Original Article

Antibiotic Susceptibility Pattern of *E. coli* Isolates from Cases of Pediatric Meningitis

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Abstract

Background: Bacterial meningitis (BM) is a dangerous illness with a high death rate. Among the children, Escherichia coli (*E. coli*) is the leading cause of BM. **Objectives:** This study aims to investigate the antibiotic resistance of *E. coli* isolated from BM samples in Babylon. **Materials and Methods:** The current study collected 200 CSF samples from patients suspected of meningitis at hospitals in Babylon from November 2023 to April 2024. All samples underwent chemical and agricultural tests to confirm the presence of the infection. Using Vitek 2 for a confirmed diagnosis, the disk diffusion method was employed to test the sensitivity of the isolates to antibiotics, and positive samples were tested for resistance genes using polymerase chain reaction (PCR). **Results:** *E. coli* was found in 4% of CSF samples, and with low glucose levels of less than 40 mg/dL, the white blood cell count is greater than 100 cells/mm^3, and the protein level is more than 60 mg/dL. The positive isolated samples were tested for their sensitivity to antibiotics, revealing 75% resistance to amoxicillin-clavulanic acid, 62.5% resistance to ciprofloxacin, gentamicin, and cefotaxime, 50% resistance to erythromycin, ceftazidime, and ceftriaxone, 87.5% sensitivity to imipenem, meropenem, and amikacin, 75% sensitivity to aztreonam, and 62.5% sensitivity to levofloxacin and tobramycin. PCR analysis for resistance genes indicated 87.5% *fim*H, 62.5% *bla*CTX-M, and 100% *neu*C. **Conclusions:** The study found several antibiotic susceptibility patterns in *E. coli* isolates. The reason for this resistance may be attributed to the presence of the *neu*C, *fim*H, and CTX-M genes. This output provides treatment options for *E. coli* in the cerebrospinal fluid.

Keywords: Antibiotic sensitivity, CTX-M, *E. coli*, *fim*H gene, meningitis

Introduction

Until recently, neonatal meningitis (NM) caused by *Escherichia coli* (*E. coli*) represented a major health problem in many countries.[1] There is few data on the epidemiology of NM and antibiotic sensitivity in developing countries. One study examined the antimicrobial susceptibility of *E. coli* in NM in a perinatal center in China over a 20-year period.[2] *E. coli* causes extraintestinal infections associated with significant morbidity and health problems.[3] Studies indicate that *E. coli* has a chance of causing bacterial meningitis (BM) in children, and Gram-negative bacteria are the most common infection in this age period.[4] *E. coli* is one of the types of Gram-negative bacteria, as it causes many diseases in children and infants. Moreover, it causes infections in infants and blood bacteria (bacteremia).[5] *E. coli* is one of the most frequent causes of meningitis infections in children from birth to five years.[6] Research examining BM relies on a small number of *E. coli* K1 strains isolated from CSF, and this may be related to the general *E. coli* K1 strain found in CSF, but we need a detailed explanation of the patterns of expression of virulence factors in these the breeds.[7] *E. coli* bacteria have a variety of resistance to antibiotics.[8] This resistance is usually controlled by multiple genes that control the manifestation of these characteristics and is either carried on the plasmid or carried on the chromosome.[9]

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β-lactamases have an extended-spectrum *β*-lactam species, one of which, CTX-M, was developed in *E. coli*. Extended-spectrum beta-lactamase (ESBL)-producing bacteria are resistant to several classes of antibiotics. It can degrade broad-spectrum cephalosporin and is inhibited by tazobactam, sulbactam, and clavulanic acid.[10] ESBL-producing bacteria have resistance to several classes of antibiotics, due to plasmids encoding the ESBL gene, and resistance may come through genetic transmission by transfer, conjunction, or transformation. This, therefore, renders *β*-lactam treatments useless. The predominant ESBL enzymes are those encoded by the *bla*CTX-M genes. *E. coli* strains efficiently produce CTX-M.[11]

This article aims to examine the resistance of *E. coli* bacteria to antibiotics isolated from BM samples in children from newborns to 15 years. It also aims to detect the presence of the genes of the isolated bacteria for further confirmation, which are the *fim*H, *neu*C, and CTX-M genes.

Materials and Methods

Samples collection and bacterial isolation

Cerebrospinal fluid samples were collected from 200 patients suspected of having meningitis who were admitted to Babylon hospitals in the period from November 2023 to April 2024. The samples were distributed among age groups under 15 years. Samples were taken by experienced doctors under sterile conditions and collected in sterile plastic tubes. The samples were then transferred to the hospital laboratory for analysis. Following the established procedure for cerebrospinal fluid analysis, the samples were divided into three small tubes. The first tube was used for microbiological analysis; the second tube was used for chemical analysis screening for glucose and proteins; the third tube was used for cytological analysis.[6]

All samples were grown on MacConkey plates immediately after collection. The plates were incubated under the appropriate conditions at a temperature of 37°C for 18–24 h.[12] After incubation, bacterial colonies were observed. The culture was performed to separate *E. coli* bacteria from other organisms. Laboratory tests were conducted on *E. coli* to isolate pure culture based on culture media, microscopic examination, and biochemical tests. After overnight incubation, the isolates showed a pink color on MacConkey plates. differentiating between bacteria unable to undergo lactose fermentation and those able to ferment lactose. On Eosin methylene blue medium, colonies with green fluorescence were observed. The isolates were confirmed by subjecting the isolates to the Vitek 2 test.[13,14]

Cytological and chemical analysis of CSF

Microscopic inspection was conducted on all samples to determine the leukocyte count. The quantities of total protein and glucose in the samples were determined by chemical analysis.[15]

Antimicrobial susceptibility testing

To test the susceptibility of *E. coli* isolated from cerebrospinal fluid, the Kirby-Bauer (disk diffusion) technique was used. Isolates were tested for 13 antibiotics against *E. coli*: Amikacin (AK) 5 μg, amoxicillin + clavulanic acid (AMC) 30 μg, ceftazidime (CAZ) 30 μg, ceftriaxone (CRO) 30 μg, ciprofloxacin (CIP) 5 μg, erythromycin (E) 10 μg, gentamicin (CN) 30 μg, levofloxacin (LVO) 10 μg, imipenem (IPM) 10 μg, meropenem (MEM) 10 μg, aztreonam (ATM) 10 μg, tobramycin (TOB) 10 μg, and cefotaxime (CTX) 30 μg.

The standard turbidity level was set at 5.0 McFarland. Using Mueller-Hinton's medium on Petri dishes, the bacteria were evenly distributed on the dishes. The antibiotic tablets were then distributed on the culture plate. The plates were incubated at 37°C for 18 h, and then, the results were read.[5-16]

Determine the presence of ESBL

Cultures were established by diluting all test strains to a McFarland standard of 0.5 in sterile normal saline. The bacteria were distributed to cover the entire surface of the dish (Mueller-Hinton agar) and then left to dry in the air for 3–5 min. After the drying process, a tablet of the antibiotic amoxicillin-clavulanic acid (30 μg) was placed in the central area of the plate. A circular piece of (30 μg) ceftriaxone and (30 μg) ceftazidime was placed on opposite sides of an amoxicillin-clavulanic acid tablet (30 μg) at a distance from the center to the tablets of approximately 15 mm. The plates were placed in the incubator and kept for a duration of 16–24 h at a temperature of 35°C ± 2°C. When the growth inhibition zone for any of the tested antibacterial drugs exceeds 5 mm more than the rest of the antibiotics, this condition is considered an indication of the bacteria's ability to produce ESBL.

Molecular assay for *E. coli*

After inoculating 10 mL of nutrient broth with the bacterial culture for 24 h at 37°C in a shaker incubator, the pellet was microcentrifuge at 16,000 rpm for 2 min to obtain the pellet. DNA was extracted using a commercial purification technique genomic DNA mini kit (Promega, USA). The primers mentioned in Table 1 (Korea, Macrogen). To analyze each gene, a primer mixture (10 mol/μL) was prepared by combining 10 μL of primer stock solution (stored at −20°C) with 90 μL of nuclease-free water. The stock solution was prepared by dissolving the freeze-dried primers in an aqueous medium that

inhibits nuclease activity, resulting in a final concentration of 100 pmol/µL. Devoid of any enzymes, water was added to increase the overall volume of the reaction mixture to 20 µL. The mixture should consist of 4 µL of template DNA, 5 µL of master mix, 2 µL of reverse primer, and 2 µL of forward primer. The polymerase chain reaction (PCR) results were electrophoresed on a 1% agarose gel for 30 min, subsequently stained with ethidium bromide, and analyzed using a gel doc.[17] Table 2 shows the amplification conditions in PCR.

It was ordered to follow the instructions attached to the kit by the manufacturer. The detected virulence genes were amplified in a 25 µL mixture containing 1.5× PCR buffer, 2 mm magnesium chloride, 5 nmol of each dNTP (Promega), and 12.5 pmol of each primer. One unit of DNA polymerase Taq was combined with 50 ng of DNA. The amplification conditions specified in Table 4 were used to determine gene duplication. The PCR products were separated by 1% agarose gel electrophoresis (Hemidia, India) in 1 × TBE buffer at room temperature using a gradient of 5 V/cm. For gel analysis, a 20 µL aliquot of PCR products was placed in wells of the gel. DNA ladder (USA, Promega), 100 bp for the *fim*H, CTX-M, and *neu*C genes, was used. To determine sample sizes, the gel was visualized, and the data were analyzed using computer software.

Table 1: The primers that were used with this study

Gene	Primer sequence (5'–3')	Amplicon size (bp)	Temperature (°C)
blaCTX-M	For: 5'-CGCTTTGCCATGT GCAGCACC-3'	307 bp	55
	Rev: 5'-GCTCAGTACGAT CGAGCC-3'		
fimH	For: 5' TGCAGAACGGATAAG CCGTGG-3'	508 bp	63
	Rev: 5'GCAGTCACCTGCCCT CCGGTA-3'		
*neu*C	For: 5'-AGGTGAAAAGCCTGG TAGTGTG-3'	676 bp	58
	Rev: 5'-GGTGGTACATCCCG GGATGTC-3'		

Ethical approval

Before samples were collected, all participants gave their consent for the study using a consent form. Pursuant to Document No. 3-39, dated 29/11/2023, the study protocols, subject information, and agreement form were examined and approved by the local ethics committee of the Department of Biology at the College of Medicine, University of Babylon.

Statistical analyzes

The data in the current study were presented as numerical values followed by their corresponding frequency expressed as a percentage. Less than 0.05 is the standard ratio. SPSS 2010 (IBM Corporation, New York) and Excel 2016 were used for statistical analysis in this study.

Results

BM is a serious disease associated with a high mortality rate. *E. coli* is considered one of the causes of this disease, especially among children. A total of 200 cerebrospinal fluid (CSF) samples from patients in Babylon hospitals suspected of having meningitis between November 2023 and April 2024. The CSF isolates had a low glucose level of less than 40 mg/dL, a protein level of more than 60 mg/dL, and a white blood cell (neutrophils) count greater than 100 cells/mm^3. *E. coli* samples accounted for eight isolates (4%). All studied samples were subjected to culture and biochemical tests.

The Vitek 2 device was used for confirmatory diagnosis, and positive samples were tested for resistance genes using PCR. During the study, isolates were subjected to antibiotic susceptibility tests using the disk diffusion method. The results were six isolates resistant to amoxicillin + clavulanic acid, five isolates resistant to ciprofloxacin, gentamicin, and cefotaxime, four isolates resistant to erythromycin, ceftriaxone, and ceftazidime, seven isolates sensitive to imipenem, meropenem, and amikacin, five isolates sensitive to levofloxacin and tobramycin, and six isolates sensitive to aztreonam [Table 3]. Table 4 shows the production of study samples for ESBL. Five isolates exhibited an increase in the zone of inhibition toward more than one antibiotic, while the remaining was the zone of inhibition toward a single antibiotic.

Table 2: The basic reaction conditions for the PCR technique used in this study

The steps of the loop	Temperature (°C)	Time (M:S)	Cycle number
Initial denaturation	94.0	03:00	1
Denaturation	94.0	01:00	30
Annealing	58.0	01:00	
Extension	72.0	01:00	
Final extension	72.0	10:00	1

Table 3: Antibiotic susceptibility of *E. coli* isolates from patients with pediatric meningitis

Antibiotic	No. of resistant (R)	% of resistant (R)	No. of sensitive (S)	% of sensitive (S)
Amikacin	1	12.5%	7	87.5%
Amoxicillin + clavulanic acid	6	75%	2	25%
Ceftazidime	2	25%	6	75%
Ceftriaxone	4	50%	4	50%
Ciprofloxacin	5	62.5%	3	37.5%
Erythromycin	4	50%	4	50%
Gentamicin	5	62.5%	3	37.5%
Levofloxacin	3	37.5%	5	62.5%
Imipenem	1	12.5%	7	87.5%
Meropenem	1	12.5%	7	87.5%
Aztreonam	2	25%	6	75%
Tobramycin	3	37.5%	5	62.5%
Cefotaxime	5	62.5%	3	37.5%

Table 4: Response of *E. coli* isolates from patients with pediatric meningitis to ESBL test

No. of isolate	Cefotaxime	Ceftazidime	Ceftriaxone
1	+	+	−
2	+	−	+
3	+	+	+
4	−	+	+
5	−	−	+
6	+	+	−
7	−	−	+
8	+	−	−

+ indicates an increase in the Müller-Hinton inhibition zone; − indicates a decrease or none in the Müller-Hinton inhibition zone

PCR was used to detect the presence of resistance genes. The results indicated the presence of the CTX-M gene by 62.5%, the *neu*C gene by 100%, and the *fim*H gene by 87.5%, [Figures 1–3], respectively.

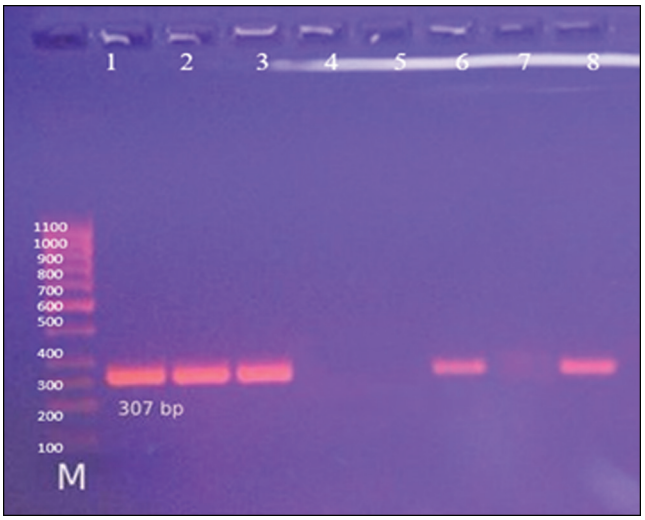

Figure 1 : Agarose gel electrophoresis of PCR product analysis of CTX-M gene in *E. coli* isolates. M (Marker ladder 1000-100bp). Lanes 1, 2, 3, 6, and 8 showed positive CTX-M gene at (307bp) PCR product size

Discussion

The susceptibility test for *E. coli*

Susceptibility testing was performed on eight isolates using traditional methods for 13 antibiotics, using the Kirby-Bauer method. Among the isolates tested, one isolate (12.5%) showed resistance to amikacin. These results are consistent with the results from Peng *et al.* which indicated a low level of resistance to this drug. Susceptibility testing in this study showed that isolates were susceptible to amikacin with a sensitivity rate of 100%.[18] Six (75%) of the isolates showed resistance to amoxicillin + clavulanic acid. The mentioned percentage was confirmed by sensitivity tests conducted by Akbar *et al.,* and the results were similar to Hassan *et al.*

Four isolates (50%) showed resistance to ceftazidime, four isolates (50%) showed resistance to ceftriaxone, and five isolates (62.5%) showed resistance to ciprofloxacin. The results are very consistent with Peng *et al*., where the percentages of isolates examined were (35.4%), (49.4%), and (55.7%) for the antibiotics ceftazidime, ceftriaxone, and ciprofloxacin, respectively.[18] Among the isolates, four isolates (50%) were resistant to erythromycin. Furthermore, among the isolates, five isolates (62.5%) were resistant to the antibiotic gentamicin. The results contrast with the study by Akbar *et al*. for gentamicin and are consistent for erythromycin, which reported resistance rates to erythromycin (44.8%) and gentamicin (26.5%).[19]

The isolates showed low resistance to levofloxacin, with only three isolates (37.5%) showing resistance. These results are consistent with the results from Hassan *et al*.[20] Both imipenem and meropenem showed significant activity against bacterial isolates, with only one isolate showing resistance (12.5%), and seven isolates (87.5%)

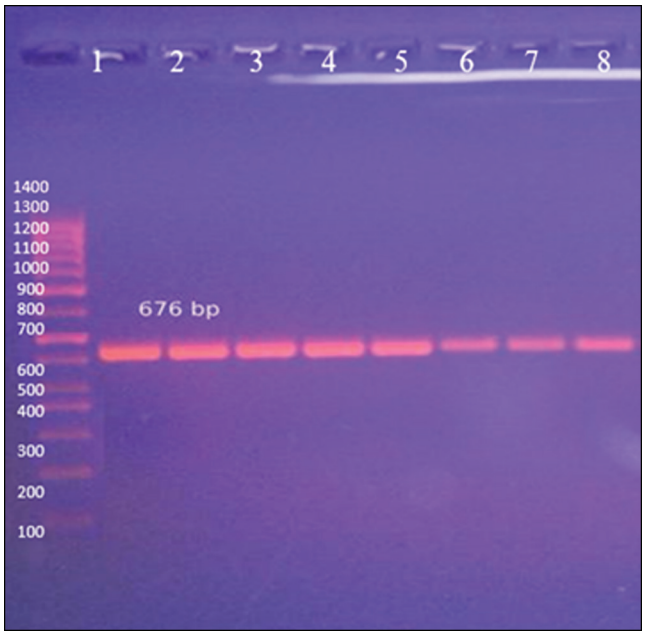

Figure 2 : Agarose gel electrophoresis of PCR product analysis of *neu*C gene in *E. coli* isolates. M (Marker ladder 1000-100bp). Lanes 1–8 showed positive *neuC* gene at 676 bp PCR product size

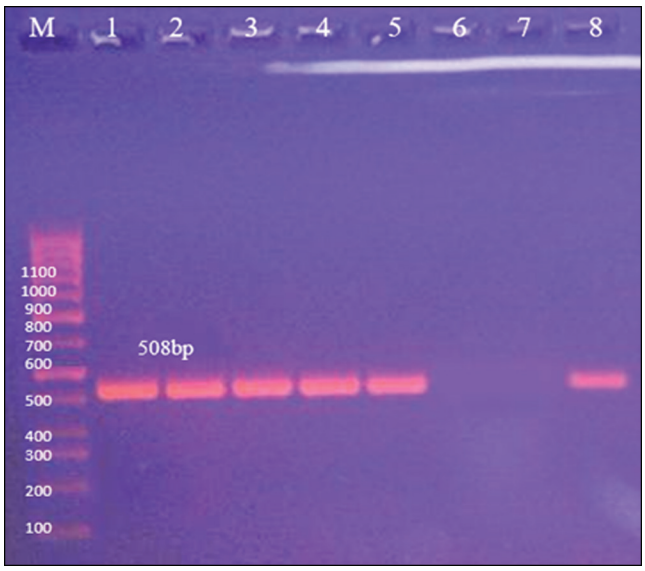

Figure 3 : Agarose gel electrophoresis of PCR product analysis of *fim*H gene in *E. coli* isolates. M (Marker ladder 1000-100bp). Lanes 1, 2, 3, 4, 5, and 8 showed positive *fimH* gene at 307 bp PCR product size

showed sensitivity to both drugs. These results are in close agreement with those results reported by Peng *et al*. for these two drugs.[18]

At the same time, the resistance of the isolates to the examined antibiotics continued to decline. There were only two isolates, representing 25% of the total *E. coli* isolates. Resistance to aztreonam was observed, and three isolates (37.5%) showed resistance to tobramycin. The study agreed with Peng *et al*., which indicated that the rate of resistance to aztreonam reached 34.7%. The rate of resistance to tobramycin in the same study reached 41.1%.[18] In the end, it showed 62.5%, as five out of eight isolates showed resistance to cefotaxime. The results are consistent with Awulachew *et al*. and Hassan *et al*. Table 3 displays the results of the susceptibility testing of the study samples.[6-18,20,21]

Detection of ESBL

The study isolates showed in the ESBL test that five out of eight samples had a wide zone of inhibition toward cefotaxime, four of which showed an increase in the inhibition zone with the ceftazidime tablet, and five samples showed inhibition of bacterial growth with the ceftriaxone disk.[22]

Detection of resistance genes

Detection of CTX-M gene: CTX-M-β-lactamases are not closely associated with TEM or SHV ESBLs. Organisms that generate CTX-M-β-lactamases are becoming prevalent globally as a cause of resistance to oxyiminocephalosporins, specifically CTX.[23] Consequently, these strains are regarded as a significant health issue. The amplification results showed that three samples out of eight (37.5% of the total) did not contain the *bla*CTX-M gene, while five samples (62.5% of the total) contained *bla*CTX-M gene. Abbasi *et al*. and Dirar *et al*. found similarly close findings. Figure 1 shows gel electrophoresis of the CTX-M gene.

Detection of *neu*C gene: *E. coli*, the pathogenic bacterium that causes diseases beyond the intestines, has a collection of 50 different capsular polysaccharides (K antigen) antigens. These antigens are generated by the transcription and translation of several genes. The K1-polysaccharide is a linear-homopolymer composed of *N*-acetylneuraminic-acid (also known as sialic-acid or NeunAc) molecules, which are linked together by α-2,8 links. The practical laboratory results of the study samples were positive, as the results of the PCR test indicated the presence of the *neu*C gene in all study isolates (100%). Studies indicating the diagnosis of this gene in *E. coli* confirm the isolation of samples from meningitis patients Proquot *et al*. Figure 2 shows gel electrophoresis of the *neu*C gene.[24]

Detection of *fim*H gene: *E. coli* type I fimbriae are multiportion hair-like appendages encoded by the *fim* cluster. The mannose-binding protein moiety helps in the synthesis of *fim*H which affects the host cell membrane and bacterial adhesion. *fim*H is expressed by most strains of *E. coli*.[25] The PCR results for the examination of the *fim*H gene indicated that the *E. coli* isolates being studied possess the *fim*H gene. *fim*H gene was detected in 87.5% of isolates, specifically in seven out of eight samples. These results are consistent with the findings by Hassan

et al., which showed a 71% detection rate. Figure 3 shows gel electrophoresis of the *fim*H gene.[20]

This study indicated that *E. coli* can evade the usual activity of antibiotics. The isolates exhibited resistance to the antibiotics under study, as the study samples were resistant to 13 antimicrobials under study at the same time. Isolate No. 5 was resistant to eight antibiotics, isolates No. 2 and 8 showed resistance to seven antibiotics, isolate No. 4 was resistant to six antibiotics, isolate No. 7 was resistant to five antibiotics, isolate No. 6 was resistant to four antibiotics, isolates No. 1 was resistant to four antibiotics, and isolate No. 3 was resistant to three antibiotics. Resistance of bacteria to multiple antibiotics is due to their possession of various virulence factors, mechanisms, and genes encoding resistance traits. All of this enables them to evade the effects of drugs, the most important of which are biofilm formation, capsules, LPS, efflux pumps, and other factors.[26-30]

Conclusions

Bacteria that exhibit a wide range of resistance patterns to antibiotics are considered dangerous health problems. The results showed that the study samples had many resistances to antibiotics. Seven of the *E. coli* samples were resistant to two or more of the drugs under study. Upon genetic examination, the isolates showed that they contain the capsule gene *neu*C, the gene encoding fimbriae type I *fim*H, and the CTX-M-β-lactamases gene. The current results indicate that bacteria possessing these genes exhibit resistance to the antibiotics under study.

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The funding for this study was provided by the researcher.

Conflicts of interests

There are no conflicts of interest.

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