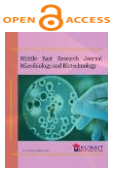

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Distribution of Antibiotics Resistance Patterns among Enteropathogenic *Escherichia Coli* Isolated from Infants in Nineva, Iraq

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Abstract: ***Background:*** The wide spread use of antibiotics is a major factor that accounts for the increase in their incidence of antibiotic resistance among Gram-negative bacteria especially *Escherichia coli* both in hospitals and the general environment. Many strains of *E. coli* carry plasmids which determine resistance to antibiotics (R factors) and which are transmissible to other bacteria. ***Materials and Methods****:* A total of 614 children feces samples (363 diarrhetic from children hospital and 251 healthy children from Mosul nurseries). All children were under three years of age. Fecal samples were transported to research laboratory using nutrient broth as transport medium for rectal swabs. EPEC identification and biotics resistance patterns were carried out. ***Results:*** The most frequent pattern was (CR, S3, AMP, TE, CAR, S, SXT, K, N, C) with 45 times disseminated among bacteria tested. There were 22 different resistant patterns detected among *E. coli* isolated from infants in children hospital with three times or more of frequency. The present study revealed that the number of resistant patterns among *E. coli* isolates of infants in nursery was 15. ***Conclusions:*** The present study revealed that among 550 resistant isolates of enteropathogenic *E. coli* (EPEC) collected from faeces of infants, there were 278 isolates resistant to five or more of antibiotics tested at the same time. The predominance of resistance combinations containing 9–11 determinants demonstrate intense antimicrobial selection pressure in the hospital environment and highlights the widespread occurrence of multidrug-resistant EPEC strains among hospitalized infants. Low-level resistance patterns (1–3 determinants) were present but less dominant. The distribution suggests progressive acquisition of resistance genes, resulting in complex MDR phenotypes.

Keywords: Infants, *Escherichia coli*, EPEC, hospital, nursery, Nineva.

Research Paper

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INTRODUCTION

Resistance to antibiotic is considered as a virulence factor for the pathogenic microorganisms to cause the infections. The first cases of antimicrobial resistance occurred in the late 1930s and in the 1940s, soon after the introduction of the first antibiotic classes, sulfonamides and penicillin. Common bacteria such as strains of *Escherichia coli* became resistant to these classes of antibiotics at record speed. Antimicrobial resistance is not new, but the number of resistant organisms, the geographic locations affected by drug resistance, and the breadth of resistance in single organisms are unprecedented and mounting [1-4]. Diseases and disease agents that were once thought to be controlled by antibiotics are returning in new leagues resistant to these therapies. However, that antimicrobial resistance is also evident in other microorganisms namely, parasites, fungi and viruses [5-8]. Drug-resistant strains initially appeared in hospitals, where most antibiotics were being used. Sulfonamide-resistant Enterobacteriaceae emerged in military hospitals in the

1930s. The existence of antibiotic resistance among human derived bacteria has been known for many years and has been the subject of numerous reviews. The mechanisms of antibiotic resistance and other chemotherapeutic agents among Enterobacteriaceae particularly *E. coli* have been also recently reviewed. Although some strains of *E. coli* are pathogenic their importance in human environment is as an indicator of pathogens such as salmonella or *Vibrio cholerae*. The wide spread use of antibiotics is a major factor that accounts for the increase in their incidence of antibiotic resistance among Gram-negative bacteria especially *E. coli* both in hospitals and the general environment [1, 9-11]. Many strains of *E. coli* carry plasmids which determine resistance to antibiotics (R factors) and which are transmissible to other bacteria (Linton *et al.,* 1981). There have been many surveys of the occurrence of antibiotic-resistant *E. coli* in man, sewage and freshwater [12-14], but little work has been done locally on this subject [7]. Almost all the strains tested were resistant to one or more antibiotics. Most strains revealed multiple resistance, and the resistance patterns were so different that 202 patterns- were recorded [15]. There is a steady increase in antibiotic resistance among *E. coli* compared to those observed previously. Penicillin resistant *S. aureus* confronted London civilian hospitals very soon after the introduction of penicillin in the 1940s [16]. Similarly, *Mycobacterium tuberculosis* with resistance to streptomycin emerged in the community soon after the discovery of this antibiotic [17,18]. Resistance to multiple drugs was first detected among enteric bacteria namely, *E. coli*, *Shigella* and *Salmonella* in the late 1950s to early1960s [19-22]. Such strains posed severe clinical problems and cost lives, particularly in developing countries. Nevertheless, the resistance problem was perceived by some, most notably those in the industrialized world, as a curiosity of little health concern confined to gastrointestinal organisms in distant countries fueled by increasing antimicrobial use and the frequency of resistance escalated in many different bacteria, especially in the developing countries where antimicrobials were readily available without prescription [23,24]. Poor sanitation conditions aided spread and small healthcare budgets prevented access to new effective but more expensive antibiotics. This study aims to assess the infection of male genital tract particularly if it happens with antibiotic- resistant bacteria and its impact on male infertility in our locality.

MATERIALS AND METHODS

Patients

This study was carried out in hospital of Children hospital and nursey of Mosul city, Iraq. 602 infants were included and they were 351 infants with diarrheic diseases and 251 infants from nursery. The acceptance for participation in the present study was taken from all the participants families whose native language is Arabic. They were not mentally retarded and they were completely healthy considering hearing and speaking.

Sampling

Rectal swabs were taken from each infant. Swabs were rinsed with sterile nutrient broth as a transport media. 351 samples of paediatric hospital patients and 251 swabs were from nursery infants [15,25,26].

Isolation

All samples were inoculated on MacConkey agar (Oxoid). Inoculated plates were incubated at 37 ^{0}C for 24 hours [27-29].

Identification of Escherichia coli

The purified isolates of suspected *E. coli* were conventionally. All plates of MacConkey agar were examined for the presence of lactose fermenting colonies. One to three suspected colonies were selected from each plate and tested for indole, acid and gas production from lactose and for ability of growth at 44 ^{0}C. This suspected E. coli were further identified by IMViC tests; H_2S production; fermentation of sucrose, maltose, xylose, rhamnose, sorbose, raffinose, sorbitol, dulcitol and salicin; decarboxylation of ornithine, arginine and lysine [15,30-33].

Antibiotic susceptibility testing

A loopful growth from isolates of *Escherichia coli* were inoculated into nutrient broth and incubated at 37°C for 18 hours. The bacterial suspensions were diluted with ringer solution. The proportion of dilution was 1:1000 [34]. Diluted bacterial suspension were poured onto the surface of the Muller-Hinton agar plates. The excess of bacterial suspensions were discarded using Pasteur pipette and plates were left for one hour at room temperature to dry. The antibiotic discs which are shown in Table 1 were selectively applied by using sterile forceps which was flamed after being cleansed with alcohol. The plates were incubated at 37 0C for over-night. The size of zones of inhibition were measured from edge of disc to the edge of inhibition of growth and the result was compared with standard diameter of inhibition zones for each antibiotic utilizing the method of Bauer et al. [35]. The following standard strain *Escherichia coli* ATCC25922 was used as a reference strain [36,37].

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics such as means, standard deviations, and frequency distributions were computed to summarize the data. A paired sample t test correlation coefficient (R) and coefficient of determination (R2) were calculated. [38-41].

RESULTS

The present study revealed that among 550 resistant isolates of enteropathogenic *Escherichia coli* (EPEC) collected from faeces of infants, there were 278 isolates resistant to five or more of antibiotics tested at the same time (Table 1). The most frequent pattern was (CR, S3, AMP, TE, CAR, S, SXT, K, N, C) with 45 times disseminated among bacteria tested. This pattern composed of 10 antibiotics commonly used in local. These patterns were collectively recorded among infants of children hospital and nursery. 19 different resistant patterns were detected during the present study. These different patterns distributed among 278 *E.coli* tested. Resistance patterns containing multiple antibiotics (9–12 determinants) were markedly more frequent than simple resistance profiles The highest frequency occurred in the 10-determinant pattern (45 isolates; 8.2%), indicating substantial multidrug resistance. Most common profiles consistently included CR (cephaloridine), S3 (sulfonamide), AMP (ampicillin), TE (tetracycline), CAR (carbenicillin) and SXT (co-trimoxazole). This suggests these antibiotics contributed strongly to MDR selection among EPEC isolates. The distribution of resistance profiles is highly heterogeneous, with a small number of multidrug-resistant patterns accounting for a large proportion of isolates. Resistance combinations involving β-lactams, sulfonamides, tetracycline, streptomycin, and co-trimoxazole predominate, indicating extensive antimicrobial selection pressure and widespread MDR among EPEC isolates (Figure 1).

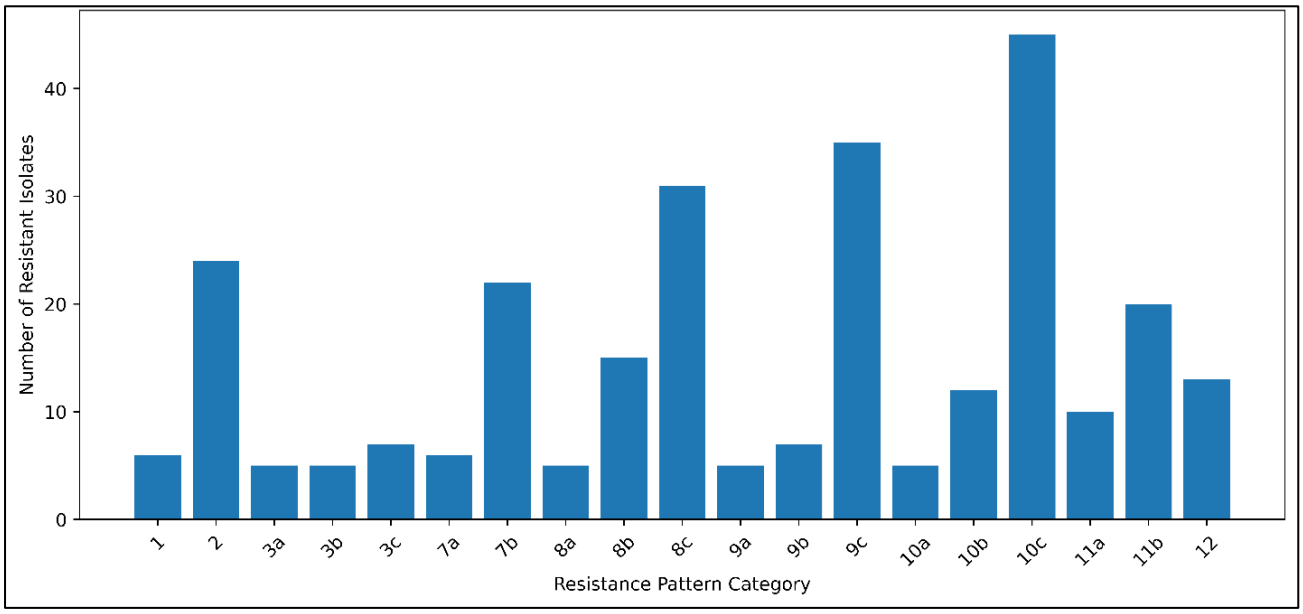

Figure 1: Frequency distribution of major multidrug-resistance patterns among enteropathogenic Escherichia coli (EPEC) isolates

Table 1: Frequency of antibiotic resistance patterns occurred five times or more among 550 resistant isolates of enteropathogenic *Escherichia coli* from faeces of infants

Number of R determinants	Resistance pattern	Resistant isolates	
		No.	%
1	CR	6	1.1
2	CR, S3	24	4.4
3	CR, S3, S	5	0.9
	CR, S3, A MP	5	0.9
	CR, S3, TE	7	1.3
7	CR, S3, AMP, TE, CAR, S, C	6	1.1
	CR, S3, AMP, TE, CAR, S, SXT	22	4
8	CR, S3, AMP, TE, CAR, S, SXT, RD	5	0.9
	CR, S3, AMP, TE, CAR, SXT, K, N	15	2.7
	CR, S3, AMP, TE, CAR, S, SXT, C	31	5.6
9	CR, S3, AMP, TE, CAR, S, SXT, C, RD	5	0.9
	CR, S3, AMP, CAR, S, SXT, K, N, C	7	1.3
	CR, S3, AMP, TE, CAR, S, SXT, E, N	35	6.4
10	CR, S3, AMP, TE, CAR, S, SXT, K, N, C	5	0.9
	CR,S3,AMP,TE,CAR,S,SXT,K,N,RD	12	2.2
	CR,S3,AMP,TE,CAR,S,SXT,K,N,C	45	8.2

Number of R determinants	Resistance pattern	Resistant isolates	
		No.	%
11	CR,S3,AMP,TE,CAR,S,SXT,K,N,C,CN	10	1.8
	CR,S3,AMP,TE,CAR,S,SXT,K,N,C,RD	20	3.6
12	CR,S3,AMP,TE,CAR,S,SXT,K,N,C,RD,CN	13	2.4
Total		278	

CR, cephaloridine; S3, Sulfonamide; AMP, ampicillin; TE, tetracycline; CAR, carbenicillin; S, streptomycin; SXT, co-trimoxazole; K, kanamycin; N, neomycin; c, chloramphenicol; RD, rifampicin; CN, gentamicin.

There were 22 different resistant patterns detected among *E. coli* isolated from infants in children hospital with three times or more of frequency (Table 2). The most common pattern was composed of 10 R determinants and occurred 26 times; this pattern was (CR, S3, S, AMP, CAR, TE, SXT, C, N, K) as shown in Table 2. These different patterns were detected among 182 isolates of *E.coli* tested. The resistance profiles were highly heterogeneous, but most common patterns shared resistance to cephaloridine, sulfonamide, ampicillin, tetracycline, carbenicillin, streptomycin, and co-trimoxazole. The predominance of resistance combinations containing 9–11 determinants demonstrate intense antimicrobial selection pressure in the hospital environment and highlights the widespread occurrence of multidrug-resistant EPEC strains among hospitalized infants. The data reveal a marked concentration of resistance among complex multidrug-resistant phenotypes. Patterns containing resistance to nine or more antimicrobial agents accounted for the majority of isolates, suggesting extensive dissemination of MDR determinants and emphasizing the need for continuous antimicrobial surveillance and stewardship programs in pediatric hospitals (Figure 2).

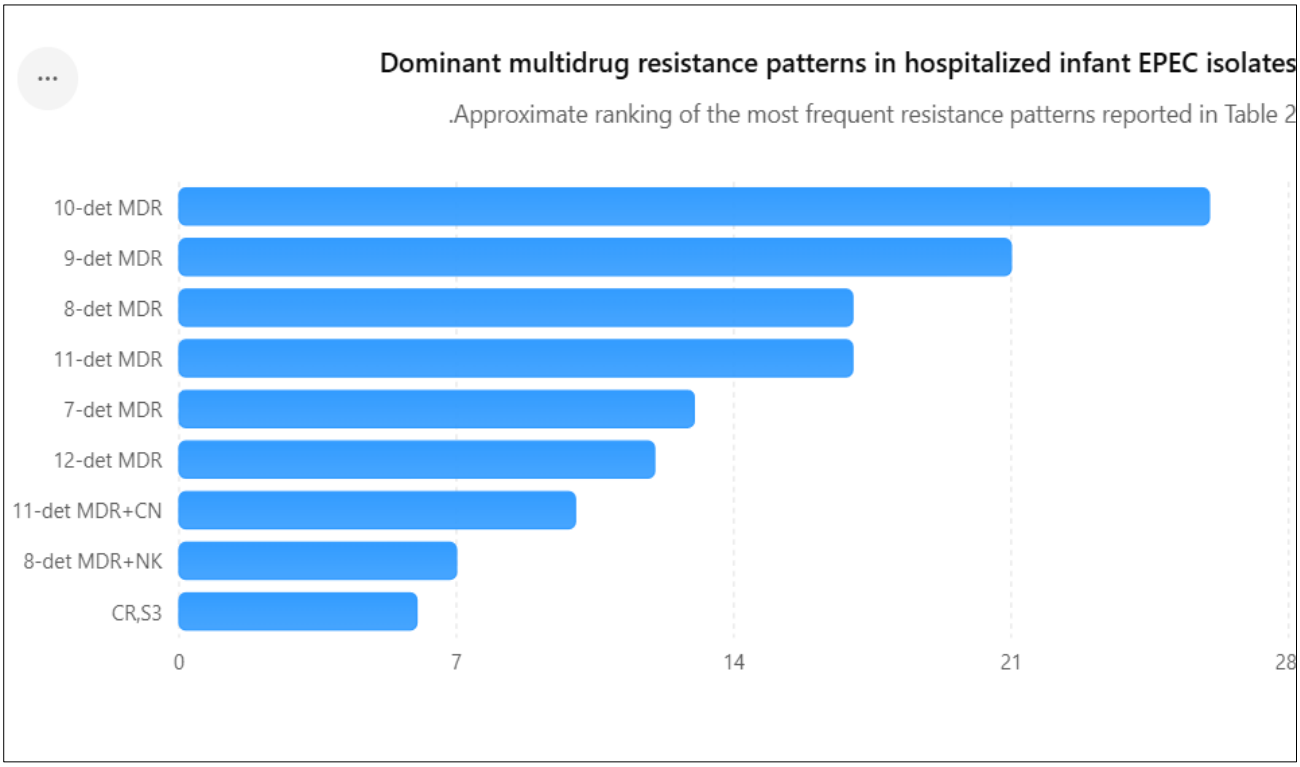

Figure 2: Distribution of multidrug antibiotic resistance patterns among enteropathogenic Escherichia coli isolates recovered from hospitalized infants

Table 2: Frequency of antibiotic resistance patterns occurred three times or more among 337 resistant isolates of enteropathogenic *Escherichia coli* isolated from of infants in children hospital

Number of R determinants	Resistance pattern	Resistant isolates	
		No.	%
3	CR,S3	6	1.1
3	CR,S3,S	3	0.9
7	CR,S3,S,AMP,CAR,SXT,C	3	0.9
	CR,S3,S,AMP,CAR,SXT,RD	4	1.2
	CR,S3,S,AMP,CAR,TE,RD	4	1.2

Number of R determinants	Resistance pattern	Resistant isolates	
		No.	%
	CR,S3,S,AMP,CAR,TE,C	4	1.2
	CR,S3,S,AMP,CAR,TE,SXT	13	3.9
8	CR,S3,S,AMP,CAR,TE,SXT,RD	4	1.2
	CR,S3,AMP,CAR,TE,SXT,N,K	7	2.1
	CR,S3,S,AMP,CAR,TE,SXT,C	17	5.1
9	CR,S3,S,AMP,CAR,TE,SXT,C,N	3	0.9
	CR,S3,S,AMP,CAR,TE,SXT,C,RD	4	1.2
	CR,S3,S,AMP,CAR,SXT,C,N,K	4	1.2
	CR,S3,S,AMP,CAR,TE,SXT,N,K	21	6.3
10	CR,S3,S,AMP,CAR,TE,C,N,K,RD	3	0.9
	CR,S3,S,AMP,CAR,TE,SXT,N,K,CN	4	1.2
	CR,S3,S,AMP.CAR,TE,SXT,N,K,RD	10	3
	CR,S3,S,AMP,CAR,TE,SXT,C,N,K	26	7.8
11	CR,S3,S,AMP,CAR,TE,SXT,C,N,K,CN	10	3
	CR,S3,S,AMP,CAR,TE,SXT,C,N,K,RD	17	5.1
12	CR,S3,S,AMP,CAR,TE,SXT,C,N,K,RD,CN	12	3.6
13	CR,S3,S,AMP,CAR,TE,SXT,C,N,K,RD,CN,F	3	0.9
Total		182	

CR, cephaloridine; S3, Sulfonamide; AMP, ampicillin; TE, tetracycline; CAR, carbenicillin; S, streptomycin; SXT, co-trimoxazole; K, kanamycin; N, neomycin; c, chloramphenicol; RD, rifampicin; CN, gentamicin; N, nitrofurantoin; N, neomycin.

The present study revealed that the number of resistant patterns among E.coli isolates of infants in nursery was 15. The pattern (CR, S3, TE, AMP, SXT, CAR, S, K, N,CN) which was composed of 10 R determinants was the most frequent type found among 19 resistant isolates of EPEC. Other patterns detected among infants of nursery were variable in their R determinant composition (Table 3). All different patterns detected were found among 114 isolates of *E.coli.* Multidrug resistance (MDR) was highly prevalent, with many isolates resistant to 8–10 antibiotics simultaneously The highest frequency occurred in the 10-drug resistance pattern (8.8%), indicating substantial accumulation of resistance determinants among nursery EPEC isolates. Low-level resistance patterns (1–3 determinants) were present but less dominant. The distribution suggests progressive acquisition of resistance genes, resulting in complex MDR phenotypes (Figure 3).

Table 3: Frequency of antibiotic resistance patterns occurred once or more among 217 resistant isolates of enteropathogenic *Escherichia coli* isolated from of infants in nursery

Number of R determinants	Resistance pattern	Resistant isolates	
		No.	%
1	CR	6	2.8
2	CR,S3	18	8.3
3	CR,S3,RD	3	1.4
	CR,S3,AMP	3	1.4
	CR,S3,SXT	3	1.4
	CR,S3,TE	6	2.8
5	CR,S3,TE,SXT,S	3	1.4
	CR,S3,TE,AMP,CAR	4	1.8
7	CR,S3,TE,AMP,SXT,CAR,S	9	4.1
8	CR,S3,TE,AMP,SXT,CAR,K,N	8	3.7
	CR,S3,TE,AMP,SXT,CAR,S,C	14	6.5
9	CR,S3,AMP,SXT,CAR,S,K,N,C	3	1.4
	CR,S3,TE,AMP,SXT,CAR,S,K,N	14	6.5
10	CR,S3,TE,AMP,SXT,CAR,S,K,N,CN	19	8.8
11	CR,S3,TE,AMP,SXT,CAR,S,K,N,C,RD	3	1.4
Total		114	

CR, cephaloridine; S3, Sulfonamide; AMP, ampicillin; TE, tetracycline; CAR, carbenicillin; S, streptomycin; SXT, co-trimoxazole; K, kanamycin; N, neomycin; c, chloramphenicol; RD, rifampicin; CN, gentamicin; N, nitrofurantoin; N, neomycin.

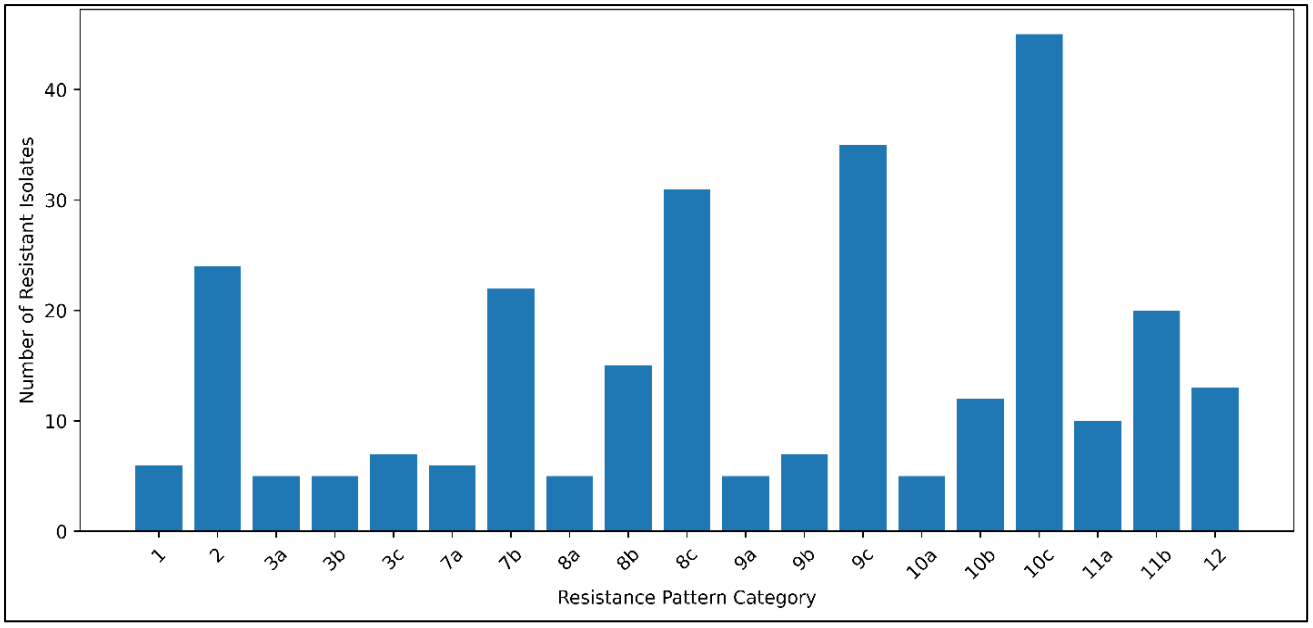

Figure 3: Frequency Distribution of Antibiotic Resistance Patterns among Enteropathogenic Escherichia coli Isolates from Nursery Infants

DISCUSSION

The present study revealed that among 550 resistant isolates of enteropathogenic *Escherichia coli* (EPEC) collected from faeces of infants, there were 278 isolates resistant to five or more of antibiotics tested at the same time. The most frequent pattern was (CR,S3,AMP,TE,CAR,S,SXT,K,N,C) with 45 times disseminated among bacteria tested. This pattern composed of 10 antibiotics commonly used in local. These patterns were collectively recorded among infants of children hospital and nursery. 19 different resistant patterns were detected during the present study. These different patterns distributed among 278 *E.coli* tested. On the other hand, it was revealed previously that *E.coli* was highly susceptible to amikacin, nalidixic acid and gentamicin and these results were almost similar to other concluded elsewhere [42-44]. On the other hand, the isolates were highly sensitive to amikacin, nitrofurantoin, cefotaxime and ciprofloxacin. These results were almost similar to those concluded by other workers. Other bacterial isolates like *Kl. Pneumoniae, E. aerogenes, Ps. aeruginosa, C. freundii* and *S. marcescens* were variable in their susceptibility to various antibiotics [2,45,46]. In addition, presence of multiple antibiotic resistance at the same time might be due to the presence of multiple R-determinants on the same plasmids carried by the organism [47,48]. It is natural for bacteria to develop antibiotic resistance which is encoded by the antibiotic resistance genes (ARGs) which is not more than production of billions of years of evolution. It has been found that bacteria living in the environment already possess ARGs which are responsible for resistance to newly approved antibiotics before using of these drugs [49-51]. Inherited structural and / or physiological properties lead to intrinsic resistance to antibiotics. These functional properties including efflux to actively eliminate antibiotics from bacterial cells which entered through porin which is the mechanism by which the antibiotics unable to pass the outer membrane and by this mechanism cannot reach the target site [49,52,53]. Moreover, it was also concluded in the present study that the most common pattern was composed of 10 R determinants and occurred 26 times ; this pattern was(CR,S3,S,AMP,CAR,TE,SXT,C,N,K) as shown in Table 2.These different patterns were detected among 182 isolates of *E.coli* tested. The resistance profiles were highly heterogeneous, but most common patterns shared resistance to cephaloridine, sulfonamide, ampicillin, tetracycline, carbenicillin, streptomycin, and co-trimoxazole. The predominance of resistance combinations containing 9–11 determinants demonstrates intense antimicrobial selection pressure in the hospital environment and highlights the widespread occurrence of multidrug-resistant EPEC strains among hospitalized infants. The data reveal a marked concentration of resistance among complex multidrug-resistant phenotypes. Furthermore, It was found that, in the case of *E. coli*, the loss of phosphatases and heptose in the lipopolysaccharide resulted in increased susceptibility to antibiotics, such as novobiocin, spiramycin, and actinomycin D [1,54]. It was found also that a few disinfectants exposed strains became resistant to neomycin and kanamycin, but not to gentamicin or amikacin [55-58). Moreover, the present study showed that The present study revealed that the number of resistant patterns among *E.coli* isolates of infants in nursery was 15. The pattern (CR, S3, TE, AMP, SXT, CAR, S, K, N, CN) which was composed of 10 R determinants was the most frequent type found among 19 resistant isolates of EPEC. Other patterns detected among infants of nursery were variable in their R determinant composition. All different patterns detected were found among 114 isolates of *E.coli.* Multidrug

resistance (MDR) was highly prevalent, with many isolates resistant to 8–10 antibiotics simultaneously The highest frequency occurred in the 10-drug resistance pattern (8.8%), indicating substantial accumulation of resistance determinants among nursery EPEC isolates. Furthermore, The mechanisms of antibiotic resistance in these variants are still under investigation. One hypothesis would be that the disinfectants altered the target site in the bacterial ribosome, making it less susceptible to neomycin and kanamycin but not to gentamicin or amikacin. Sivaji, Mandal and Agarwal [59] observed that disinfectant derived variants of *Staphylococcus aureus* became resistant to streptomycin but not to gentamicin or kanamycin. Destruction of various periplasmic enzymes by disinfectants has also been reported [60,61,62,63]. Decreased uptake of antibiotics can also be another contributory factor to the resistance. However, microorganisms exposed to subinhibitory concentrations of antimicrobials tend to adopt an adaptative response or develop resistance mechanisms in order to overcome this selective pressure [64,65]. It may occur as a horizontal gene transfer, thus enabling the spread of antimicrobial resistance genes and alteration of antimicrobial susceptibility profiles [65]. However, *E. coli, Proteus mirabilis, Ps. Aeruginosa, S. aureus, K. pneumoniae and S. marcesens* were still highly sensitive to amikacin, ciprofloxacin and chloramphenicol. However, 188hloramphenicol is not preferred to be commonly used for medication as it might because ablastic anemia. Furthermore, the most common UTI pathogens and highly resistant to antibiotics emphasize the need for judicious u use of antibiotics [2]. Furthermore, it was found elsewhere that The present study revealed that *E.coli* was highly susceptible to amikacin, nalidixic acid and gentamicin and these results were almost similar to other concluded elsewhere. [66-68] On the other hand, the isolates were highly sensitive to amikacin, nitrofurantoin, cefotaxime and ciprofloxacin. These results were almost similar to those concluded by other workers [69-72]. Other bacterial isolates like *Kl. Pneumoniae, E. aerogenes, Ps. Aeruginosa, C. freundii* and *S. marcescens* were variable in their susceptibility to various antibiotics [73-78]. The existence of antibiotic resistance among human derived bacteria has been known for many years and has been the subject of numerous reviews. The mechanisms of antibiotic resistance and other chemotherapeutic agents among Enterobacteriaceae particularly *E. coli* have been also recently reviewed [79-83]. Although some strains of *E. coli* are pathogenic their importance in human environment is as an indicator of pathogens such as salmonella or *Vibrio cholerae* [84-91].

CONCLUSIONS

The present study revealed that among 550 resistant isolates of enteropathogenic *Escherichia coli* (EPEC) collected from faeces of infants, there were 278 isolates resistant to five or more of antibiotics tested at the same time. The predominance of resistance combinations containing 9–11 determinants demonstrate intense antimicrobial selection pressure in the hospital environment and highlights the widespread occurrence of multidrug-resistant EPEC strains among hospitalized infants. Low-level resistance patterns (1–3 determinants) were present but less dominant. The distribution suggests progressive acquisition of resistance genes, resulting in complex MDR phenotypes.

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Statement of Ethics

All the procedures involving human participation were conducted in strict accordance with ethical standards of Institutional Research Committee, Department of Scientific Research, Mosul University as well as the 1964 Helsinki Declaration and its subsequent amendments or equivalent ethical norms.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Statement

The author declares that he has no conflicts of interest, financial or otherwise.

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