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The Emergence of Multidrug-Resistant (MDR), Extensively Drug-Resistant (XDR), and Pandrug-Resistant (PDR) In Iraqi Clinical Isolates of Escherichia coli

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ABSTRACT

Antimicrobial-resistant bacteria consider one of the world's most pressing health issues. One of the most significant bacteria that can show multiple antimicrobial resistance patterns e.g., Resistance to many drugs, including XDR and PDR strains (PDR) is Escherichia coli, which, has been shown to increase in the rates of resistance to different categories of antimicrobial agents. The current study aims to investigate the emergence of antimicrobial resistance patterns in the clinical isolates of E.coli among patients in Baghdad city. The results showed that out of 500 different clinical specimens, 113 (22.60%) isolates were identified as E.coli, and the percentage of E.coli isolation in females (54.87%) when compared to men (45.13%). More than half (51.33%) of the isolates obtained came from urine. Patients younger than 20 years old accounted for 33% of all E. coli samples taken. Most E. coli isolates revealed a pattern of strong resistance to the majority of the commonly used antibiotics, as determined by antimicrobial susceptibility testing. Piperacillin, amoxicillin, ceftazidime, ceftriaxone, cefepime, ciprofloxacin, azithromycin, and tetracycline were all antibiotics that the isolates exhibited extreme resistance. and the percentage of resistance were 96.46%, 95.58%, 92.92%, 92.04%, 90.27%, 84.96%, 84.07%, 82.30%, and 80.53% respectively. However, most E.coli isolates were highly sensitive to Meropenem (82.30%), followed by Piperacillin-tazobactam and Imipenem which both had susceptible rates reach to 69.91%. Most E.coli isolates (98.23%) were classified as MDR, 21.24% were classified as XDR, and 1.77% were classified as possibly PDR. Concludingly, most E.coli isolates were MDR, additionally, XDR and possibly PDR have been emerged. Which revealed a severe and critical health problem that could threaten the community and healthcare settings.

Keywords: *Antimicrobial-resistant bacteria, Escherichia coli, Extensively drug-resistant (XDR), Multidrug-resistant (MDR), Pandrug-resistant (PDR).*

INTRODUCTION

Escherichia coli is a kind of rod-shaped, Gram-negative bacterium in the Enterobacteriaceae family and it is commonly inhabited human and animals intestinal tract, and considered one of the main normal flora of the guts1,2,3,4. E.coli strains are harmless but some of them can cause a series infections and diseases5,6. Pathogenic strains of Many types of infections outside the intestines may be brought on by E. coli, particularly in immune-compromised patients7.

Regarding intestinal infections, E.coli is considered a main cause of water and foodborne diarrhea all around the world, particularly in developing countries, which the reason of deaths for children below five years old 2, 3, 8, 9. Intestinal infections involve diarrhea or dysentery caused by six pathogenic strains of E.coli; Enteropathogenic E.coli (EPEC), Enteroinvasive E.coli (EIEC), Enteroaggregative E.coli (EAEC), Enterotoxigenic E.coli (ETEC), Enterohemorrhagic E.coli (EHEC) [Verocytotoxigenic E.coli (VTEC) or E. coli that produces Shiga toxins (STEC)], and Diffusely adhering E.coli (DAEC) 5, 10. While extraintestinal infections of E. coli include, urinary tract infection, wound infections, bloodstream infections, meningitis, otitis media, and pneumonia. Additionally, E.coli consider the major cause of nosocomial infections 2, 3, 7.The prevalence of antimicrobial drug-resistant bacteria become one of the major health problems worldwide because these bacteria can lead to the limitation in efficient antimicrobial agents that can be used to treat the infections and diseases caused by these pathogens 5 ,11,12. The infection and diseases caused by antimicrobial drug-resistant pathogens led to the increase the morbidity and mortality in health-care settings5, 8,13. As this problem increased dramatically, standardized definitions to explain and categorize bacteria that are resistant to many classes of antimicrobial agents are required, to facilitate collected epidemiological data and compared in a reliable mannor across countries11. Multidrug-resistant (MDR) is defined as “non-susceptibility to at least one agent in three or more antimicrobial categories”. Moreover, extensively drug-resistant (XDR) is defined as “non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories)”. While, Pandrug-resistant (PDR) is defined as “non-susceptibility to all agents in all antimicrobial categories (i.e., no agents tested as susceptible for that organism)” 11,14. One of the most significant bacteria that can show multiple antimicrobial resistance patterns, e.g., MDR, XDR, and PDR are E. coli, which, has been shown increase in rates of resistant to different categories of antimicrobial agents 8,13,15. E. coli is one of "the key priority antibiotic-resistant microorganisms," according to the World Health Organization (WHO). Furthermore, MDR E. coli has been identified as a top pathogen 7,16,17. From this vantage point, the current study seeks to learn more about the development of MDR, XDR, and PDR in E. coli bacteria isolated from hospitalized patients in Baghdad.

MATERIAL AND METHODS

Collection of clinical specimens

From November 2020 to March 2021 about 500 clinical specimens were gathered from Baghdad, Iraq, hospital patients (Teaching Laboratories in Baghdad Medical City, Hospital for Surgical Specialties at the Ghazi Al-Hariri Teaching Hospital in Baghdad, and Al Elwya Teaching Hospital for Children). The clinical specimens included; urine, stool, blood, wound swabs, ear swabs, pus, abscess, sputum, and body fluids (e.g., ascitic fluid, intrabdominal fluid, and CSF).

Isolation and identification of bacterial isolates

The clinical specimens were transported to the laboratory and cultured on plates of MacConkey agar (Liofilchem, Italy) which were used to first separate E. coli bacteria, followed by an incubation period during which the plates at 37ºC for 24h. The E.coli isolates appear smooth, round, and lactose fermented colonies on MacConkey agar. For identification of the bacterial isolates, a single isolated colony was transferred to EMB agar (Liofilchem, Italy) the E.coli isolates showed metallic green sheen on EMB agar. Additionally, biochemical tests such as IMViC test, oxidase and catalase assays were used to verify the presence of E. coli in the isolated bacteria.

Molecular Detection of E.coli

The uidA gene, which codes for the -D-glucuronidase enzyme, was the target of a polymerase chain reaction (PCR) used to verify the identity of E. coli isolates. Firstly, the genomic DNA (gDNA) from each bacterial isolate was extracted using ABIOpureTM Total DNA kit (ABIOpure, USA). Then the PCR reaction was performed using the universal

primers: (the forward primer 5´-ATCACCGTGGTGACGCATGTCGC-3´ and the reverse primer 5´-CACCACGATGCCATGTTCATCTGC-3´) for amplifying the uidA gene fragment with 486bp amplicon size 9. Each 20 µl of the PCR reaction mixture contained; 10µl of green master mix (Promega, USA), 1µM of both primer in both directions, 3 µl of gDNA (the template), in addition to 5 µl of water without nucleases (Promega, USA). The following describes the settings of the thermal cycler: The annealing temperature was 50 degrees Celsius, while the denaturation temperatures ranged from 95 to 5 minutes., During 30 seconds, the temperature was set to 72 degrees Celsius, and for 7 minutes, the temperature was maintained at 72 degrees Celsius. Finally, PCR amplification was verified by running a sample through 1.5% agarose and 10mg/ml ethidium bromide solution on an agarose gel electrophoresis apparatus. (Promega, USA).

Antimicrobial susceptibility test

All E. coli isolates were subjected to an antibiotic susceptibility test using the Kirby-Bauer disk diffusion technique on Muller-Hinton agar (Oxoid, UK) following CLSI standards 18. The test was performed using twenty antimicrobial agents that fall within thirteen different antimicrobial categories. The antimicrobial agents include; Piperacillin-tazobactam (100/10µg), Ampicillin (10µg), Piperacillin (100µg), Cefotaxime (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefepime (30µg), Cefoxitin (30µg), Imipenem (10µg), Meropenem (10µg), Aztreonam (30µg), Gentamicin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Tetracycline (30µg), Azithromycin (15µg), Chloramphenicol (30µg), Trimethoprim-sulfamethoxazole (1.25/23.75µg), and Nitrofurantoin (300µg). The antimicrobial disks were provided by Bioanalyse, Turkey.

Statistical analysis

Graph Pad Prism, in its fifth iteration, was used for the statistical analysis. Percentages were utilized for comparing research samples. During the data analysis, Chi-square was used to compare the categorical variables. All of the data was compared using the One-way ANOVA (Kruskal-Wallis test) and the Paired t-Test. The level of statistical significance used in all analyses was 5%. *p 0.05; **p 0.01; ***p 0.001 are significant at the 0.05 level or below in the posttest.

Ethical approval

The College of Science Research Ethics Committee provided its permission for this study at the University of Baghdad. Patients also filled out consent forms for specimen collection.

RESULTS AND DISCUSSION

Specimens collection

From November 2020 to March 2021 (5 months) about 500 clinical specimens were collected from hospitalized patients in Baghdad city (Teaching Laboratories in Baghdad Medical City, Baghdad Teaching Hospital, Ghazi Al-Hariri Surgical Specialties Hospital, and Al Elwya Teaching Hospital for Children). The clinical specimens were collected from different patients with different age groups, included different source of infections: urine, stool, blood, wound swabs, ear swabs, pus, abscess, sputum, and body fluids (e.g., ascitic fluid, intrabdominal fluid, and CSF). The results showed that out of 500 different clinical specimens, 113 (22.60%) isolates were identified as E.coli while 387 (77.40%) belonged to the other bacterial species (Fig. 1). This result was close to the result of a study conducted by Kibret and Abera 8 who found that 14.2% of bacterial isolates that collected from various clinical samples belong to the E.coli. However, the results of AL-khazraji 19 found that the percentage of E.coli isolates was 49% of the total number of clinical specimens, which disagreed with the current study's results. The difference in the isolation ratio may be attributed to the difference in the size and types of original clinical specimens, in addition to the difference in time and geographic area of the specimens' collection.

Cultural and biochemical tests for identification of E.coli

MacConkey agar, used for primary isolation of E. coli bacteria, was used to culture the clinical specimens, and the plates were incubated at 37oC for 24 hours. The E.coli isolates appeared as smooth, round, and lactose fermented colonies on MacConkey agar 20. To confirm bacterial isolates, a single isolated colony was transferred to EMB agar, the E.coli isolates on EMB agar 21, it glistened with a shiny green color. To be sure, we ran certain biochemical tests like the IMViC (Indole, Methyl Red, Voges-Proskauer, and Citrate) test, some oxidase tests, and some catalase tests to be sure of the E.coli that thrive in MacConkey and EMB agar. Bergey's Manual 22 was used for all of the biochemical analyses. All 113 E. coli isolates passed the Indole and Methyl Red tests but failed the Voges-Proskauer and Citrate tests (biochemical assays). In addition, catalase tests were positive in every single isolation and negative for oxidase test. These results were typical for identification of E.coli bacteria 21.

Molecular Detection of E.coli by PCR

Phenotypic identification of the collected isolates was genetically confirmed by focusing on the -D-glucuronidase encoding uidA gene. The uidA gene is one of E. coli's eight housekeeping genes (the others being dinB, icdA, pabB, polB, putP, trpA, trpB, and uidA). The uidA gene is often employed for the definitive identification of E. coli (species-specific gene) 23,24. All primary isolates (n=113) were verified by polymerase chain reaction as E.coli by cultural and biochemical tests have been possessing the uidA gene (Fig. 2). This result agreed with the result of Alsanjary and Sheet 23, who found that every positive E.coli isolate obtained using traditional methods contained the species-specific gene uidA. As a result, the outcomes of traditional methods (biochemical tests) and the outcomes of the PCR test have been consistent. Consequently, the PCR assay could be considering a new approach utilized to verify that all isolates discovered by conventional methods were E.coli.

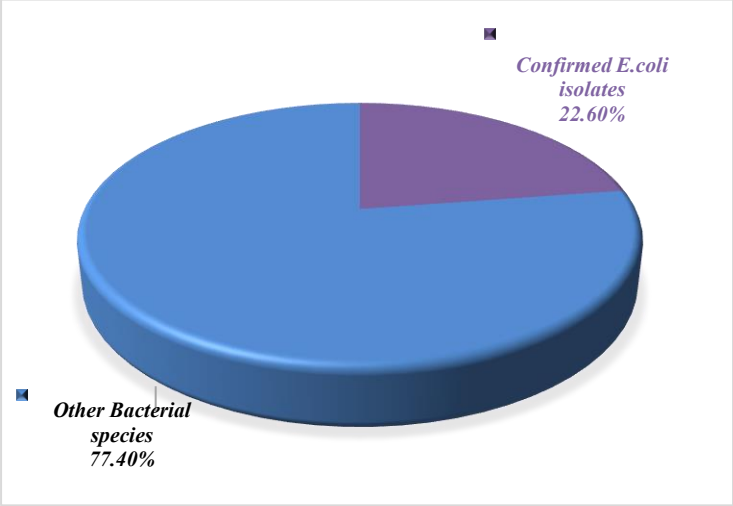

FIGURE 1: The percentage of E.coli bacteria isolated from total of 500 different clinical specimens

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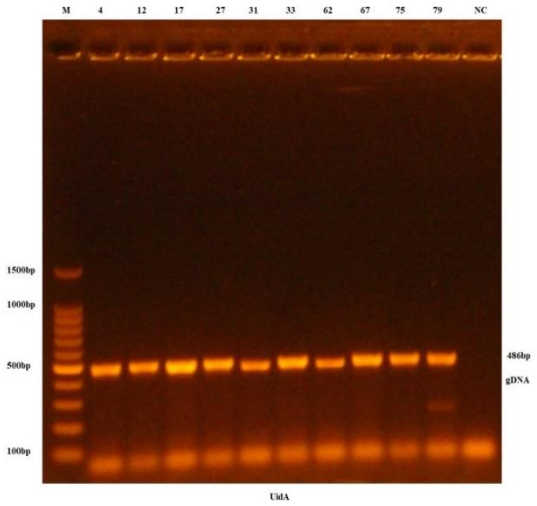

FIGURE 2: Gel electrophoresis of amplification UidA gene on 1.5% agarose stained with Ethidium Bromide, M: 100bp ladder marker, Lane 4-79: product size 486bp, NC: Negative Control .

Distribution of E.coli Across Various Clinical Specimens

The results of E.coli isolation showed that E.coli isolates were distributed differently among various sources of infections. Additionally, the detected isolates were distinguished in a different ratio according to both gender and age groups of patients. The results showed (Table 1) that from total 113 confirmed E.coli isolates, 62 (54.87%) isolates were collected from females, while 51 (45.13%) isolates were collected from males (Fig. 3). This result indicates that the percentage of E.coli isolation in females was higher than in males, with no significant differences (P< 0.9), this is may be due to that females are more susceptible to infections than males specially urine tract infections (UTI). Similar result was obtained from the study of AL-khazraji 19 who found that females (53.8%) had a higher percentage of E.coli than males (46.2%). Additionally, the result was agreed with the study conducted by Naqid et al. 2 who observed that females were more likely to have E.coli bacteria (70.7%) than males (29.3%). Moreover, The results showed (Table 1) that out of the total 113 E.coli isolates, 58 (51.33%) isolates were collected from urine samples with higher frequency of females (63.79%) than males (36.21%) with significant differences (P<0.03). This result indicated that the highest rate of E.coli bacteria was isolated from urine samples and females had a higher infection rate with UTIs in comparison to males. This result agreed with many studies that showed a high frequency of E.coli isolates in urine samples in comparison to other clinical samples, for instance, Kibret and Abera 8 found that urine samples represent the highest isolation rate of E.coli (45.5%). However, Naqid et al.2 revealed that the vast majority of the E.coli isolates were collected from urine, but the rate of isolation was 92.2%, which is higher than the rate of the current study. Nevertheless, the result of the current study was similar to the study conducted by Naqid et al.2, who found that in urine sample, female had high frequency of E.coli isolates (73.9%) than males (26.1%). Moreover, the result of the current study agreed with the finding of Assafi et al. 1, which indicated that the rate of E.coli isolation was higher in females (21.4%) than males (18.5%), but statistically not significant. Many other studies demonstrated that E.coli was more common in UTIs in females 3,25,26. The high incidence of E.coli in females can be attributed to a variety of factors, including the shorter urethra in females, which reduces the distance that bacteria must travel to reach the bladder. Additionally, a change in the vaginal microbiota may significantly contribute to the promotion of coliform colonization of the vagina, which is connected to UTIs. Additionally, the amount of bacteria that enters the bladder increases during

sexual activity. Poor hygiene and low socioeconomic level increase these UTI-causing variables 2,3,25 . Table 1 and Fig. 4 showed that the rate of E.coli bacteria from stool came after the urine samples with 24 of 113 (21.24%) E.coli isolates, however, the E.coli isolated from stool was higher in males (54.17%) than females (45.83%), but the difference was not significant. Wound infections and pus come after urine and stool samples in 11.50% and 7.08% respectively with no distinct difference between males and females. Additionally, E.coli bacteria were collected from other sources of infections such as; body fluids [ascitic fluids, intrabdominal fluids] (2.65%), sputum (2.65%), blood (1.77%), and finally both ear swabs and CSF (1%) which represented the lowest rate of collection, without any significant variation between males and females. Fig. 4 demonstrated the distribution of E.coli bacteria among various clinical specimens in general, while Fig. 5 showed the distribution of E.coli isolates across various clinical specimens according to gender.

TABLE 1: Distribution of E. coli among various clinical specimens according to gender

Source of clinical specimens	Male No. (%)	Female No. (%)	Total No. (%)	P value
Urine	21 (36.21%)	37 (63.79%)	58 (51.33%)	0.03
Stool	13 (54.17%)	11 (45.83%)	24 (21.24%)	0.05
Wound swabs	7 (53.85%)	6 (46.15%)	13 (11.50%)	0.07
Pus, Abscess	4 (50.00%)	4 (50.00%)	8 (7.08%)	-
Body fluids	1 (33.33%)	2 (66.67%)	3 (2.65%)	0.2
Sputum	2 (66.67%)	1 (33.33%)	3 (2.65%)	0.2
Blood	2 (100%)	0 (0.00%)	2 (1.77%)	0.9
Ear swab	1 (100%)	0 (0.00%)	1 (1%)	0.8
CSF	0 (0.00%)	1 (100%)	1 (1%)	0.8
Total	51 (45.13%)	62 (54.87%)	113 (100%)	0.9

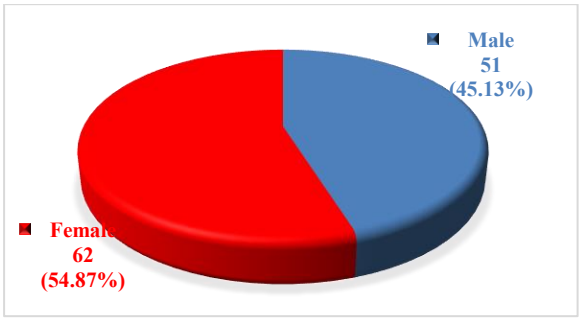

FIGURE 3: The percentage of E.coli isolates according to gender

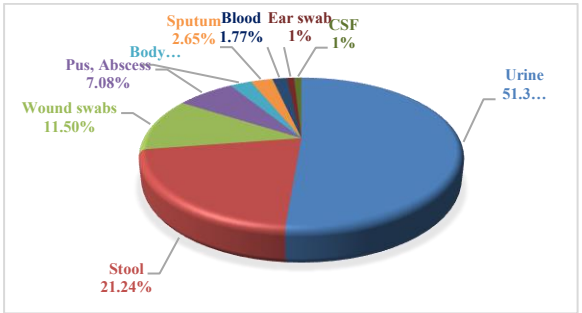

FIGURE 4: Distribution of E.coli isolates among various clinical specimens

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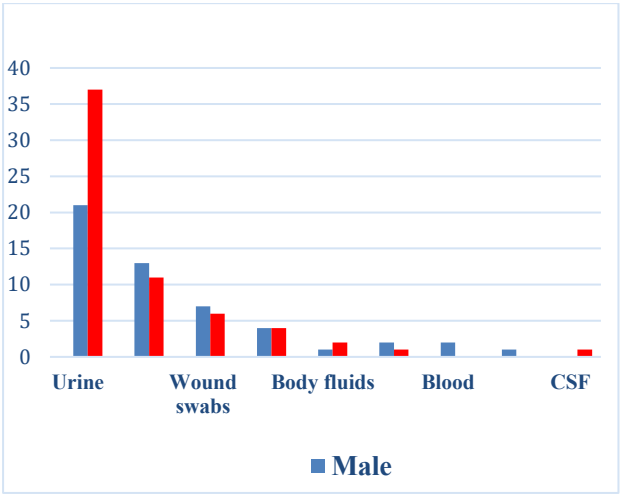

FIGURE 5: Distribution of E. coli among various clinical specimens according to gender

Tables 2 and 3 shown how E. coli bacterial isolates were distributed throughout patients of varying ages and sexes. Women outnumbered men, although the difference was not statistically significant (P=0.3), and patients less than 20 years old (mainly children) had the greatest percentage of E. coli bacteria collected (32.74%).Whereas, the lowest rate of E.coli bacteria (19.47%) was collected from the age group more than 60 years (elderly or aging patients) with no different between males and females. The patients in the age group 20-40 (adults) had the percentage of isolation reach to the 25.66% followed by the patients in the age group 41-60 with 22.12%. This result agreed with the study conducted by Odongo et al.25, who observed that, when compared to other age groups, the prevalence of E. coli isolates was greater in the group of people under the age of 17. These results can be explained by the immunological status of the children (children have an immune system which still not fully maturation), which make children more vulnerable to infectious agents including E.coli bacteria than adults. Additionally, children are more exposed to a greater number of infectious agents than adults. Furthermore, malnourished children, and living of children in poor sanitation 25,27. (Fig. 6), (Fig. 7).

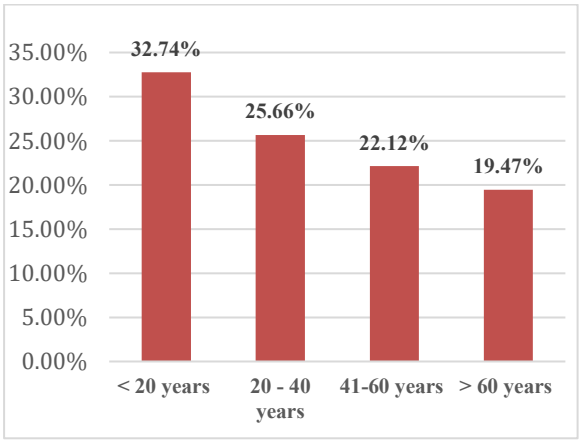

FIGURE 6: Distribution of E.coli isolates according to age groups of patients

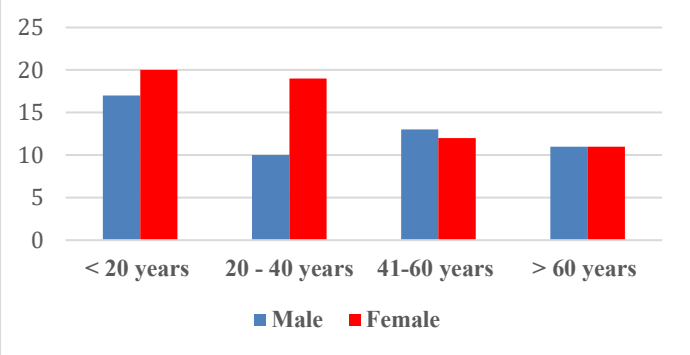

FIGURE 7: Distribution of E.coli isolates according to age groups and gender of patients

TABLE 2: Distribution of E.coli bacterial isolates according to age groups of patients

	Age groups				**P value**	**Total**
	< 20 years	20 - 40 years	41-60 years	> 60 years		
Bacterial isolates	37	29	25	22	0.4	113
	32.74%	25.66%	22.12%	19.47%		100%

TABLE 3: Distribution of E.coli bacterial isolates according to age groups and gender of patients

Gender	**Age groups**				**Total**
	< 20 years	20 - 40 years	41-60 years	> 60 years	
Male	17 (45.95%)	10 (34.48%)	13 (52.00%)	11 (50.00%)	51 (45.13%)
Female	20 (54.05%)	19 (65.52%)	12 (48.00%)	11 (50.00%)	62 (54.87%)
Total	37	29	25	22	113 (100%)
P value	<0.3	<0.05	<0.4	-	

Antimicrobial susceptibility test

Each of the 113 E. coli isolates was put through an antibiotic susceptibility test on Muller-Hinton agar using the Kirby-Bauer disk diffusion technique and the standards set out by the Clinical and Laboratory Standards Institute the CLSI guidelines18, 21. The test was performed using twenty (20) antimicrobial agents that fall within thirteen (13) different antimicrobial categories or classes. The results (Table 4) and (Fig. 8) showed that most E.coli bacteria demonstrated high resistance to Piperacillin, Ampicillin, Cefotaxime, Ceftazidime, Ceftriaxone, and the percentage of resistance were 96.46%, 95.58%, 92.92%, 92.04%, and 90.27% respectively. Additionally, the isolates showed high resistance to Cefepime, Ciprofloxacin, Levofloxacin, Azithromycin, and Tetracycline with resistance rates reach to 84.96%, 84.07%, 84.07, 82.30%, and 80.53% respectively. While the resistance of isolates against Trimethoprim-sulfamethoxazole, Aztreonam, and Nitrofurantoin was 76.11% 70.80%, and 60.18% respectively. However the findings revealed that the vast majority of isolates were highly susceptible to Meropenem (82.30%), which could be described as the drug of choice, followed by Piperacillin-tazobactam and Imipenem which both had susceptible rates reached to 69.91%. Moreover, 62.83% of isolates were susceptible to Gentamicin, and Chloramphenicol and finally, results showed that 61.95% Cefoxitin was effective against of the isolates tested. These findings corroborated those of a research by AL-khazraji19, who also showed that most E. coli isolates gathered from various infection sources had great resistance to the commonly used antibiotics Nitrofurantoin, Amoxicillin, Ceftriaxone Ceftazidime, and Cefixime. Whereas, the highest sensitivity rate

was observed against Imipenem, Meropenem, and Amikacin. Additionally, Naqid et al.2 indicated that E.coli bacteria were resistant to Ampicillin, Cefepime and Ceftriaxone, while, sensitive to Nitrofurantoin, Imipenem, and Ertapenem. Moreover, according to the review conducted by O.M. Al-Dahmoshi et al.7, who gathered data from about 100 studies in Iraq regarding antimicrobial-resistant E.coli, the study exposed that Cefotaxime and Ceftriaxone had the highest resistance rates with 75.9% and 76.5% respectively, followed by Gentamycin (41.65%) and Ciprofloxacin (32.13%), while, Amikacin, Levofloxacin and Imipenem had the lowest resistance rate by 17.3%, 15%, and 5.14% respectively. Many other studies showed similar results with little variation in the percentage of susceptibility3,7,25,26. Nevertheless, differences and variations in the rate of resistance and sensitivity among these studies and the current study were observed, Naqid et al. 2 mentioned that the period between studies, population fluctuations, and dramatic variations in sample sizes and types could all be responsible for variations in the susceptibility pattern containing antimicrobials. Antibiotic resistance in E. coli has risen worldwide, and there is substantial heterogeneity in susceptibility patterns across different regions, populations, and ecological settings. Moreover, many studies carried out in many regions of the world had indicated rises in rates of resistance to various antimicrobial agents 8. Assafi et al.1 stated that E.coli has been found to have high antimicrobial resistance rates against the majority of β-lactamase drugs. More than 90% of isolates were found to be resistant to both recently introduced drugs like Amoxicillin-Clavulanic acid, Cephalexin, and Ceftazidime as well as commonly used antibiotics like Ampicillin, Ampicillin-Cloxacillin, and Cloxacillin. Approximately, 80–90% of isolates were also resistant to the other β-lactamase drugs, such as Amoxicillin, Ceftazidime, Ceftriaxone, Cefixime, Cefotaxime, and Nalidixic acid. Beside the β-lactamase agents, most tested isolates (90%) were also highly resistant to Erythromycin and Clindamycin. However, the high sensitivity to both Imipenem and Meropenem was observed in the E.coli isolates. One of the most important factors that cause resistance to β-lactams antibiotics (Penicillins and Cephalosporins) is production of β-lactamase, especially, ESBLs 28. Most of the ESBLs' E.coli are resistant to a wide range of β-lactams antibiotics, and non β-lactams including Fluoroquinolones, Trimethoprim and Gentamycin26,29. Additionally, Mohammad13 established that high resistance rate to β-lactams antibiotics maybe as a result of the prolonged and widespread usage of these antibiotics on a global scale. In recent years, Fluoroquinolones, particularly Ciprofloxacin, have been utilized to treat E.coli infections. The present study found that E.coli isolates showed a high resistance rate for both Ciprofloxacin and Levofloxacin (84.07%), which is consistent with the previous studies13,25,26. However, this result disagreed with the study of Kibret and Abera 8 which demonstrated high degree of sensitivity rates to Ciprofloxacin. These results reveled that through 10 years, E.coli isolates developed resistance to Ciprofloxacin, which suggested a serious health problems. Ciprofloxacin has always been used in the treatment of UTIs. The extensive use of Fluoroquinolone particularly Ciprofloxacin can contribute to the increase in Fluoroquinolone resistance. Likewise, Fluoroquinolones are widely accessible and relatively inexpensive. This might have made them more available to the patients, raising the risk that they would abuse and overuse them and developing resistance1,25. Furthermore, the current study showed rise the resistance rate of Nitrofurantoin to reach 60.18%, which disagreed with previous study of Mohammad13 who found that Nitrofurantoin was the most successful drugs against E.coli with a sensitivity rate of 90.4%. Nitrofurantoin resistance may have been triggered by the widespread misuse of antibiotics. Persister cells, which are metabolically dormant and do not develop or die when exposed to bactericidal doses of antibiotics, are another major roadblock. Treatment failure, recurrence, and persistent infections are often associated with these cells. because they continue to reproduce even after antibiotic therapy has been stopped. It can also be as a result of the inexpensive prices and easily accessible to this medication25. Moreover, the present study showed higher sensitivity of E.coli

isolates to Meropenem, Piperacillin-tazobactam, and Imipenem which agreed with many other studies7,13,25, 26. This can be explained by that these drugs are designed to be effective against the β-lactamase producing strain, which can overcome the resistance by this mechanism. In addition, the combination of Piperacillin and β-lactamase inhibitor (Tazobactam), are difficult for the organism to resist, Tazobactam targets the β-lactamase enzyme that causes resistance to β-lactam antibiotics. One of the most frequent bacterial pathogens that causing infection is E.coli. E. coli strains that have developed resistance to many antibiotics remain a major public health threat across the world and can result in serious health issues like longer hospital stays and failed treatment 2. E.coli is becoming increasingly resistant to routinely prescribed antimicrobial medications for a variety of reasons, including inadequate and improper antimicrobial agent delivery in empiric treatment and a lack of effective infection control measures. This issue highlights the value of conducting antibiotic susceptibility testing prior to receiving antibiotic therapy13,30. It is well known that using these antibiotics incorrectly or excessively will certainly result in selection pressure and raise the resistance rate. Additionally, improper antibiotic prescriptions due to incorrect disease diagnosis may result in bacterial resistance in patients 1.

TABLE 4: The antimicrobial susceptibility test results of 113 E.coli isolates against 20 antimicrobial agents that fall within 13 different antimicrobial classes

Antimicrobial agents	**Resistant %**	**Intermediate %**	**Susceptible %**
Piperacillin-tazobactam – PIT (100/10μg)	15.04	15.04	69.91
Ampicillin – AMP (10 μg)	95.58	0.88	3.54
Piperacillin – PRL (100 μg)	96.46	1.77	1.77
Cefotaxime – CTX (30 μg)	92.92	4.42	2.65
Ceftazidime – CAZ (30 μg)	92.04	7.08	0.88
Ceftriaxone – CRO (30 μg)	90.27	0.88	8.85
Cefepime – FEP (30μg)	84.96	0.00	15.04
Cefoxitin – FOX (30 μg)	33.63	4.42	61.95
Imipenem – IMP (10 μg)	14.16	15.93	69.91
Meropenem – MEM (10 μg)	11.50	6.19	82.30
Aztreonam – ATM (30 μg)	70.80	15.93	13.27
Gentamicin – CN (10 μg)	32.74	4.42	62.83
Amikacin – AK (30 μg)	21.24	30.97	47.79
Ciprofloxacin – CIP (5 μg)	84.07	15.04	0.88
Levofloxacin – LEV (5 μg)	84.07	15.04	0.88
Tetracycline – TE (30 μg)	80.53	3.54	15.93
Azithromycin – AZM (15μg)	82.30	0.00	17.70
Chloramphenicol – C (30 μg)	23.89	13.27	62.83
Trimethoprim- sulfamethoxazole – SXT (1.25/ 23.75 μg)	76.11	1.77	22.12
Nitrofurantoin – NI (300 μg)	60.18	31.86	7.96

The distribution of MDR, XDR, and PDR among clinical E.coli isolates

The antimicrobial susceptibility test of the present study exhibited that out of 113 E.coli isolates, 111 (98.23%) were classified as Multidrug-resistant (MDR).While, only 2 (1.77%) isolates were susceptible to almost all antimicrobial agents (not classified as MDR). Furthermore, the results showed that out of 113 isolates, 24 (21.24%) were classified as Extensively drug-resistant (XDR), and only 2 isolates (1.77%) were classified as possibly

Pandrug-resistant (PDR) (Fig. 9). These results agreed with the results of a study conducted by Aabed et al.5 who found that almost all isolates (95.8%) of E.coli collected from urine were multidrug-resistant (MDR). Another study by Saeed et al.9 revealed that all (100%) of Consistent with these findings, all E. coli isolates tested positive for resistance to at least three antimicrobial agents from various antimicrobial classes. AL-khazraji19 showed that 58.2% of the E. coli isolates were MDR, however the present investigation revealed a far lower percentage. In addition, research by Sabir et al.26 demonstrated that 81% of E.coli isolates were MDR and 8.7% of the isolates were XDR. The variations in population, geographic location, duration between investigations, and clinical specimen types and sizes can all be used to explain why the results of different studies have different findings.

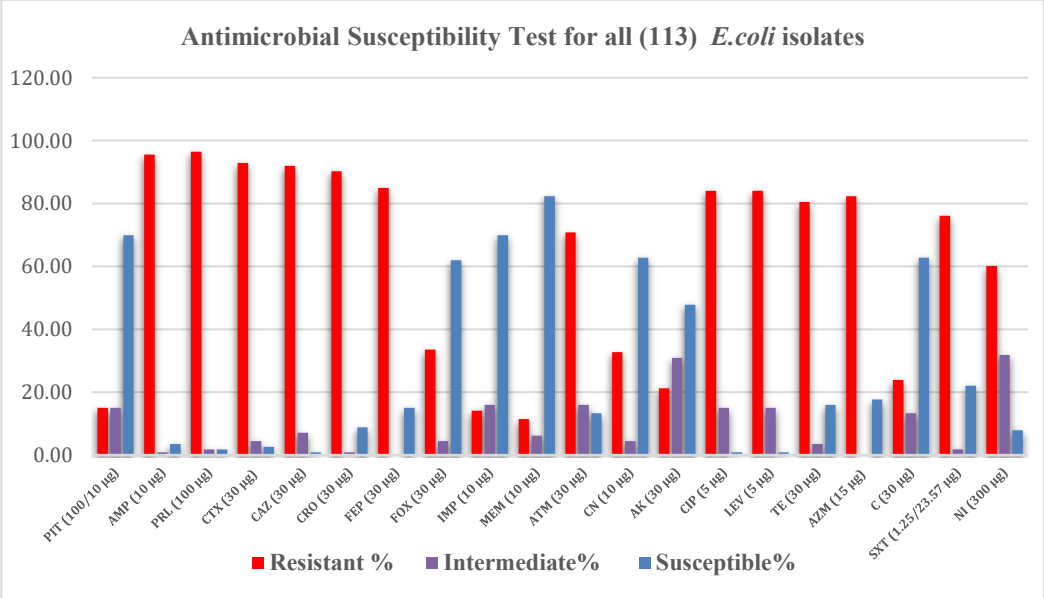

FIGURE 8: The antimicrobial susceptibility test results of 113 E.coli isolates against 20 antimicrobial agents that fall within 13 different antimicrobial classes

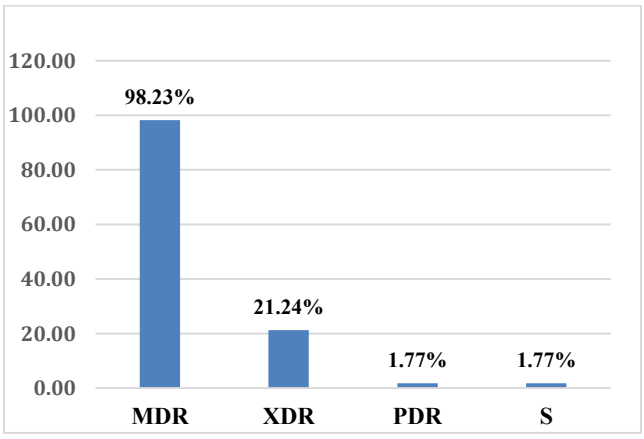

FIGURE 9: Susceptibility of 113 E.coli isolates to 20 antimicrobial agents that fall within 13 different antimicrobial classes. Where MDR: Multidrug-resistant, XDR: Extensively drug-resistant, PDR: Pandrug-resistant, and S: Sensitive

CONCLUSION

The current results indicated that most E.coli isolates collected from patients in Baghdad exhibited highly antimicrobial-resistant patterns and most of them were classified as MDR, as well as the emergence of XDR and possibly PDR E.coli isolates. Which revealed a severe and critical health problem that could threaten the community. Accordingly, all healthcare units, hospitals, physicians, and scientists should draw attention to this problem and consider a suitable solution to control it. The antimicrobial resistance of E.coli can be attributed to the over and misused of antimicrobial agents by patients in the community and health care units. Usually, most physicians prescribed a broad-spectrum antibiotic to patients without cultural or antimicrobial susceptibility tests which could accelerate the growth of germs that are resistant to antibiotics. Antibiotic-resistant bacteria may also spread since most patients do not complete their prescribed antibiotic treatment cycle.

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Authors' declaration

No potential biases were found.

All of the figures and tables in the text are original work by us and are confirmed here.

The authors agree to the study's ethical review and sign off on it.

The University of Baghdad's ethics board has given the proposal the go light..

Author's contributions statement

All writers worked together to complete this project. Halah M.H. Al-Hasani designed and performed the experiments, collected and preprepared samples, conducted the research, performed the analyses, and wrote the paper. Dalal S. Al-Rubaye and Alyaa Abdelhameed were involved in planning and supervising the work. Dalal S. Al-Rubaye conceived the original idea, supervised the project, and contributed to the revision, and proofreading of the manuscript. Alyaa Abdelhameed helped supervise the project and performed the statistical analysis.

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