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REVIEW ARTICLE

Antibiotic-resistant diarrheagenic *Escherichia coli* isolated from patients under 5 years of age in Al-Diwaniyah city

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Abstract:

Background: Escherichia coli, a common bacterium, is becoming increasingly resistant to antibiotics, making diarrhea treatment difficult. The situation is especially concerning for multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL)-producing strains. These "superbugs" can resist multiple antibiotics and spread among bacteria. Traditional treatments like ampicillin and tetracycline are becoming less effective. The Centers for Disease Control and Prevention (CDC) recommends fluoroquinolones, macrolides, and rifaximin for some cases, but these have limitations.

Aim of the study: The present study explores the antimicrobial resistance of diarrheagenic E. coli pathotypes to determine the antibiotic resistance pattern.

Methodology :Twelve isolates of diarrheagenic E. coli isolated from children under five years old with diarrhea in Al-Diwaniyah city, Iraq, all isolates examined antibiotic susceptibility with Kirby's Bauer method after isolation and identification by conventional and molecular methods.

Results Isolates were resistant to multiple antibiotics, including penicillins (amoxicillin, ampicillin), cephalosporins (ceftazidime, ceftriaxone, cefotaxime, cefepime), and 1 type of aminoglycoside (amikacin). Isolates were sensitive to colistin, nitrofurantoin, and azithromycin. Fluoroquinolones (Ciprofloxacin, Ofloxacin, Levofloxacin), Carbapenems (Imipenem, Meropenem), and Nalidixic acid.

Keywords: diarrheagenic E. coli, multidrug-resistant (MDR), antibiotic resistance

Introduction

The global incidence of antimicrobial resistance in E. coli over the years is alarming and underlines the need for proper interventions to prevent transmission [1]. Moreover, the emergence of multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL)-producing diarrheagenic E. coli (DEC) strains has become a serious threat to public health, as the existing antibiotics are increasingly ineffective [2]. MDR and ESBL-producing DEC strains are superbugs that have the ability to resist at least two different classes of antibiotics and to hydrolyze the third-generation cephalosporins such as cefotaxime, ceftazidime, ceftriaxone, cefuroxime, and cefepime or monobactams such as aztreonam [3]. The most common beta-lactamase (bla) genes reported in the ESBL-producing DEC isolates include blaTEM, blaSHV, and blaCTX-M, with the highest frequency of ESBL producers found in the EAEC pathotype [4][5]. The fact that these resistance genes are easily transferable among bacterial species via mobile genetic elements (e.g., plasmids, integrons, insertion sequences, and transposons) has made disease management for MDR and ESBL-producing DEC more challenging [6]. In the absence of new quality-assured antimicrobials, these groups of strains may cause untreatable infections and death. Hence, at present, updates on the antimicrobial resistance in DEC are essential for effective treatment to reduce the morbidity and mortality rates associated with diarrhea [7][1].

However, the global emergence of drug-resistant strains, limiting the choice of effective antimicrobial drugs for diarrhea treatment, has become the main challenge in the treatment of DEC infections. For instance, in recent years, ETEC has been reported to be becoming resistant to many first-line drugs, such as ampicillin, nalidixic acid, tetracycline, sulfonamides, and azithromycin [8][9].

Concerning the antimicrobial drugs for the treatment of DEC, the Centers for Disease Control and Prevention (CDC) Yellow Book 2020 recommends that antibiotics used to treat non-STEC DEC include fluoroquinolones such as ciprofloxacin, macrolides such as azithromycin, and rifaximin (or other rifa-

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mycin derivatives, such as rifampicin), although they are not recommended for the treatment of STEC due to the possible risk of hemolytic uremic syndrome [10]. [11]. Resistance was common among DEC isolates, particularly against the penicillin class of antibiotics. While the prevalence of resistant DEC was high against the penicillin antibiotics, combinations with other antimicrobial agents somehow increased the susceptibility of DEC to amoxicillin and ampicillin. For instance, the combination of amoxicillin with clavulanic acid rendered DEC more susceptible to the antibiotic [12]. Similarly, the combination of ampicillin with sulbactam, on the other hand, decreased the resistance of DEC [13].

Additionally, the use of piperacillin in the treatment of DEC recorded resistance, while the combination of piperacillin with tazobactam reduced the prevalence substantially [14]. It is possible that utilizing more than one β-lactam inhibitor in the treatment of DEC increases the susceptibility of the pathogen significantly. High rates of resistance to amoxicillin, ampicillin, and piperacillin are unfortunate events in the majority of Asian countries and could reflect the excessive and unjustified use of antibiotics in general care [15][1][16].

2. Materials and Methods

Ninety-seven patients under five years of age with diarrhea were examined in several hospitals (Women's and Children's General Hospital and Imam Hussein Hospital for Children) in Al-Diwanyiah city.

Stool samples were collected from patients (0-5 years of age). A fresh stool sample was collected in a sterile container. A stool sample is usually collected with a swab of the rectum or by using a clean diaper or a disposable stool collection cup [17]. All samples are cultured on nutrient agar and incubated for 18-24 hours at 37°C to activate the bacteria, then the bacteria are isolated with conventional and molecular methods.

The disc diffusion method for in vitro antibiotic susceptibility tests was done according to the method described by [18]. The antibiotic disc agents, concentrations, and the interpretation of zones of inhibition for E. coli were performed according to the National Committee for Clinical Laboratory Standards [19]. The preparation of the test was done by using a Mueller-Hinton agar plate that was inoculated by a 0.5 McFarland tube dilution of bacterial culture, which was spread by a sterile cotton swab. The antibiotic discs included gentamicin (10 µg), amikacin (30 µg), netilmicin (30 µg), imipenem (10 µg), meropenem (10 µg), ceftazidime (30 µg), cefepime (30 µg), ofloxacin (10 µg), levofloxacin (5 µg), piperacillin (100 µg), aztreonam (30 µg), colistin (10 µg), and polymyxin B (300 U). Then the plate was incubated at 37ºC for 24 hours. The presence or absence of a zone of inhibition around each of the discs after the period of incubation was explained by antibacterial action, and the diameter of the zone of inhibition produced by each antibiotic was measured to determine patterns of antibiotic susceptibility.

3. Results and Discussion

The present study examined the susceptibility of DEC isolates to antibiotics using disc diffusion method (Table 1). Significantly, the findings revealed that the isolates were resistant to Amoxicillin, Amikacin, Ampicillin, Ceftazidime, Ceftriaxone, Cefotaxime and Cefepime; while intermediate resistance was identified with Gentamicin. However, significant sensitivity was recorded to Colisitin, Nitofurantion, Azithromycin, Ciprofloxacillin, Ofloxacine, Imipenem, Meropenem, Levofloxacine, and Nalidixic acid. However, the findings of multidrug resistance revealed that there was significant resistance to two types of Penicillin (Amoxicillin and Ampicillin), four types of Cephalosporins (Ceftazidime, Ceftriaxone, Cefotaxine, and Cefepime), and 1 type of Aminoglycosides (Amikacin), However there is marginally relationship between the isolated pathotypes were isolated in this study and the multiple drug resistance at p-value = 0.074, and there is no association between pathotypes, virulence factors and multiple drug resistance at p-value = 1 . (Table 2) (Table 3) (Figure 1).

Table (1): Results of antibiotic susceptibility to DEC

Anti-Biotic Group (Family)	Types of antibiotic	Total No. of tested isolate	Standard zone of inhibition		Susceptibility pattern			p-value
			Resistant	Sensitive	Resistant	Intermediate	Sensitive	
Penicillin (Amino-penicillin)	Amoxicillin	12	≤ 15	≥ 20	12 (100%)	0 (0%)	0 (0%)	0.009 S
	Ampicillin	12	≤ 10	≥ 14	12 (100%)	0 (0%)	0 (0%)	0.009 S
Cephalosporins	Ceftazidime	12	≤ 20	≥ 21	12 (100%)	0 (0%)	0 (0%)	0.009 S
	Ceftriaxone	12	≤ 14	≥ 20	11 (91.67%)	1 (8.33%)	0 (0%)	0.0113 S
	Cefotaxime	12	≤ 17	≥ 22	12 (100%)	0 (0%)	0 (0%)	0.009 S
	Cefepime	12	≤ 17	≥ 25	12 (100%)	0 (0%)	0 (0%)	0.009 S
Aminoglycosides	Amikacin	12	≤ 14	≥ 17	10 (83.33%)	2 (16.67%)	0 (0%)	0.0188 S
	Gentamicin	12	≤ 11	≥ 15	0 (0%)	9 (75%)	3 (25%)	0.0361 S
Macrolides	Azithromycin	12	≤ 12	≥ 15	1 (8.33%)	2 (16.67%)	9 (75%)	0.0249 S
Polymyxins	Colistin	12	≤ 10	≥ 11	0 (0%)	0 (0%)	12 (100%)	0.009 S

Nitrofurans	Nitrofurantoin	12	≤ 14	≥ 17	0 (0%)	3 (25%)	9 (75%)	0.0361 S
Monocarboxylic acid (Quinolone)	Nalidixic acid	12	≤ 13	≥ 19	0 (0%)	0 (0%)	12 (100%)	0.009 S
(Fluoroquinolones) Quinolones	Ciprofloxacillin	12	≤ 21	≥ 26	0 (0%)	0 (0%)	12 (100%)	0.009 S
	Ofloxacine	12	≤ 12	≥ 16	0 (0%)	0 (0%)	12 (100%)	0.009 S
	Levofloxacine	12	≤ 16	≥ 21	0 (0%)	1 (8.33%)	11 (91%)	0.0113 S
Carbapenems	Meropenem	12	≤ 14	≥ 18	0 (0%)	0 (0%)	12 (100%)	0.009 S
	Imipenem	12	≤ 19	≥ 23	0 (0%)	1 (8.33%)	11(91.67%)	0.0113 S
	p-value				0.0097**	0.0082**	0.0092**	-
	S: Significance * (P<0.05), ** (P<0.01)							

Table (2): Association between isolated Diarrheagenic E. coli pathotypes with Anti-Biotic resistance

Isolate Number	Pathotypes	Class of Antibiotic	Multi-Drugs resistance
2	Enterohaemorrhagic	Penicillin, Cephalosporins, Aminoglycosides	+
3	Enterotoxagenic	Penicillin, Cephalosporins	+
4	Enterotoxagenic	Penicillin, Aminoglycosides	+
6	Enterohaemorrhagic	Penicillin, Cephalosporins, Aminoglycosides	+
9	Enterotoxagenic	Penicillin, Cephalosporins, Aminoglycosides	+
10	Enterotoxagenic	Penicillin, Cephalosporins, Aminoglycosides	+
11	Enterotoxagenic	Penicillin, Cephalosporins, Aminoglycosides	+
14	Enterotoxagenic \ Enteroaggregative	Penicillin, Cephalosporins, Aminoglycosides, Macrolides	+
16	Enterotoxagenic	Penicillin, Cephalosporins, Aminoglycosides	+
17	Enterotoxagenic	Penicillin, Cephalosporins, Aminoglycosides	+
19	Enterotoxagenic	Penicillin, Cephalosporins, Aminoglycosides	+
20	Enterotoxagenic	Penicillin, Cephalosporins	+
p-value: 1; Chi-square test statistic: 0			

* There is not a statistically significant association (p > 0.05) between pathotype and antibiotic.

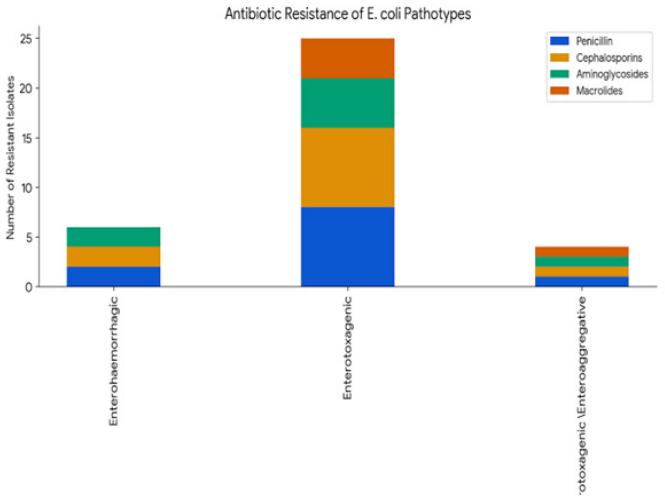

Figure (1) Association between isolated Diarrheagenic E. coli pathotypes with Anti-Biotic resistance

Table (3) Association between virulence factors genes of isolated pathotypes and Anti-Biotic resistance

Pathotypes	Virulance Factors Genes	Antibiotc Resistance
Enterohaemorrhagic	Intimin (eae) gene Shiga toxin (stx) gene	Penicillin, Cephalosporins, Aminoglycosides
Enterotoxagenic	Heat stable toxin (est) gene Heat labile toxin (elt) gene	Penicillin, Cephalosporins, Aminoglycosides
Enterotoxagenic \ Enteroaggregative	Heat stable toxin (est) gene Heat labile toxin (elt) gene Adhesin (aggR) gene Transporter system (CVD432) gene	Penicillin, Cephalosporins, Aminoglycosides, Macrolides
p-value: 0.0002; Odds Ratio: infinity		

* The statistic results (Fisher's exact test) suggest a strong association between the presence of all tested genes (CVD432, aggR, elt, est, and eae) and resistance to Penicillin, Cephalosporins, Aminoglycosides, and Macrolides at p-values are less than 0.05

Antibiotics are very useful against pathogenic bacteria and as such the use of these drugs has decreased the rate of death from bacterial infection globally [20]. However, because of the misuse and incorrect prescription of antibiotics, genetic factors and environmental factors, the rate of antibiotic resistance is increasing and so is the threat to health [21].

The treatment of DEC with antibiotics is not routinely recommended; however, understanding the antibiotic susceptibility of these pathogens is important as intestinal E. coli strains may serve as a reservoir of antibiotic resistance genes [22]. In addition, antimicrobial therapy may be indicated in children with diarrhea due to DEC once identified, and in children with persistent diarrhea. DEC may be twice as likely to be resistant to different antibiotic [23], which agrees with observed with the results of other studies as most DEC isolates are resistant to wide range of antibiotics that commonly used for treating diarrhea and other pediatric diseases [24][25][26]. The high resistance levels observed here already described as an emerging problem of DEC isolated from children in other developing countries and for other enteric bacteria worldwide could be therefore the result of its widespread use and development the selective pressure favoring resistant isolates due to treatment with this antibiotics [27][28].

[29] found a high rate of drug resistance in E. coli to the common antimicrobial agents used in the treatment of

diarrhea. All the isolates were resistant to ampicillin, imipenem and cotrimoxazole and were sensitive to amikacin. The prevalence of drug resistance to ampicillin, chloramphenicol, cotrimoxazole, imipenem, nalidixic acid, norfloxacin was "very high" in our study. This may be due to the indiscriminate use of first-line inexpensive antibiotics in our country.

The resistance to ampicillin and imipenem may be due to production of beta-lactamases enzymes, and the most common mechanism for resistance to cotrimoxazole is acquisition of plasmid-encoded, variant diamino-pyrimidine folate reductase enzymes [30]. This may be chromosomal or plasmid mediated. Recently resistances to third-generation cephalosporins have emerged as a major concern, as seen in this study. In India, the emergence of multidrug resistant strains and its variation over the years have been increasing [31]. Appropriate antibiotic therapy for diarrhea reduces mortality and also shortens the duration of symptoms. Increased frequency of drug-resistant E. coli strains is remarkable, since resistance to first-line drugs will require more expensive drugs for effective treatment and may pose a major challenge to the health care system [32].

[33] mentioned that Azithromycin resistant strains were also more frequent in Southeast Asia/India than in Africa and Latin America, with resistance rates of 33.3%, 25%, and 9.1%, respectively, for EAEC and 28.6%, 11.1%, and 0%, respectively, for ETEC. It is important to highlight that 58% of the patients from Southeast Asia/India visited India, and among these, the percentages of resistance to nalidixic acid were 75% and 71.4% for EAEC and ETEC, respectively; ciprofloxacin resistance rates were 62.5% and 43% for EAEC and ETEC, respectively; and rates of resistance to azithromycin were 37.5% and 28.6% for EAEC and ETEC, respectively. However, statistical analysis was not performed due to the low population size obtained when stratifying the strains according to pathotype and geographical origin.

Even though colistin is an old antimicrobial substance, its use in human medicine has augmented the last decade, largely due to the appearance of multidrug resistant Pseudomonas, Klebsiella and Acinetobacter spp. [34][35] . Due to its excellent intrinsic activity against E. coli, the low prevalence of acquired resistance and the poor absorption after oral administration, colistin is a frequently used antimicrobial agent for the prevention and treatment of neonatal or weaning-associated E. coli infections [36]. Even though acquired resistance to colistin in E. coli strains was seen only occasionally in the past, the last few years, this is becoming more common [37][38].

4. Conclusions

Diarrheagenic E. coli isolates characterized here were highly resistant to Amoxicillin, Amikacin, Ampicillin, Ceftazidime, Ceftriaxone, Cefotaxime and Cefepime. There is marginally relationship between the isolated pathotypes were isolated in this study and the multiple drug resistance and there is no association between pathotypes, virulence factors and multiple drug resistance.

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