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Antibiotic Susptipility Profile and Virulence Factors Profile of E.Coli Isolated from Otitis Media

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Abstract

The current study aimed to find Antibiotic susptipility profile and virulence factors profile of E.coli isolated from otitis media, for this purpose 200 ear swabs collected from patients suffering from otitis media whom attending to the ENT consulting clinic at Tikrit Teaching Hospital and the private outpatient clinic in Tikrit city. Bacterial isolation and identification were done, virulence factors profile detected by classical and genetic methods, and Antibiotic susptipility were done.

The result shows that out of 200 ear sample collected from otitis media, E.coli in rate of 10 % (20 out of 200), virulence factor detection by classical methods shows that he results of the current study showed that E.coli possessed protease, lecithinase, Dnase, Hemolysin and urease were 10%, 5%,20%, 20% and 0% respectively, while the enzymes denase and hemolysin appeared by 45.6% and 71.7%, respectively. When using genetic methods, the detection of virulence genes IroN, IutA, and F17 genes by 55%, 70%, and 45%, respectively. The results of the current study showed high resistant rate were for Ampicillin (90%) and for Cefotaxime and Amoxicillin (55%), while the low resistant is for Impenem (10%) and Ceftazidime (15%).

Introduction

Otitis media is defined as inflammation of the membrane lining the middle ear in whole or in part due to various types of microorganisms such as bacteria, fungi or viruses that reach the middle ear through the outer ear or after infection of the respiratory system through the nose or throat through a tube (1).

The first to isolated Escherichia coli was by the German scientist Theodor Escherich in 1885 from the feces of newborns, and he called it Bacterium coli, then it was remained as Escherichia coli by the two scientists Gastellani and Chalmers in 1919 (2). E.coli is Gram-negative bacteria, with shape varies from spherical to ciliated rod-shaped non-forming spores, mobile by flagella (peritricheus flagella). E.coli have may virulence factor such as somatic antigen, flagellar antigen, fimberal antigen, endotoxin, enterotoxin, Verotxine or shiga like toxine, chelating enzyme and hemolysin, these factor play important role in the pathogensis of bacteria (3,4)

Materials and methods :

- 200 ear swabs were collected from patients suffering from otitis media, the Patients attending the ENT consulting clinic at Tikrit Teaching Hospital and the private outpatient clinic in Tikrit city in period from September 2021 until the end of April 2022.
- Bacterial isolation and identification: each swab put in tube contain nutrient broth, then transport to bacteriology laboratory, then sub culturing on macConkey agar and EMB agar cultivation on 37C for 48hours, then sub culturing in same agar and cultivation condition. Gram stain and group of biochemical tests were done as to (5).

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- Detection of virulence factors by classical methods which inculde

a- Protease production : conducted according to (6)

b- Lecithinase production: : conducted according to (6)

c- DNAas: conducted according to (6)

d- Hemolysin production: conducted according to (6)

e- Urease production: conducted according to (6)

- Detection of virulence factors by PCR test:

a- DNA extraction: by using a ready-made kit (G-spin™ Total DNA Extraction) and according to manufacturer instructions.

b- Primer: primers us in current study as in table (1)

Table (1): primers us in current study

Primer	Sequence	Size of DNA product	Annealing temp	Annealing time (second)	References
iroN	F:AATCCGGCAAAGAGACGAACCGCC R:GTTCGGGCAACCCCTGCTTTGACTT	500	61	60	Johnson et al.,2008 (7)
iutA	F: GGCTGGACATCATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG	300	63	30	Johnson& Stell,. (2000). (8)
F17	F: GGGCTGACAGAGGAGGTGGGGC R:CCCGGCGACAACTTCATCACCGG	410	60	60	Salvadori et al.,2003 (9)

c- Components of the PCR master mix for one sample as in table (2)

Table (2): mixture compound and amount

Compound	Amount (microliter)
DNA Template	2.5
2X Master Mix	12.5
For word primer	1
Reverence primer	1
DNAse free water	8
Total	25

d- Thermocyclar program: as in table (3).

Table (3): thermocyclar program

Step	Temperature	Time (mints)	Cycles
First Denaturation step	94	4	1
Denaturation step	94	1	30
Primer-annealing step:	According to primers type		

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DNA extension step	72	1	
Final DNA extension step	72	10	1
End Temperature	4	--------	------

Antibiotic susceptibility test: conducted by disc diffusion methods and according to (10).

Results and discussion

Out of 200 ear sample collected from otitis media E.coli isolated in rate of 10% (20 out of 200) Escherichia coli appeared as pink colonies fermenting lactose sugar on macConkey agar (figure 1), while it appeared as bright green colonies on the Eosin-Methylene Blue medium (figure 2).

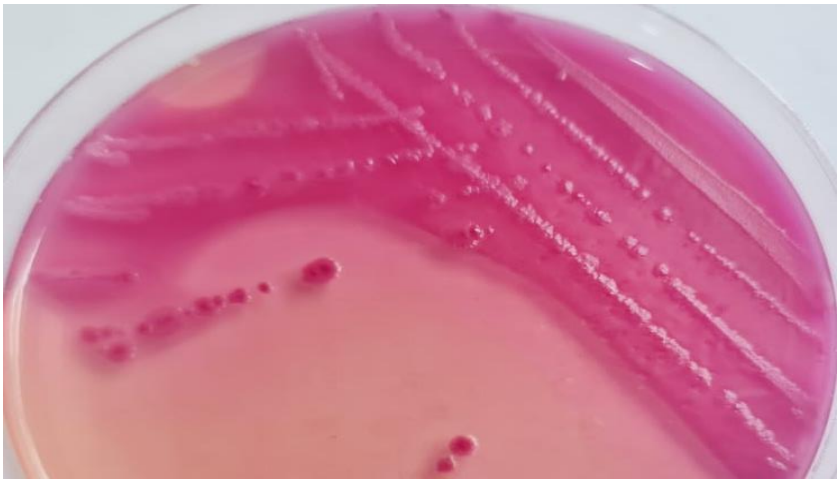

Figure (1): Escherichia coli grow on the medium of MacConkey agar. appeared as lactose-fermented colonies after 24 hours of incubation at 37 °C.

Figure 2. E. coli growing on EMB agar media and appearing as bright green colonies after 24 hours of incubation at 37°C

virulence factor detection by classical methods shows that he results of the current study showed that E.coli possessed protease, lecithinase, Dnase, Hemolysin and urease were 10%, 5%,20%, 20% and 0% respectively, while the enzymes denase and hemolysin appeared by 45.6% and 71.7%, respectively.

as from table (4)

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Table (4): virulence factor detected by classical methods

Virulence factors	Number of positive isolates	Rate
Protease	2	%10
Lecithinase	1	%5
DNase	4	%20
Heamolysin	4	%20
Urease	0	%0

All isolates were negative for urease, and this result coincided with what Al-Obaidy (2012) concluded, which indicated that all Escherichia coli isolates were negative for urease (11), while Yousif, (2011) indicated in a study that the percentage of urease enzyme appearance is 10%.(12) Hemolysin appeared at a rate of 20%, which is lower than the percentage recorded by Al-Obaidy (2012) and Yousif, (2011), which amounted to 60% and 66%, respectively (11,12). Hemolysin production is an important virulence factor for Escherichia coli because it decomposes erythrocytes, causing anemia and weakening of the host's defenses, and this provides a large amount of iron that the bacteria benefit from in their metabolic activity. Calcium ion concentration plus temperature and incubation period.(13).

In the current study, proteases was recorded at a rate of 10%, and this percentage is lower than the percentage previously recorded by Al-Obaidy (2012) and Yousif, (2011), which amounted to 20%.(11,12)

The polymerase chain reaction test showed that Escherichia coli possessed the IroN, IutA, and F17 genes by 55%, 70%, and 45%, respectively (Table 5) (figure 3,4,5)

Table (5) Virulence factors of E.coli bacteria detected by genetic methods

Gene	Rate of positive isolates	Rate of positive isolates
IroN	11	%55
IutA	14	%70
F17	9	%45

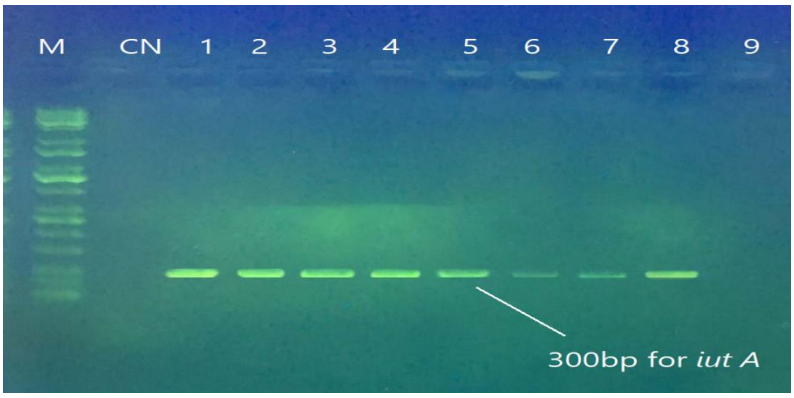

Figure (3) Escherichia coli electrophoresis results for the detection of the iutA gene. M indicates a molecular weight parameter, CN: negative control, pits 1–8: positive results.

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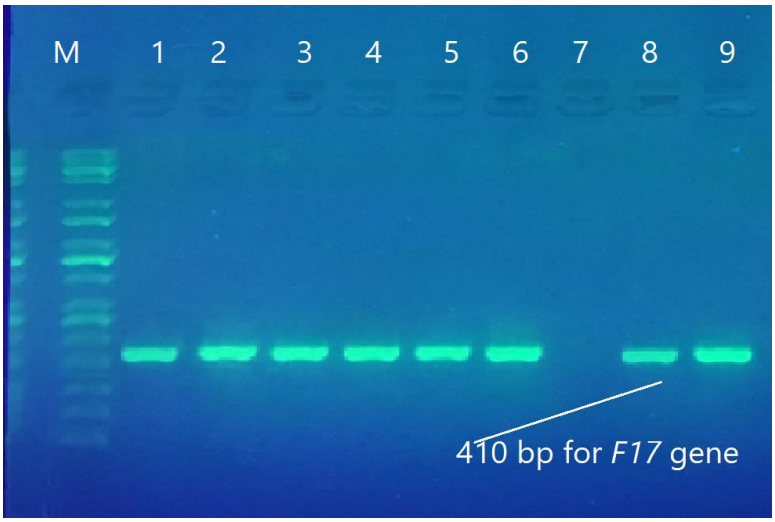

Figure (3) Escherichia coli electrophoresis results for the detection of the F17 gene, M indicates a molecular weight parameter, CN: negative control, pits 1-6, 8, 9: positive results

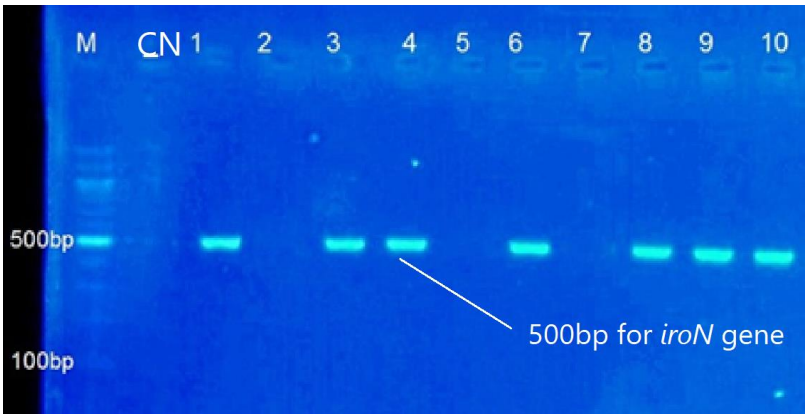

Figure (5): Escherichia coli electrophoresis results for detection of iroN gene, M denotes molecular weight parameter, CN: negative control, etch 1,3,4,6,8,9,10: positive results.

Iron is a necessary element as an oxidizing agent for proteins that contribute to electron transfer processes in the respiratory chain. It is also one of the most important elements for bacteria to cause infection (14). The gene iutA is a gene that encodes siderophores iron carriers (8).iroN gene, it encodes surface proteins that are considered to be receptors for iron (7,9). In general, iroN and iutA genes are among the most important genes refer to bacteria's ability to benefit from the iron available in the medium. The F17 gene that encodes the fermentation enzyme It is a major factor in bacterial adhesion and colonization of host cells (9).

From table (6) showed that high resistant rate were for Ampicillin (90%) and for Cefotaxime and Amoxicillin (55%), while the low resistant is for Impenem (10%) and Ceftazidime (15%)

Table (6)Antibiotic susceptibility test

Antibiotic	Resistant	Intermitted	Sensitive
Amoxicillin	11 (%55)	1 (%5)	(%40) 8
Tetracycline	10 (%50)	2 (%10)	8 (%40)
Chloramphenicol	9	1	10

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	(45%)	(%5)	(%50)
Gentamicin	5 (%25)	3 (%15)	12 (%60)
Impenem	2 (%10)	0 (%0)	18 (%90)
Vancomycin	9 (%45)	1 (%5)	10 (%50)
Erythromycin	9 (%45)	0 (%0)	11 (%55)
Azithromycin	8 (%40)	2 (%10)	10 (%50)
Ceftazidime	3 (%15)	2 (%10)	15 (%75)
Ciprofloxacin	4 (%40)	5 (%25)	11 (%55)
Trimethoprim	6 (%30)	1 (%5)	13 (%65)
Cefotaxime	11 (%55)	0 (%0)	9 (%45)
Ampicillin	18 (%90)	0 (%0)	2 (%10)

The characteristic of multiple resistance to bacterial isolates and to more than one antibiotic is one of the major and serious problems from a medical point of view, due to the difficulty of testing the appropriate treatment for the patient, and one of the main reasons for the emergence of multiple resistance is the indiscriminate use of antibiotics without relying on conducting a sensitivity test for them, which increases the chances of adaptation Bacteria and their resistance to antibiotics used in treatment (15) Bacteria may appear resistant to many antibiotics, as a result of transmission of the R-Factor resistance factor, which may be responsible for increasing resistance, especially in patients who are hospitalized for a long time. Most studies indicate the resistance of Gram-negative bacteria, especially members of the enteric family, to antibiotics of all kinds, especially beta-lactam antibiotics (16). Resistance to these antibiotics. The genetic basis for antibiotic resistance is the result of the presence of genes responsible for this resistance that are carried on the chromosome or on the plasmid. These genes possess enzymes responsible for destroying antibiotics and converting them into an ineffective form (17,18).

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