-M}} \) N (%) * | \( \text{bla}_{\text{SHV}} \) N (%) * | \( \text{bla}_{\text{IMP}} \) N (%) * | \( \text{aac-(6')-Ib-cr} \) N (%) * |
|-------------------|-----------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Wound infections  | 25                          | 14 (56%)                        | 23 (92%)                        | 14 (56%)                        | 15 (60%)                        | 12 (48%)                        | 9 (36%)                          |
| UTI               | 28                          | 17 (60.7%)                      | 27 (96.4%)                      | 11 (39.2%)                      | 12 (42.8%)                      | 16 (57%)                        | 6 (21%)                          |
| Gastro-enteritis  | 15                          | 1 (7.6%)                        | 7 (46.6%)                       | 0 (0%)                          | 0 (0%)                          | 0 (0%)                          | 0 (0%)                           |
| Blood             | 20                          | 7 (35%)                         | 20 (100%)                       | 13 (65%)                        | 20 (100%)                       | 7 (35%)                         | 10 (50%)                         |
| Chest infection   | 4                           | 1 (25%)                         | 3 (75%)                         | 0 (0%)                          | 4 (100%)                        | 0 (0%)                          | 0 (0%)                           |
| Ear infection     | 2                           | 1 (50%)                         | 2 (100%)                        | 1 (50%)                         | 0 (0%)                          | 0 (0%)                          | 0 (0%)                           |
| Eye               | 1                           | 1 (100%)                        | 1 (100%)                        | 1 (100%)                        | 0 (0%)                          | 0 (0%)                          | 0 (0%)                           |
| Total             | 95                          | 42 (44.2%)                      | 76 (80%)                        | 40 (42%)                        | 52 (54.7%)                      | 35 (36.8%)                      | 25 (26.3%)                       |

* percent was correlated to the total number of β-lactam resistant isolates in each type of infection. ** Significant \( p \) value at \( p \leq 0.05 \).

2.8. Genotypic-Phenotypic Agreement of the Tested Genes

Out of 48 MBL phenotypic positive samples, 38 (79%) isolates were confirmed genotypically as MBL producers. It was found that 15.78% of isolates were phenotypically positive and harbored both \( \text{bla}_{\text{NDM}} \) and \( \text{bla}_{\text{IMP}} \) (Figure 3). Furthermore, there was a significant decrease in MAR index when isolates were both \( \text{bla}_{\text{NDM}} \) and \( \text{bla}_{\text{IMP}} \) negative (\( p \) value < 0.001). The \( \text{bla}_{\text{NDM}} \) producers had higher MAR index than those which were only \( \text{bla}_{\text{IMP}} \) producers. However, isolates harbored both \( \text{bla}_{\text{NDM}} \) and \( \text{bla}_{\text{IMP}} \) was observed resisting higher number of antibiotics (Figure 4). Moreover, positive correlations between MBLs phenotype, genotypes and carbapenem resistance were observed. Statistically significant correlations between detected MBL genotypes and meropenem resistance were observed. The MBLs phenotypes were more significantly associated with \( \text{bla}_{\text{NDM}} \) than \( \text{bla}_{\text{IMP}} \) (Table 5).
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ESBLs phenotypes showed strong positive correlation with the presence of \textit{bla}TEM. However, all correlations were significant at the 0.01 level. Among the detected ESBL genes, presence of \textit{bla}TEM and \textit{bla}CTX-M types had the upper hand on the isolates’ resistance followed by \textit{aac(6$'$Ib-cr} gene (Table 6).

The current study identified that \textit{aac(6$'$Ib-cr} gene was mainly related to aminoglycoside antibiotics than fluoroquinolones. It was found that 47.2% of amikacin resistant isolates and 32.4% of ciprofloxacin resistant isolates harbored \textit{aac(6$'$Ib-cr} gene. The presence of \textit{aac(6$'$Ib-cr} gene was least correlated to ofloxacin resistance. Significant moderate positive correlation was observed between \textit{aac(6$'$Ib-cr} and the resistance to amikacin and tobramycin, \(p\) values < 0.001 and 0.003, respectively (Table 7).

**Table 5.** Relation between MBLs phenotype, detected genotype and carbapenem resistance across the \textit{E. coli} isolates.

| MBLs Phenotype | \textit{bla}_{NDM} | \textit{bla}_{IMP} | Imipenem Resistance | Meropenem Resistance |
|----------------|---------------------|-------------------|---------------------|---------------------|
| MBLs phenotype | 1                   | 0.372 **          | 0.232 *            | 0.237 *            |
| \textit{bla}_{NDM} | 1                   | 0.155             | 0.054              | 0.212 *            |
| \textit{bla}_{IMP} | 1                   | 0.122             | 0.275 **           | 0.292 **           |
| Imipenem resistance | 1                   |                   | 0.292 **           |                     |

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). \(p\) values calculated by Fisher’s exact test.
ESBLs phenotypes showed strong positive correlation with the presence of blaTEM. However, all correlations were significant at the 0.01 level. Among the detected ESBL genes, presence of blaTEM and blaCTX-M types had the upper hand on the isolates’ resistance followed by aac(6’)-Ib-cr gene (Table 6).

Table 6. Correlation matrix ($r^2$) between phenotypes, genotypes and antibiotic resistance across the E. coli isolates.

| MAR index | ESBLs Production | MBLs Production | blaNDM | blaIMP | blaTEM | blaCTX-M | blashv | aac(6’)-Ib-cr |
|-----------|------------------|-----------------|--------|--------|--------|----------|--------|-------------|
| 0.611 **  | 0.342 **         | 0.330 **        | 0.289 **| 0.366 **| 0.365 **| 0.251 * | 0.360 **| 0.205 *     |
| ESBLs phenotype | 1               | 0.347 **        | 0.305 **| 0.262 * | 0.696 **| 0.293 ** | 0.377 **| 0.365 **    |

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). p values calculated by Fisher’s exact test.

The current study identified that aac(6’)-Ib-cr gene was mainly related to aminoglycoside antibiotics than fluoroquinolones. It was found that 47.2% of amikacin resistant isolates and 32.4% of ciprofloxacin resistant isolates harbored aac(6’)-Ib-cr gene. The presence of aac(6’)-Ib-cr gene was least correlated to ofloxacin resistance. Significant moderate positive correlation was observed between aac(6’)-Ib-cr and the resistance to amikacin and tobramycin, p values < 0.001 and 0.003, respectively (Table 7).

Table 7. Correlation between aac(6’)-Ib-cr, aminoglycoside and fluoroquinolone resistance.

| Antibiotics     | Number of Resistant Isolates | Number of aac(6’)-Ib-cr Positive Isolates (%) | Person Correlation ($r^2$) | p Value |
|-----------------|-----------------------------|-----------------------------------------------|--------------------------|---------|
| Streptomycin    | 61                          | 19 (31)                                      | 0.135                    | 0.193   |
| Tobramycin      | 43                          | 17 (39.5)                                    | 0.301                    | 0.003 **|
| Gentamycin      | 43                          | 13 (30.2)                                    | 0.044                    | 0.670   |
| Amikacin        | 36                          | 17 (47.2)                                    | 0.374                    | <0.01 ***|
| Ofloxacin       | 39                          | 9 (23)                                       | 0.018                    | 0.866   |
| Norfloxacin     | 42                          | 14 (33.3)                                    | 0.1450                   | 0.162   |
| Ciprofloxacin   | 37                          | 12 (32.4)                                    | 0.152                    | 0.142   |

*percent correlated to no. of resistant isolates of each antibiotic. p values were calculated by Fisher’s exact test. ** p value is significant at 0.05 level (2-tailed). *** p value is significant at 0.01 level (2-tailed).

2.9. Association of Different Resistance Genotypes

Most isolates harbored more than one resistance gene (81%). The resistance frequency has significantly increased with the increased number of the co-existed genes ($p < 0.01$). The most frequent association was of the five genes blaNDM, blaIMP, blaTEM, blaCTX-M and blashv (8.4%) (Table 8).

The correlation matrix of the detected genes indicated overall positive correlations. The strongest and most significant correlation was observed between blaCTX-M and blashv ($r = 0.519$). Moreover, aac(6’)-Ib-cr gene was significantly associated with blaCTX-M (Table 9).

Studying the association of blaNDM gene with class A ESBL genes (blaTEM, blaCTX-M and blashv) among the blaNDM positive isolates indicated that the association of the blaNDM with the three ESBLs (blaTEM + blashv + blaCTX-M) genes was the highest, accounting for 40.47% of the isolates harboring blaNDM (Figure 5).

Furthermore, the spectrum of antibiotics to which the isolates were resistance is significantly increased ($p$ value < 0.01) with the number of positive genes. The isolate harbored five genes showed the highest mean of antibiotic resistance (21 antibiotics), as indicted in Figure 6. As indicated in Table S5 (Supplementary Data) the resistance of β-lactam drugs is significantly associated with presence of higher number of genes. Also, significant moderate correlation of number of positive genes with ciprofloxacin, norfloxacin and aminoglycoside were observed.
Table 8. Co-existence of different genotypes.

| Genotype                        | Number of Isolates (%) |
|---------------------------------|------------------------|
| One gene                        |                        |
| blaTEM                          | 8 (8.5)                |
| blaSHV                          | 1 (1)                  |
| Two genes                       | 20 (21.1)              |
| blaTEM, aac(6′)Ib-cr            | 1 (1)                  |
| blaIMP, blaTEM                  | 5 (5.2)                |
| blaTEM, blaSHV                  | 5 (5.2)                |
| blaNDM, blaTEM                  | 7 (7.3)                |
| blaTEM, blaCTX-M                | 2 (2.1)                |
| Three genes                     | 21 (22.1)              |
| blaIMP, blaTEM, blaSHV          | 4 (4.2)                |
| blaNDM, blaTEM, blaCTX-M        | 1 (1)                  |
| blaNDM, blaTEM, aac(6′)Ib-cr    | 2 (2.1)                |
| blaTEM, blaCTX-M, blaSHV        | 5 (5.2)                |
| blaTEM, blaSHV, aac(6′)Ib-cr    | 1 (1)                  |
| blaNDM, blaCTX-M, blaSHV        | 1 (1)                  |
| blaNDM, blaIMP, blaTEM          | 2 (2.1)                |
| blaNDM, blaTEM, blaSHV          | 5 (5.2)                |
| Four genes                      | 21 (22.1)              |
| blaTEM, blaCTX-M, blaSHV, aac(6′)Ib-cr | 5 (5.2) |
| blaNDM, blaIMP, blaTEM, aac(6′)Ib-cr | 3 (3.1) |
| blaNDM, blaTEM, blaCTX-M, aac(6′)Ib-cr | 2 (2.1) |
| Five genes                      | 12 (12.6)              |
| blaNDM, blaIMP, blaTEM, blaCTX-M, blaSHV | 8 (8.5) |
| blaNDM, blaIMP, blaTEM, blaCTX-M, aac(6′)Ib-cr | 2 (2.1) |
| Six genes                       | 3 (3.2)                |
| Total                           | 95                     |

Percentages were correlated to the total number of isolates.

Table 9. Correlation matrix ($r^2$) between the different genotypes.

|          | blaNDM | blaIMP | blaTEM | blaCTX-M | blaSHV | aac(6′)Ib-cr |
|----------|--------|--------|--------|----------|--------|--------------|
| blaNDM   | 1      | 0.155  | 0.211 * | 0.185    | 0.086  | 0.190        |
| blaIMP   | 1      | 0.290 ** | 0.233 * | 0.081    | 0.089  |              |
| blaTEM   |        | 1      | 0.196  | 0.227 *  | 0.155  |              |
| blaCTX-M |        |        | 1      | 0.519 ** | 0.265 **|              |
| blaSHV   |        |        |        | 1        | 0.159  |              |
| aac(6′)Ib-cr |      |        |        |          |        | 1            |

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). $p$ values were calculated by Fisher’s exact test.
3. Discussion

The last two decades have witnessed a conspicuous increase in the number of infections caused by multi-drug resistant strains of *E. coli*, and this has impacted the outcomes of different infections [11].

The present study demonstrated that the prevalence of *E. coli* pathogens isolated from patients suffering from different infections in El-Minya hospitals accounted for 47%. This result was in accordance with results reported by Amer et al. (45%) [12] and Fam et al. (56%) [13].
Among extraintestinal infections, *E. coli* was the most common among urine isolates (62%) followed by blood infections (52%). Likewise, in Saudi Arabia Alanazi et al. [14] reported that *E. coli* was isolated from 60.24% of the urine samples and in Greece Koupetori et al. [15] reported high incidence of *E. coli* accounting for 48% of blood isolates. In contrary, many studies showed lower *E. coli* accounting for 48% of blood isolates. On the other hand, *E. coli* was least isolated from eye infections (10%). This was higher than results reported in USA by Miller et al. (5.9%) [18].

Moreover, intestinal *E. coli* was serotyped to ensure its pathogenicity. Diarrheagenic *E. coli* (DEC) serotypes accounted for 54.1% which was considered very high in comparison to results obtained by Zhou et al. (7.9%) [19]. *E. coli* O157:H7 accounted for 12.5% of total DEC serotypes. Comparably, in the same region of study Abd El Gany et al. [20] reported incidence rate of 15.72%. The variations between the different studies may be ascribed to many socioeconomic, demographical and geographical factors. It was obviously noted that *E. coli* were relatively high in the present study compared to other studies indicates poor hygienic attitudes that correlated to the mentioned factors.

Antimicrobial resistance (AMR) emphasizes an overwhelming health and economic burden in both developed and developing countries. As Resistance narrows the therapeutic options leading to increased morbidity and mortality [21]. Our results showed high prevalence of MDR *E. coli* (73%) which were higher than results obtained by Siwakoti et al. [22] and Abdelaziz et al. [23] (28% and 60%, respectively). Although carbapenem resistance is considered low, it is higher than previous studies done at the same government [9,20,24]. This may be attributed to the availability and the usage of the drugs when the studies were held. Concerning our results, the drugs were more available and highly used but in previous studies there was a shortage in many antibiotics inside the hospitals. Moreover, the resistance to meropenem was higher than imipenem which may be attributed to that meropenem is cheaper than imipenem, so it is commonly used while some other studies reported complete meropenem sensitivity [9,23].

The continuous spread of ESBLs and carbapenemase mediated resistance has dramatically increased in both hospital and community infections. It was found that 89.4% of the tested isolates were ESBL producers. Abd El-Baky et al. [24] in earlier study in our area reported that 46.8% of isolates were phenotypically ESBL producers. It seems very alerting as the prevalence is almost doubled in short period.

The incidence of the carbapenemase and MBLs producers accounted for 77.8% and 64.8%, respectively. These rates were very high when compared to results obtained by Ibrahim, et al. [25] who reported that carbapenemase and MBLs incidences were 37.6% and 46.3% respectively. A previous study in our area showed that 52.3% of *P. aeruginosa* were MBL producers [26]. The differences in prevalence may be due to strains and time variations but overall indicate high incidence of MBLs among bacteria in our area.

There was a significant difference in the distribution of detected genes among the different sample sources *p* values < 0.05 which was in agreement with many studies [27,28]. The most prevalent genotype was *bla*<sub>TEM</sub> (80%) followed by *bla*<sub>SHV</sub> (54.7%), *bla*<sub>NDM</sub> (44.2%) and *bla*<sub>CTX-M</sub> (42%). Similarly, *bla*<sub>TEM</sub> was predominant in results reported by Mohamed et al. [28] and Maamoun et al. [29]. On the other hand, a study on *Escherichia coli* causing sepsis among Egyptian children reported that *bla*<sub>SHV</sub> was the most common ESBL (61.22%), followed by *bla*<sub>TEM</sub> (38.78%) and *bla*<sub>CTX-M</sub> (20.41%) [30]. Furthermore, the higher incidence of *bla*<sub>TEM</sub> gene reported by the current study or other studies in our region suggests that *bla*<sub>TEM</sub> gene may be endemic. In contrast to our study, several studies in Asia reported that *bla*<sub>CTX-M</sub> was the most frequent indicating that *bla*<sub>CTX-M</sub> is a predominant genotype in Asia [31–33]. Also, reports from Qatar stated that *bla*<sub>CTX-M</sub> type genes evolved through mutations in *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes and it is a recent endemic [34].

Similar to our study, studies in UK have reported 44% of isolates as *bla*<sub>NDM</sub> producers, most of them were from urine samples [35]. The number of *bla*<sub>NDM</sub> producers is increasing in Egypt which is reflected by many studies conducted in this area [30,36,37]. Lower *bla*<sub>IMP</sub> incidence reported in previous studies in Egypt compared to this study (36.8%) suggesting...
an increasing rate of MBLs producers [36,37]. However, the current reported high prevalence of MBLs may be attributed to the ability of E. coli to acquire novel resistance genes through horizontal transfer or the increased use of carbapenems in the clinical treatment. 

blaOxa-48 like and blaKPC were not detected in any isolate. Quite higher prevalence of blaOxa-48 like and blaKPC (38.46% and 23%, respectively) was reported in Bangladesh [38]. In accordance with the current study, blaKPC wasn’t detected in several previous studies in Egypt or detected in very low rate [37,38]. In addition, blaKPC wasn’t detected in countries such as Saudi Arabia [39] or those of the Arabian Peninsula [40]. These data confirmed that blaKPC genes does not predominate in this geographical region, where it is frequently detected in the United States [41] and endemic in Israel [42].

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4.2. **Antimicrobial-Susceptibility Testing**

The antimicrobial susceptibility of the isolates was tested by the Kirby-Bauer Disk Diffusion method [61]. The used antibiotics discs were ready cartilages purchased from Oxoid; Basingstoke, UK. The following antibiotic discs were used: Cefpodoxime (10 µg), Streptomycin (10 µg), Aztreonam (30 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), Amoxicillin/clavulanic (20/10 µg), Piperacillin/Tazobactam (100/10 µg), Ceftazidime (30 µg), Imipenem (10 µg), Meropenem (10 µg), Cefoperazone (75 µg), Doxycycline (30 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Nalidixic acid (30 µg), Ceftazidime (30 µg), Ceftazidime (30 µg), Ampicillin/sulbactam (10/10 µg), Norfloxacin (10 µg), Tobramycin (10 µg), Sulfamethoxazole/trimethoprim (23.75/1.25 µg), Chloramphenicol (30 µg). Isolates classified as sensitive, intermediate and resistant according to inhibition zones interpretation standards of Clinical Laboratory standards Institute (CLSI) 2018 (Table S2) [62].

4.3. **Serotyping of Intestinal E. coli**

*Escherichia coli* recovered from gastroenteritis infections were sent to the Animal Health Research Institute, Giza, Egypt to be serotyped. The isolates serotyped through detection of isolates agglutination with O and H antisera using the slide agglutination method according to the manufacturer instructions (Pro-Lab Diagnostics, Round Rock, TX, USA).

4.4. **Phenotypic Detection of ESBLs and MBLs Production**

Detection of ESBL in *E. coli* isolates was carried out by combined disc test (CDT). Isolates defined positive when the difference between the inhibition zones of cefotaxime and cefotaxime/clavulanic or ceftazidime and ceftazidime/clavulanic disks is ≥5 mm [62]. Carbapenemases were detected in carbapenem resistant isolates by Modified Hodge test (MHT). MHT positive isolates were further tested for MBL production using EDTA-combined disk synergy test. An increase in zone diameter of at least 7 mm around the imipenem–EDTA or meropenem–EDTA disks were recorded positive result [63].

4.5. **Amplification of Resistance Genes**

The DNA template was extracted by available commercial kit QIAprep® Spin Miniprep Kit (QIAGEN, Germany) by following the manufacturer instruction. Resistance genes were detected using conventional PCR technique. Amplification was done using 25 µL PCR reaction mixture consisting of 12.5 µL master mix (BIOMATIk, Kitchener, Canada) 1 µL of each forward and reverse primers (BIOMATIk, Canada), 2 µL DNA template and 8.5 µL nuclease-free water. PCR cycling conditions are indicated in Table S5 (Supplementary Data).

4.6. **Statistical Analysis**

Data were analyzed using IBM SPSS version 20.0. First, normal distribution of data was tested by normality tests as Kolmogorov–Smirnov and Shapiro–Wilk *p*-values in addition to histograms. Descriptive analysis was done to analyze prevalence of *E. coli* isolates among different infections and hospitals, percentage of resistance and prevalence of ESBL and MBL producers and prevalence of the different genes. To compare differences in distribution between different groups chi-square (X²) test was done but when more than 20% of cells were less than 5, Fisher’s exact test was done to be more accurate. One-way ANOVA tests was done to compare mean values between different groups as MAR index mean values in different sample sources. Non parametric tests were used for non-parametric data as Kruskal–Wallis. To study associations between phenotypes, genotypes and resistance, correlations were established using Pearson's correlation coefficient (r²) in bivariate. *p*-values are significant if they are ≤0.05.

5. **Conclusions**

High resistance reported in our study indicates poor awareness of the microbiological laboratory test importance, high empirical antimicrobial prescription and high patient compliance. Moreover, the massive co-existence of the detected genes strongly supports
the presence of one or more circulating plasmids that harbors different resistance genes. Finally, the study highlighted the importance of continuous surveillance of the resistance trends and the direct need to strictly apply the infection control policies, implementing a national antimicrobial stewardship plan.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/antibiotics10070835/s1, Table S1: The different tested antibiotics and their different biological processes targets, Table S2: The different thresholds of inhibition zones according to CLSI (2018), Table S3: Different serotypes of the intestinal *E. coli*, Table S4: Resistance pattern of ESBLs and MBLs producers, Table S5: Correlation coefficient of the number of detected genes in isolates with the resistance of the antibiotics, Table S6: Primers used in the current study. References [63–70] are cited in Supplementary File.

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**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Commission on the Ethics of Scientific Research of Faculty of Pharmacy, Minia University (protocol code HV06/2020 and date of approval 17-6-2020).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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