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Molecular ,Identification Bacterial causing Chronic Suppurative otitis media using 16srRNA

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Abstract :

Middle ear infection is a common problem that can occur in people of all ages. It happens as a result of fluid buildup behind the eardrum, which can cause the eardrum to burst if the inflammation persists, leading to the discharge of fluids and pus from the ear. Bacteria that cause middle ear infection can spread in two ways: either by moving from the nasal cavity to the middle ear through the Eustachian tube, or due to a problem or defect in the eardrum, allowing opportunistic pathogens to enter the middle ear and cause infection. Middle ear infection is a significant public health issue, with otitis media (OM) being the most common reason for children to visit doctors, take antibiotics, or undergo surgical procedures. It is an inflammatory condition affecting the mucous membrane lining the middle ear and its associated cavity. Accurate identification of the bacterial causative agent in such diseases is crucial for prescribing appropriate treatment to prevent recurrence or exacerbation. Moreover, precise identification of the bacteria responsible for the disease reduces the likelihood of indiscriminate antibiotic use, which can lead to the emergence of bacterial strains that are more resistant to antibiotics, making them harder to treat and eradicate.

Key Word : Otitis media , Discharge , CSOM , Middle ear , Perforation

الجزيئية، تحديد البكتيريا المسببة لالتهاب الأذن الوسطى القيحي المزمن باستخدام srRNA16

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الملخص:

تعد عدوى الأذن الوسطى مشكلة شائعة يمكن أن تحدث للأشخاص من جميع الأعمار. وتحدث نتيجة لتراكم السوائل خلف طبلة الأذن، مما قد يتسبب في انفجار طبلة الأذن إذا استمر الالتهاب، مما يؤدي إلى إفراز السوائل والقيح من الأذن. يمكن أن تنتشر البكتيريا المسببة لعدوى الأذن الوسطى بطريقتين: إما بالانتقال من تجويف الأنف إلى الأذن الوسطى عبر قناة استاكيوس، أو بسبب مشكلة أو عيب في طبلة الأذن، مما يسمح

لمسببات الأمراض الانتهازية بالدخول إلى الأذن الوسطى والتسبب في العدوى. تعد عدوى الأذن الوسطى مشكلة صحية عامة كبيرة، حيث يعد التهاب الأذن الوسطى السبب الأكثر شيوعًا لزيارة الأطفال للأطباء أو تناول المضادات الحيوية أو الخضوع للإجراءات الجراحية. إنها حالة التهابية تؤثر على الغشاء المخاطي المبطن للأذن الوسطى والتجويف المرتبط بها. يعد التعرف الدقيق على العامل المسبب للبكتيريا في مثل هذه الأمراض أمرًا بالغ الأهمية لوصف العلاج المناسب لمنع تكرار حدوثها أو تفاقمها. علاوة على ذلك، فإن التعرف الدقيق على البكتيريا المسؤولة عن المرض يقلل من احتمالية استخدام المضادات الحيوية بشكل عشوائي، مما قد يؤدي إلى ظهور سلالات بكتيرية أكثر مقاومة للمضادات الحيوية، مما يجعل علاجها والقضاء عليها أكثر صعوبة.

الكلمات المفتاحية: التهاب الأذن الوسطى، إفرازات، التهاب الأذن الوسطى، الأذن الوسطى، ثقب

Introduction:

Chronic suppurative otitis media (CSOM) is a common problem in the ear characterized by the discharge of fluids, pus, and blood from the ear due to perforation of the tympanic membrane (Orji& Dike,2015;Chiappini *et al*.,2019;Hayashi *et al*.,2020). This often leads to hearing loss in both children and adults, accompanied by severe inflammation of the middle ear mucosa, leading to the discharge of fluids and pus (Bhutta *etal* .,2024; Wan Draman *etal*.,2021).CSOM occurs as a result of prolonged acute otitis media lasting more than 12 weeks (Gupta *etal*.,2020) CSOM is considered a significant health issue, especially in developing and low-income countries (van der Veen *etal*.,2006;Madana *etal*.,2011; Taipale *etal*.,2011; Sakulchit, & Goldman ,2017). Chronic suppurative otitis media affects all age groups, but children are more prone to it due to their weakened immune systems (Taipale etal.,2011; Sakulchit, & Goldman ,2017; Minovi.,2014; Wahid etal.,2014) and shorter Eustachian tubes (Luo *etal*.,2014)), which facilitate the transfer of pathogens from the nasopharynx to the middle ear (Lechien *etal*.,2021).

. According to the World Health Organization in 2015, more than 5% of the world's population suffers from middle ear infection, equivalent to 328 million adults and 32 million children(Elmanama *etal*.,2014). The causative agents responsible for middle ear infections are often bacterial. The most important types of bacteria causing middle ear infections are: *Pseudomonase aeruginosa ,Staphlococcus aureus ,Staphlococcus epidermidis ,Streptococcus pneumonia ,Proteus mirabilis ,k.pneumonia ,E. coli*(Marom *etal*.,2012;Protasova etal.,2017; Ubukata *etal*.,2019; Chonmaitree *etal*.,2016).

Polymerase Chain Reaction (PCR) technology is a modern method for accurately diagnosing bacterial species causing diseases, allowing for rapid detection through the amplification of a small portion of the DNA(Sune *etal*.,2020). This technique is particularly important for strains that are difficult to culture using traditional

methods such as Haemophilus and Moraxella(Hayashi *etal*.,2020). Additionally, traditional methods take much longer compared to PCR technology(Wasfi *etal*.,2020). The infection may occur in one ear, or it may occur in both ears of the same person. The infection may be caused by one type of bacteria, or a group of species may be responsible for causing the infection(Jensen *etal*.,2011)

Material & Method :

The study included 50 samples from patients with chronic suppurative otitis media, collected from the ENT clinic at Al Salam Teaching Hospital in Mosul city. Samples were taken from patients diagnosed with the condition by specialist doctors at the clinic. Swabs were inserted into the ear after cleaning with povidone-iodine and saline solution to eliminate contamination from natural flora in the ear canal. Samples were then transported to the laboratory for culture and necessary biochemical tests. Bacteria were cultured on Blood agar, MacConkey agar, and Chocolate agar, and incubated at 37°C for 18-24 hours. Cultures showed positive growth in 43 out of 50 samples, while 7 showed no growth, possibly due to prior antibiotic use, non-bacterial pathogens, or anaerobic bacteria requiring specific conditions for growth. Isolates were subcultured on individual plates using cross-streaking technique to obtain pure cultures. Biochemical tests were performed, followed by DNA extraction for further analysis. The DNA was extracted from the collected samples following their cultivation on agar media, subsequent isolation, purification, and biochemical assays. The DNA extraction was performed for 100 samples using a DNA isolation kit according to the manufacturer's instructions. The procedure was as follows: a loopful of pure, single colony was taken and placed in a 200 µL microcentrifuge tube, to which 1 mL of PBS solution was added. After 5 minutes, centrifugation was carried out for 2 minutes using a refrigerated microcentrifuge. The supernatant was decanted, and the pellet was resuspended in either 20 µL of Protenis K and 180 µL of GT buffer (for Gram-negative samples) or 200 µL of Lysozyme and 20 µL of Protenis K (for Gram-positive samples). The sample was then transferred to a water bath at 60°C (Elution buffer was also preheated at this stage). The sample remained in the water bath for 10 minutes with gentle mixing every 3 minutes. After 10 minutes, 200 µL of GB buffer was added, and the water bath temperature was raised to 70°C. The sample was kept in the water bath for another 10 minutes with mixing every 3 minutes. Afterward, 200 µL of absolute ethanol was added, mixed quickly, and transferred directly to a filter provided by the company. Centrifugation was performed for 2 minutes, and the supernatant was decanted, leaving the pellet on the filter. Then, 400 µL of Wash 1 buffer was added, followed by another centrifugation for 2 minutes. The supernatant was decanted, leaving the pellet on the filter. Next, 600 µL of Wash 2 buffer was added, followed by centrifugation for

2 minutes. The supernatant was decanted, leaving the pellet on the filter. The filter was transferred to a new microcentrifuge tube, and 50 µL of preheated Elution buffer was added in two stages, with 25 µL added each time. The filter was left for a minute, followed by centrifugation. This process was repeated for the second stage. Subsequently, the purity of the sample was measured using a Nanodrop device to ensure the purity of the extracted DNA. The sample was then stored and labeled with freezing information until further use for gel electrophoresis and PCR.

Table 1 Equipment used to extract DNA

NO	The device	Company	Origin
1.	Incubator	Binder	Germany
2.	Water bath	Gall encamp	England
3.	Vortex	Heidolph	Germany
4.	Centerfuge	Baird & Tatlock	Germany

Table 2 Materials used to extract DNA

NO	Substance	Quantity
1.	GB buffer	40 ml
2.	GT buffer	30 ml
3.	Lysozyme	110 mg
4.	W1 buffer	45 ml
5.	Wash buffer	25 ml
6.	Protenase K	1.1 gm
7.	Elution buffer	30 ml
8.	GD Column	100 pcs
9.	2ml Collection	100 pcs

Prepare a 1x TAE solution, by diluting 200 ml of TAE50X (0.08M) MTris 0.08 actic acid and 0.02M EDTA, diluted to 10X by taking 200 ml of TAE 50X and adding 800 ml of deionized water (ddH2o), and this 10x buffer was restored. Dilute it to 1x (working solution) by taking 100 ml and adding it to 900 ml of deionized water. Agarose gel was prepared at a concentration of 2%, adding 600 ml of 1x TAE, which was measured using a measuring cylinder. 1.2 grams of agarose powder with a concentration of 2% and 1.8 gm with a concentration of 3% were weighed and placed in a beaker. 1X TAE was added to the agarose to be analyzed. The mixture was heated in the microwave to ensure that all the particles were dissolved in the solution and mixed. The solution was then left to cool to 70C

Celsius, then Red Jel dye was added, after which the solution was left to cool to 50 degrees Celsius. The agarose solution was then poured into the loading tray and the combs were installed. The solution was then left to cool at room temperature (20-25C) for 30 minutes, after which the gel was immersed in 400 ml of 1x TAE solution, then10 microliters of DNA was injected into the loading pits with a Ladder placed in one of the first pits, after which the device was connected to an electrical current with a voltage of 80v for 80 minutes. After completion, the gel was transferred to the ultraviolet measurement room and the beams were recorded with pictures.

Results and Discussion:

The study results showed that out of 50 samples, 35 were male and 15 were female. This study corroborated with (Wahid etal.,2014; Restuti etal.,2022; Libwea etal.,2018) possibly due to males being more exposed to external factors and disease-causing agents because of their nature of work and activities such as swimming in contaminated rivers and pools, while it differed from (Uddén *etal*.,2018; Xu *etal*.,2021; Kumar *etal*.,2011; Anifasi & Tumushime-Buturo,1989; Fairbanks,1986). The results indicated that the left ear had the highest incidence with 28 out of 50 samples, accounting for 56%, while the right ear had 22 cases out of 50, accounting for 44%. The current study also revealed that children had a higher infection rate than adults, with 32 out of 50 children infected, accounting for 64%. Additionally, 100% of the patients discharged from the hospital had perforation of the tympanic membrane, and 66% of them experienced hearing loss. Furthermore, 5% of the patients suffered from tinnitus, and 11% suffered from m. The results of using PCR technology for all samples showed that the most common bacteria causing otitis media were *Pseudomonas aeruginosa, followed by Staphylococcus aureus, Staphylococcus epidermidis,Streptococcus pneumonia ,Proteus mirabilis ,klebsella pneumonia ,E. coli*.

Table 3 The percentage of bacterial isolates from patient

No.	Bacterial Isolates	No	Percentage
1.	*Pseudomonase aeruginosa*	17	34%
2.	*Staphlococcus aureus*	8	16%
3.	*Staphlococcus epidermidis*	5	10%

4.	*Streptococcus pneumonia*	7	14%
5.	*Proteus mirabilis*	4	8%
6.	*k.pneumonia*	6	12%
7.	*E. coli*	3	6%

Conclusions:

1. Gram-negative bacteria are the most isolated and the leading cause of chronic suppurative otitis media.

2. The infection rate is higher in males than females, and children are more affected than adults.

3. The infection rate increases in rural areas and in developing and economically disadvantaged countries.

4. Using PCR diagnosis is faster and more accurate than traditional diagnostic methods

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التعرف الجزيئي على البكتريا المسببة لالتهاب الاذن الوسطى المزمن القيحي باستخدام 16s rRNA

الخلاصة : التهاب الأذن الوسطى هو مشكلة شائعة يمكن أن تحدث للأشخاص من جميع الأعمار. يحدث نتيجة تجمع السوائل خلف طبلة الأذن، مما قد يؤدي إلى انفجار الطبلة السمعية إذا استمر التهابها، مما يؤدي إلى تدفق السوائل والقيح من الأذن. يمكن أن تنتشر البكتيريا التي تسبب التهاب الأذن الوسطى بطريقتين: إما بالانتقال من التجويف الأنفي إلى الأذن الوسطى عبر قناة الاستاكيوس، أو بسبب مشكلة أو عيب في طبلة الأذن، مما يسمح للكائنات الدقيقة الانتهازية بالدخول إلى الأذن الوسطى والتسبب في العدوى.

التهاب الأذن الوسطى يعد مشكلة صحية عامة هامة، حيث يعتبر التهاب الأذن الوسطى السبب الأكثر شيوعاً لزيارة الأطفال للأطباء، وتناول المضادات الحيوية، أو إجراء عمليات جراحية. إنه حالة التهابية تؤثر على الغشاء المخاطي الذي يبطن الأذن الوسطى والتجويف المرتبط بها. وان التعرف الدقيق على المسبب البكتيري لهذا النوع من الامراض مهم جدا في وصف العلاج المناسب للمريض لمنع عودة الاصابة او تفاقمها ، كما ان التعرف الدقيق للبكتريا المسببة للمرض يقلل من احتمالية الاخذ العشوائي للمضادات الحيوية التي تسبب ظهور سلالات بكتيرية اكثر مقاومة للمضادات الحيوية مما يصعب علاجها والقضاء عليها .

الكلمات المفتاحية : التهاب الاذن الوسطى ، سوائل قيحية ، التهاب الاذن الوسطى المزمن القيحي ، انثقاب غشاء الطبلة