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Antimicrobial Susceptibility and Resistance Profile of *Escherichia coli* Isolates from Patients at Suez Canal University Specialized Hospital

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ABSTRACT

Antibiotics have increased our lifespan by more than 20 years and shielded us from dangerous microbes. However, their power is dwindling. Therefore, antibiotic resistance is a current worldwide health emergency, and it could be the following pandemic. Thus, the present study assesses the susceptibility and resistance pattern of various *E. coli* isolates collected from patients at Suez Canal University Specialized Hospital. Collectively, 50 isolates of *E. coli* out of 150 specimens were isolated from different specimens' types (urine, stool, and blood) and were identified using traditional standard protocols. After performing antibiotic susceptibility testing, the overall tested isolates showed the highest susceptibility against MRP (92%) followed by AK (90%), while the highest resistance was obtained against AMC (100%) followed by CTX (44%). On the other hand, *E. coli* isolates from urine showed the highest susceptibility against MRP and AK (91.43%) each, while the highest resistance was against AMC (100%) followed by CTX (51.43%). Moreover, the maximum susceptibility of stool isolates was against AK and MRP (83.33 %) each, whereas the isolates obtained from stool showed the highest resistance against AMC (100%) followed by CTX (50%) each. Finally, the *E. coli* isolates from blood revealed the superior susceptibility to MRP (88.89%) followed by LEV, AK, and DO (77.78%), while the highest resistance of blood specimens was found against AMC (100%) followed by CTX (33.33%). Furthermore, the resistance patterns revealed that 66% of the isolates were MDR. Finally, it is crucial to evaluate the antimicrobial sensitivity test for effective therapy.

Key Words:
Escherichia coli, Antimicrobial Susceptibility, Antibiotic Resistance, *E. coli* Infections, Multi-Drug Resistant.

1. INTRODUCTION

Escherichia coli, which belongs to the family of Enterobacteriaceae, is typically found in the flora of both human and animal digestive tracts but it is also present in vegetation, soil, and water [1]. Although

this Gram-negative bacterium can colonize the human gut without harm and serves as a symbiont to help with vitamin absorption and production, it causes serious infections outside of the gastrointestinal tract, like bacteremia and sepsis [2], [3].

According to the Centers for Disease Control and Prevention (CDC), in hospitalized patients, *E. coli* is one of the most causative agents of bloodstream infections, pneumonia, and urinary tract infections (UTIs) [4]. *E. coli* is known to be the most prevalent bacteria related to UTIs globally, leading to both community and hospital-acquired UTIs accounting for up to 35% of nosocomial infections and 80–95% of community-acquired infections, and being the second leading cause of nosocomial bacteremia. In addition, female patients with UTIs are making up 87.5% of cases as opposed to males [5], [6].

Importantly, transmitting a primary *E. coli* infection to another location frequently leads to bacteremia. While hospitalized patients are more likely to develop bacteremia because of lower respiratory tract infections, and community-acquired *E. coli*, bacteremia is most usually caused after UTI complications in elderly people [7]. On the other hand, *E. coli* caused 30% of neonatal sepsis with more than a 10% death rate. A high 90-day death rate is linked to *E. coli* bloodstream infections, and the mortality is much greater for "multidrug-resistant" (MDR) strains[8]. Collectively, 27% of sepsis cases are caused by *E. coli*, which together result in an enormous burden on healthcare systems around the world [6].

Recent studies clearly demonstrate that Enterobacteriaceae, including *E. coli,* are now a common causative agent of ventilator-assisted pneumonia. Nosocomial infections continue to be a serious risk to patients and a financial burden on the healthcare sector. In units of intensive care, ventilator-associated pneumonia is the most frequent life-threatening nosocomial infection; the mortality rate is about 13%, partially due to longer periods of mechanical ventilation and longer ICU stays leading to extra expenses [9].

E. coli is developing all its potential and virulence mechanisms because of the misuse and overuse of antibiotics [10]. Important defense mechanisms against antibiotics are shown by *E. coli*, including overexpression of efflux proteins, aminoglycoside degrading enzyme, target modification, reduced absorption, and beta-lactamase enzyme degradation [5]. Antimicrobial resistance (AMR) is receiving a lot of attention worldwide since more bacteria are acquiring resistance to the antimicrobials that are currently in use. Despite notable advances in antibacterial treatments, treatment failure of MDR bacteria has become more widespread worldwide. According to a recent study, bacterial AMR is presently estimated to be responsible for 1 million yearly deaths. The term MDR bacteria refers to bacteria that have developed a non-sensitivity to at least three different antimicrobial drug classes and it is prevalent in hospitals [8],[11],[12].

Of interest, although numerous papers have shown the resistance patterns of numerous diseases, few researchers that estimate the endemic antimicrobial resistance profile in low- and middle-income countries [13]. Fortunately, previous reports on the antibiotic resistance *E. coli* profile from various clinical sources have been conducted in Egypt [13]–[15]. In this article, we discuss an aspect of prevalent antibiotic-resistant *E. coli*, which were chosen due to their significant influence on the availability of clinical treatments for life-threatening diseases. Unfortunately, up to 95% of individuals with serious symptoms are often treated without having a bacteriological test [1]. Therefore, this susceptibility profile study is intended to identify the ideal empirical antibiotic treatment that can aid in the effective management of *E. coli* infections. Additionally, these investigations will contribute to reduce the rise in antimicrobial resistance caused by the improper use of antibiotics [16].

2. MATERIALS AND METHODS

2.1. Specimens' collection

According to standard protocols procedures [17]–[19], clinical specimens from various sources (blood, urine, and stool) were collected from patients who were admitted to Suez Canal University Specialized Hospital, Ismailia, Egypt, between May 2021 and November 2021. Briefly, sterilized wide-mouth containers were used to collect urine specimens from midstream urine. Stool specimens from diarrhea cases were collected in sterile plastic cups with tightly led screws. Venipuncture was used to obtain the blood specimens, and 10 ml were collected into blood culture bottles. The specimens were delivered in an ice box and processed as soon as possible at the Microbiology lab of the Faculty of Science at Port Said University.

2.2. Identification of the isolates

Specimens were streaked on Luria-Bertani agar and incubated for 24 hours at 37°C. All suspected clinical isolates of *E. coli* were identified using traditional techniques including, Gram stain, MacConkey agar, Sorbitol MacConkey agar (SMAC), and Eosin methylene blue (EMB) cultivation, as well as biochemically utilizing the indole test, Methyl Red- Vogus Proskauer (MR-VP) test, and Simmons's citrate agar cultivation [20].

2.3. Antibiotics used in the current study

Using the disc diffusion method, 8 antimicrobials that were purchased from (Oxoid, UK) were used as shown in Table 1.

Table 1: Antibiotic used in this study.

Antibiotic	Abbreviation	Concentration (µg/disc)
1. Levofloxacin (Quinolones)	LEV	5
2. Meropenem (Carbapenems)	MRP	10
3. Cefotaxime (Cephalosporins)	CTX	30
4. Trimethoprim/Sulfamethoxazole (Sulfonamides)	SXT	25
5. Amikacin (Aminoglycosides)	AK	30
6. Piperacillin (β-lactams)	PRL	100
7. Doxycycline (Tetracyclines)	DO	30
8. Amoxicillin/clavulanic acid (lactams)	AMC	30

2.4. Antimicrobial susceptibility testing

The antibiotic susceptibility testing of the tested isolates was performed using the disc diffusion method. In brief, the isolates' overnight cultures were diluted in tryptone soya broth (TSB) to reach the turbidity of 0.5 MacFarland's. Each isolate's aliquot of 10 µl was used to inoculate Muller-Hinton agar (MHA) plates with uniform distribution perpendicularly. On the agar's surface, the antimicrobial discs were applied using sterile forceps. The diameter of each zone of inhibition surrounding the discs was measured after overnight incubation and the results were interpreted following The Clinical and Laboratory Standards Institute's guidelines (CLSI) [21].

3. RESULTS AND DISCUSSION

3.1. Identification of *E. coli* isolates

From the patients admitted to Suez Canal University Specialized Hospital, 50 positive *E. coli* isolates were identified from the collected 150 specimens. Using selective and differential media, as well as biochemical testing, all the 50 isolates were identified as *E. coli*. The Gram staining of the examined isolates revealed Gram-negative, rod-shaped cells that were lactose fermenting on MacConkey agar, produced colorless colonies on SMAC and produced a green metallic sheen on EMB. Additionally, testing for indole and MR yielded positive results for the tested isolates while yielding negative results for VP and citrate utilization tests. The distribution of *E. coli* isolates among various specimen types is shown in Table 2. The rate of *E. coli* recovery is superior from urine specimens (45.45%) compared to the other specimens such as stool (14.29%) and blood (29.03%).

Table 2: The distribution of *E. coli* isolates among various specimen types.

Specimen type	Urine	Stool	Blood	Isolates Total Number
Total number of specimens (%)	77 (51.33%)	42 (28%)	31 (20.67%)	**150 (100%)**
Number of *E. coli* isolates (%)	**35 (45.45%)**	**6 (14.29%)**	**9 (29.03%)**	**50 (33.33%)**

The patient cohort encompassed individuals of both genders, with male and female patients constituting 27.33% and 72.76% of the total, respectively. The mean age of the individuals under investigation ranged from 1 month to 80 years, with the greatest prevalence observed among patients aged between 20 and 40 years (Table 3).

Table 3: The distribution of age and gender of patients from whom *E. coli* isolates were obtained.

Patient sex and age	Specimen type	Urine		Stool		Blood		Isolates Total Number
		Male	Female	Male	Female	Male	Female	
<1 to 20	T	4	20	5	9	0	1	**39**
	N	1 (2.86%)	3 (8.57%)	3 (50%)	2 (33.33%)	2 (22.22%)	5 (55.56%)	**16 (32%)**
20 to 40	T	2	24	1	7	4	5	**43**
	N	3 (8.57%)	20 (57.14%)	0	1 (16.67%)	0	1 (11.11%)	**25 (50%)**
40 to 60	T	3	16	3	4	8	2	**36**
	N	2 (5.71%)	4 (11.43%)	0	0	0	0	**6 (12%)**
60 to 80	T	1	7	6	7	4	7	**32**
	N	**0**	**2 (5.71%)**	**0**	**0**	**1 (11.11%)**	**0**	**3 (6%)**

T=Total number of specimens, N= Number of isolated *E. coli* strains

3.2. Antimicrobial susceptibility profile

The antibiotic susceptibility testing of the tested isolates was performed using the disc diffusion method against 8 antimicrobials (Fig. 1).

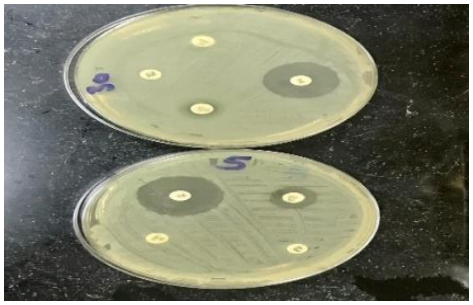

Figure 1: Representative example of antimicrobial susceptibility test of some *E. coli* isolates

In the present research, *E. coli* was isolated from different patients experiencing UTIs, gastrointestinal tract infections (diarrhea), and bloodstream infections. Collectively, the overall 50 *E. coli* isolates showed the highest susceptibility against MRP 46/50 (92%) followed by AK 45/50 (90%), on the other hand, the highest resistance was obtained against AMC 50/50 (100%) followed by CTX 22/50 (44%), the full results are shown in Fig. 2. Similarly, in an earlier study conducted in Egypt [22], MRP and AK showed the highest susceptibility against the tested *E. coli* isolates with percentages of 78.94% and 51.8% respectively, while AMC and CTX exhibited the highest resistance rate with percentages of 68.4 % and 42.1 % respectively. On the other side, in a previous study conducted in the USA [23], *E. coli* isolates showed the highest susceptibility to MRP and AK with percentages of 98.5% and 99.93%, respectively, while showed the highest antimicrobial resistance against AMC, CTX with percentages of 40% and 12%, respectively, which is to some extent in accordance with our results. Given the current results, the highest susceptibility rates of AK and MRP might be attributed to the careful selection of these antibiotics for therapy, which maintain the antibiotic's sensitivity, while the rising resistance rate of AMC and CTX may be associated with their extensive empirical usage and ineffective implementation of infection control measures [24], [25]. Considering the geographic locations from where the specimens were obtained, they may have an impact on the distribution of *E. coli* isolates in various investigations [16].

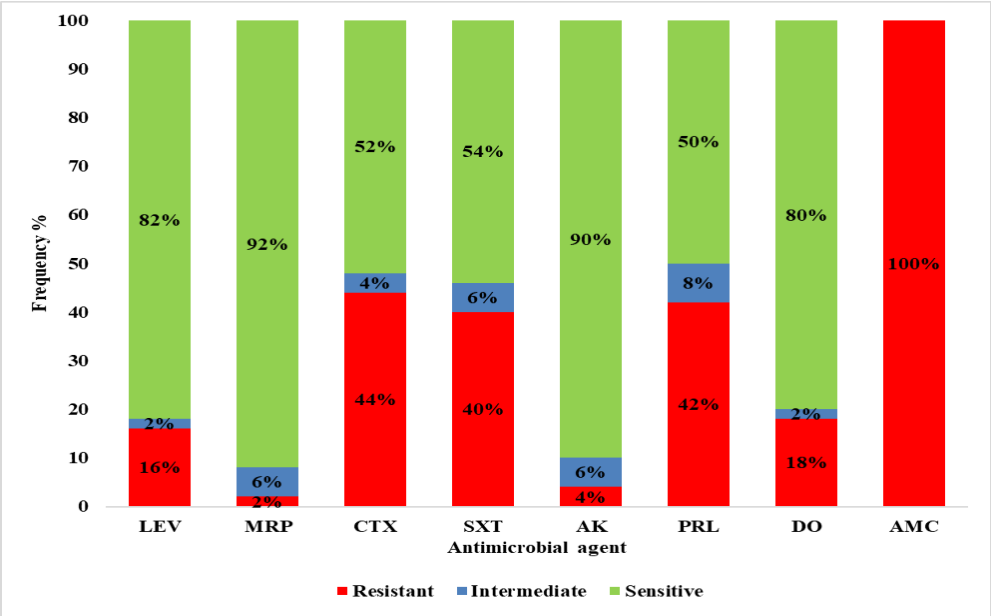

Figure 2: Frequency of antimicrobials susceptibility of overall *E. coli* isolates

According to specimens' type, the 35 *E. coli* isolates from urine specimens were shown to have the highest susceptibility to MRP and AK 32/35 (91.43%), while the highest resistance of urine isolates of *E. coli* was against AMC 35/35 (100%), followed by CTX 18/35 (51.43%), the full results are shown in Fig. 3. Similarly, in previous reports in Egypt [26] and globally [27], AK and MRP exhibited highest susceptibility rates against *E. coli* isolates from urine specimens with percentages of (72.4%, and 96.5%) in Egypt, and (95%, and 100%) globally, respectively. Earlier studies also reported that *E. coli* isolates from urine were highly resistant against AMC and CTX with a percentage of (82.5 %, and 97.5%) in Egypt [28] and (25.2%-8.1%) globally [29].

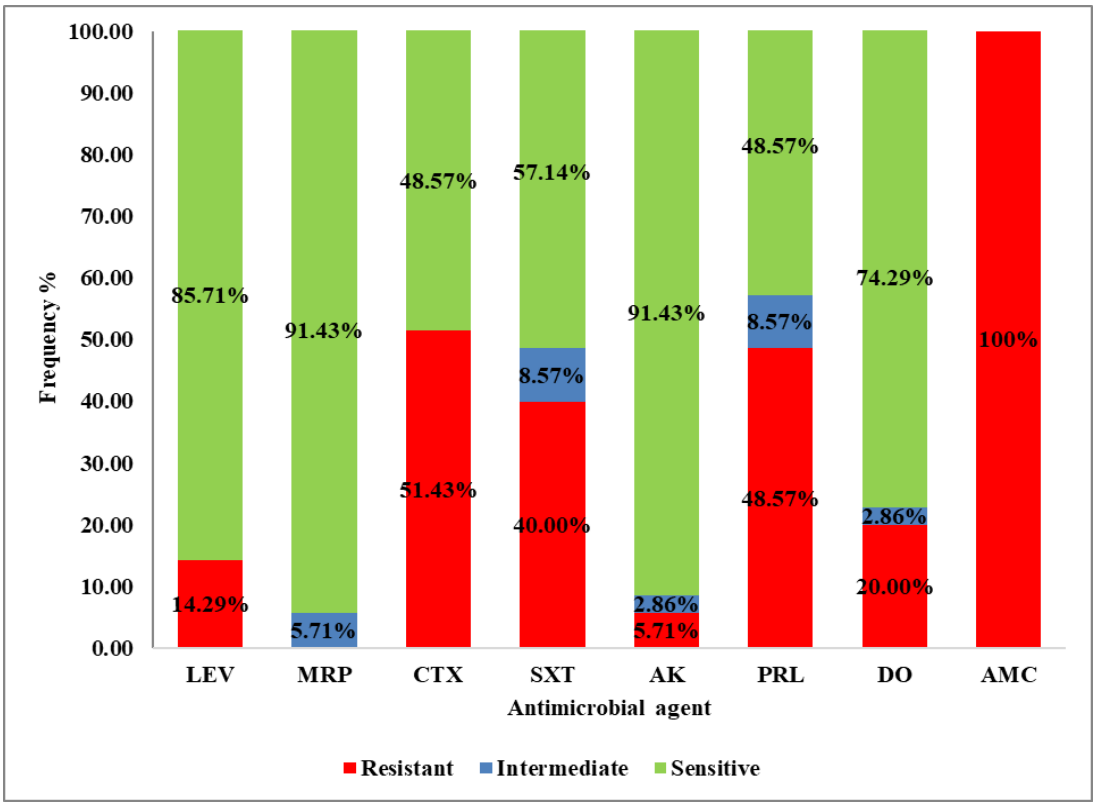

Figure 3: Frequency of antimicrobials susceptibility of *E. coli* strains isolated from urine specimens.

On the other hand, the 6 stool isolates of *E. coli* had the maximum susceptibility to both MRP and AK 5/6 (83.33%), each, whereas the most resistance was against AMC 6/6 (100%) followed by CTX 3/6 (50%), each, (Fig. 4). In accordance with the current results, in previous reports conducted in Egypt [30] and globally [31], *E. coli* isolates from stool specimens showed the highest susceptibility to MRP, AK with percentages of (89%, and 97.3%) in Egypt and (61%, and 89.7%) globally, respectively. Furthermore, previous studies also reported that *E. coli* isolates from stool were highly resistant to AMC with a percentage of 100% in Egypt [32], and 22% globally [33], while other reports exhibited that *E. coli* isolates showed high resistance against LEV and DO (18%, and 37%) respectively [34] in Egypt and (33%, and 29%) respectively, globally [35]. Notably, in the stool specimens, the DO and LEV revealed the highest resistance following AMC against the tested isolates may be because they are the drug of choice for diarrheal infections by *E. coli* leading to extensive use and a raise of resistance against these antimicrobial agents [36], [37]. Likewise, starting an empirical antibiotic therapy without performing a microbiological test such as the antimicrobial susceptibility test is common practice in many countries. Therefore, antimicrobial susceptibility test of *E. coli* infections must be used to provide recommendations for empirical therapy [29].

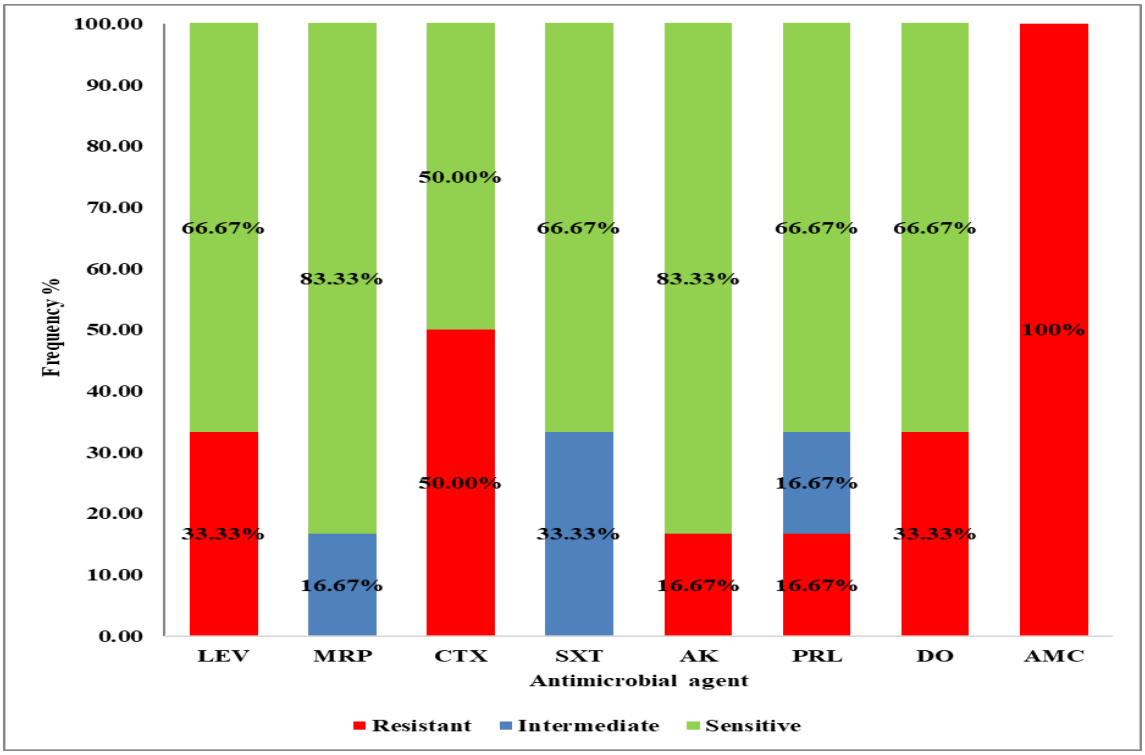

Figure 4: Frequency of antimicrobials susceptibility of *E. coli* strains isolated from stool specimens.

Moreover, the 9 isolates from blood specimens revealed the maximum susceptibility to MRP 8/9 (88.89%) followed by AK, LEV, and DO 7/9 (77.78%), while the highest resistance of blood specimens was found against AMC 9/9 (100%) followed by CTX 3/9 (33.33%) as shown in Fig 5. Matching with our results, in previous researches in Egypt [38], [39] and globally [40], MRP, AK, LEV, and DO show the highest sensitivity rates against the *E. coli* isolates from blood specimens with a percentage of (50.5 %, 81.5 %, 34.4 %, and 77.5%), respectively, in Egypt and (100 %, 71.7 %, 93.4 %, and 23%), respectively, globally. In addition, previous studies reported that *E. coli* isolates from blood were highly resistant to AMC and CTX with a percentage of (97.5%, and 90%) in Egypt [41], and (71.6%, and 62.2%) globally [42] respectively. Regarding the current results, the highest susceptibility rates of LEV and DO might be attributed to the infrequent use of these antibiotics for the therapy of blood infections. Moreover, the variation in antibiotic resistance patterns between different studies may be due to various diseases and specimen types, risk factors, the location of hospitals, and infection control procedures applied in different countries [24], [25].

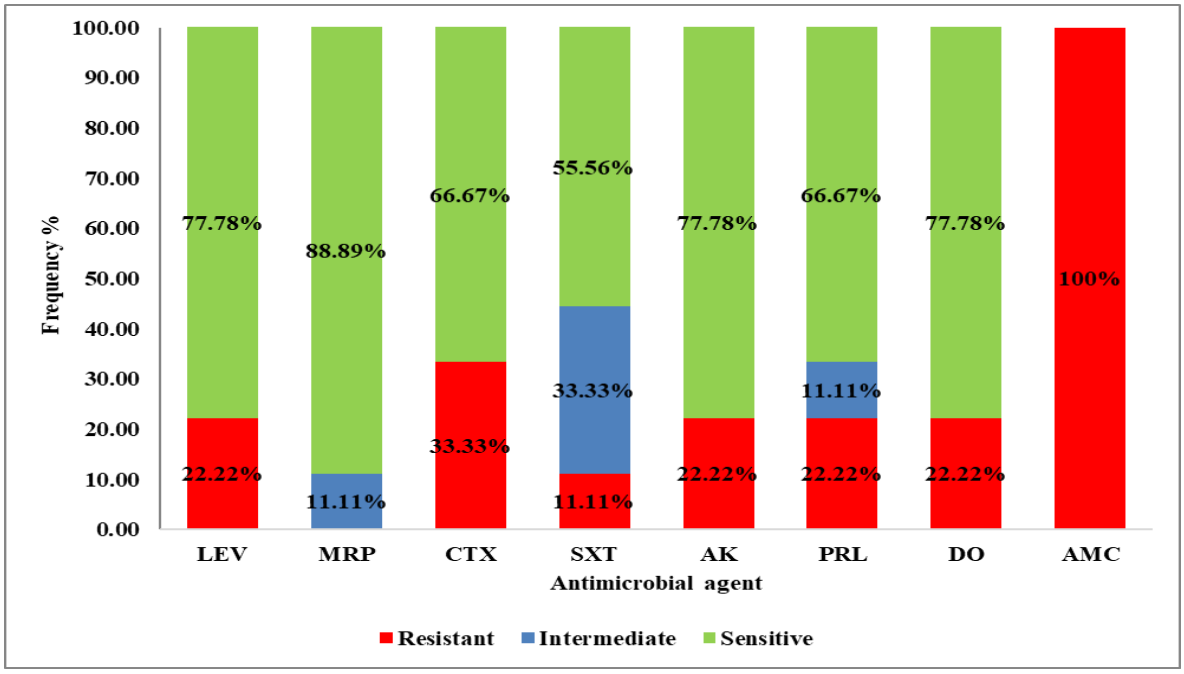

Figure 5: Frequency of antimicrobials susceptibility of *E. coli* strains isolated from blood specimens.

Another key point, as shown in Table 4, is the resistance patterns of the isolates in the current study which revealed that 33/50 (66%) of the isolates are MDR and this is attributed to an inadequate commitment to infection control policies and unnecessary usage of antimicrobials [24].

Importantly, the MDR rate in this research was 66% which is comparable to rates previously reported in Egypt, which varied from 20 to 96.07% [43],[44],[45],[46]. Conversely, other European studies exhibited lower MDR rates; 4.8% in Germany [47], 14% in the USA [48], 37.6% in France [49], and 30% in Canada [50]. Considering comparison to other nations, Egypt has a higher MDR rate, which alerts us to the need to implement strict antibiotic-prescribing strategies.

Table 4: MDR *E. coli* isolates' resistance profile

Antibiotics / Isolates	LEV	MRP	CTX	SXT	AK	PRL	DO	AMC
E1	R	S	R	R	S	R	S	R
E2	S	S	R	R	S	R	S	R
E3	R	S	R	R	S	R	S	R
E4	I	S	R	R	S	R	S	R
E5	S	S	S	R	S	R	S	R
E6	S	S	S	R	S	R	S	R
E7	S	S	R	S	S	S	S	R
E8	R	S	R	R	S	R	S	R
E9	S	S	R	R	S	R	S	R
E10	S	S	S	S	S	R	S	R

Continued, MDR *E. coli* isolates' resistance profile

E11	S	S	S	S	S	S	S	R
E12	S	S	R	R	S	R	S	R
E13	S	S	R	R	S	R	R	R
E14	S	R	S	S	S	S	S	R
E15	S	S	R	S	S	S	S	R
E16	S	S	S	S	S	R	S	R
E17	S	S	R	S	S	S	I	R
E18	S	S	R	R	S	R	S	R
E19	S	S	S	R	S	I	R	R
E20	S	S	R	S	S	R	S	R
E21	S	S	R	R	S	S	R	R
E22	S	S	R	R	S	R	R	R
E23	S	S	R	R	S	I	R	R
E24	S	S	S	S	S	R	S	R
E25	S	S	S	S	S	S	S	R
E26	R	I	R	R	S	R	S	R
E27	S	S	S	S	S	S	S	R
E28	S	S	R	S	S	S	S	R
E29	R	S	R	R	S	R	S	R
E30	S	S	S	S	S	R	S	R
E31	S	S	S	S	S	S	S	R
E32	S	S	R	S	S	S	S	R
E33	S	S	R	I	S	S	S	R
E34	S	I	R	S	S	S	S	R
E35	R	S	S	S	S	R	R	R
E36	R	S	S	I	R	I	R	R
E37	S	S	S	S	S	R	S	R
E38	S	S	S	I	R	S	S	R
E39	S	S	S	R	S	S	S	R
E40	S	S	S	S	I	S	S	R
E41	S	S	S	S	S	S	R	R
E42	S	S	I	S	S	S	S	R
E43	S	S	S	S	S	S	S	R
E44	S	S	S	R	S	S	S	R
E45	S	S	S	S	S	S	S	R
E46	S	I	I	S	S	S	S	R
E47	S	S	S	S	S	I	S	R
E48	S	S	S	R	S	S	R	R
E49	S	S	S	S	I	S	S	R
E50	R	S	S	S	I	S	S	R

4. CONCLUSION

Given the current findings, the current article presents the most recent information on the AMR profile of *E. coli* to spotlight on implementation of strict antibiotic-prescribing strategies. Therefore, we may conclude that some of the existing antibiotics such as MRP and AK are still capable of successfully

treating *E. coli* infections, despite the emergence of MDR strains that are challenging to treat. To begin a successful therapy, it is crucial to determine the antimicrobial sensitivity and resistance profile of *E. coli* isolates, particularly in cases of MDR infections. Furthermore, antibiotic resistance must be monitored continuously and frequently to prevent it from rising. Although carbapenems appear to be the most effective antibiotic, they must be used carefully, especially in developing countries like Egypt where antibiotics are accessible without prescriptions.

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Data availability: Data is available within the article.

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