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ORIGINAL ARTICLE

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The role of non-typable Haemophilus influenza in recurrent otitis media in children

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Abstract

Background When reaching their first birthday, kids in poor nations and about 62% of children in wealthier countries experience their initial occurrence of otitis media. The primary culprits for these bacterial ear infections in infants are the non-typeable Haemophilus influenzae (NTHI) strains, known for causing both acute and recurring episodes of otitis media. This study aimed to isolate and identify the non-typable Haemophilus influenzae isolates in children with recurrent otitis media and to evaluate the antibiotic sensitivity of the isolated Haemophilus influenzae strains.

Methods This cross-sectional study was conducted on 60 children (38 males and 22 females) who suffered from recurrent acute otitis media, ranging in age from 6 months to 5 years, and presented at the outpatient clinic between June 2022 and March 2023. Swabs were collected from ear discharge and examined using the Vitek system and PCR.

Results Microbiological examination of ear swabs revealed that H. influenzae was the most commonly isolated species (38.3%), followed by S. pneumoniae (26.7%). The current study found that 82.6% of Haemophilus influenzae isolates were non-typeable, whereas only 17.4% were typeable based on PCR results. The results were considered highly significant (Hs) as the *P* value was < 0.001. High-level resistance of H. influenzae isolates was observed against ampicillin (78.3%), while high-level sensitivity was noted with Amoxicillin-clavulanate (73.9%). NTHi was significantly associated with the presence of complications compared to other organisms (78.3%).

Conclusions This study concluded that NTHi plays a significant role in recurrent acute otitis media (rAOM). Therefore, we hope that significant advancements will be made in developing an efficient vaccine against NTHi in the near future.

Keywords Recurrent AOM, NTHi, Vaccines against NTHi, Vitek, PCR

Background

Acute otitis media (AOM) ranks among the most common childhood illnesses, affecting about 50% of children, with approximately 80% experiencing it at some point in their lives. Recurrent AOM (rAOM) in a child is defined as having four or more episodes within a year or at least three episodes within 6 months. Various risk factors, such as prior upper respiratory tract infections and genetic factors, can increase a child's susceptibility to developing acute otitis media. Prompt diagnostic and therapeutic measures are crucial to prevent complications such as speech, language, and cognitive delays [1].

A middle ear infection can result from viral, bacterial, or combined infections. Primary bacterial culprits causing otitis media include Streptococcus pneumoniae, non-typeable Haemophilus influenzae (NTHi), and Moraxella catarrhalis. With the widespread use of

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vaccines, there has been a shift in the prevalence of pathogens causing persistent acute otitis media (AOM), leading to a decrease in S. pneumoniae cases and an increase in NTHi occurrences. NTHi is commonly found in the middle ear of children experiencing recurrent acute otitis media (rAOM) and/or chronic otitis media with effusion [2].

There are two types of H. influenzae strains categorized based on the presence or absence of a capsule: those with a capsule (encapsulated or typeable) and those without (non-encapsulated or non-typeable). Encapsulated strains are divided into six serotypes (A to F), sharing relative similarity irrespective of their serotype. Conversely, non-encapsulated strains exhibit genetic diversity [3].

NTHi employs various strategies to evade host immunity and thrive within its host. Biofilm formation enhances antibiotic resistance in many NTHi strains and shields the bacteria from external stressors. Additionally, NTHi generates several surface elements, including outer membrane proteins (OMPs) and lipooligosaccharides (LOS) [4].

Despite extensive global efforts, there is currently no accessible vaccine for preventing NTHi infections. Antibiotics are the primary treatment for NTHi infections, but there is growing concern about the rapid increase in antibiotic and multi-drug-resistant bacteria. Hence, there is an urgent need for enhanced or alternative treatment methods for these illnesses [5, 6].

The aim of the present study is to isolate and identify non-typable Haemophilus influenzae isolates in children with recurrent otitis media and to assess the antibiotic sensitivity of the isolated Haemophilus influenzae strains.

Methods

This cross-sectional study involved 60 children (38 males and 22 females) suffering from recurrent acute otitis media, aged between 6 months and 5 years, who presented at the outpatient clinic of Benha University Hospital. Microbiological examinations were conducted at the Otolaryngology Department and Medical Microbiology and Immunology Department, Benha University, between June 2022 and March 2023.

The study received approval from the Ethical Committee of the Benha Faculty of Medicine. Research procedures adhered to the Helsinki Declaration of 1975 and its subsequent amendments, with written informed consent obtained from participants' parent or legal guardians in the case of children under 16).

Please include a statement on informed written consent from parents/guardians of patients under 16 years old.

Children meeting any of the following criteria were excluded from the study: age exceeding 5 years, patients with an intact eardrum, presence of signs of chronicity, and patients undergoing antibiotic treatment.

The patients underwent the following assessments

Comprehensive medical history evaluation, covering aspects such as fever, ear pain, feeding issues, irritability, ear discharge, frequency of attacks, identification of complications, and prior ear surgeries. Risk factor data were collected through a parent-administered questionnaire, which included family history of acute otitis media (AOM), breastfeeding practices, and exposure to tobacco smoke in the child's primary residence.

Symptoms were quantified using a five-question parent-based survey (Ear Treatment Gro5 symptom questionnaire—ETG-5), assessing ear pain, fever, irritability, feeding problems, and disrupted sleep [7].

Otoscopic examination of the ear, nose, nasopharynx, and oropharynx.

Microbiological examination

Swabs were taken from ear discharge and placed in sterile tubes containing specific transport media, then immediately delivered to the laboratory. The specimens were cultured on blood agar, chocolate agar, and MacConkey agar plates. Colonies were identified by colony morphology and microscopic examination following Gram stain staining, with further identification using VITEK® 2 Systems cards for Gram-positive and Gram-negative aerobic bacteria (BioMerieux, France). Antibiotic susceptibility testing was performed on isolated Haemophilus influenza strains using VITEK® 2 antibiotic susceptibility cards (BioMerieux, France) (Fig. 1).

Polymerase chain reaction (PCR) was employed to differentiate H. influenzae isolates by molecularly identifying the presence of the bexA and bexB genes,

Fig. 1 Microscopic observation of a specimen H. influenzae shows gram-negative coccobacillus

which indicate typeable H. influenzae isolates, allowing for the distinction between typeable and non-typeable strains.

DNA extraction

It was performed using the ABT Bacterial DNA Mini Extraction Kit following the manufacturer's instructions (Applied Biotechnology, Egypt). Purified DNA was stored at – 20 °C until used.

Gene amplification

Using the bexA and bexB gene-specific primers. The primer sequences used are shown in Table 1. PCR was conducted in a Biometra thermal cycler (Germany) using a PCR master mix, specifically the 2X TOPsimple™ DyeMIX-nTaq (Enzynomics, Korea). The procedure adhered to the manufacturer's instructions.

The PCR process included an initial denaturation at 95 °C for 2 min, followed by 30 cycles comprising denaturation at 95 °C for 30 s, primer annealing at 54 °C for 30 s, and extension at 72 °C for 45 s. The final extension phase lasted for five minutes at 72 °C. The resulting amplified products were separated using 1.5% agarose gel electrophoresis and observed after staining with ethidium bromide for visualization (Fig. 2).

Table 1 Sequences primers utilized in the study

Genes	Primer sequence	Size amplicon (bp)	Reference
bexA	Forward: 5′CGTTTGTATGAT GTTGATCCAGAC-3′ Reverse: 5′-TGTCCATGTCTT CAAAATGATG-3′	343	(Al-Buhilal et al., 2022 [8]) 7
bexB	Forward: 5′GGTGAT TAACGCGTTGCTTAT GCG-3′ Reverse: 5′-TTGTGCCTGTGC TGGAAGGTTATG3′	567	(Al-Buhilal et al., 2022 [8]) 7

Statistical analysis

The data were processed utilizing SPSSTM software, version 22.0 (IBM Corporation, Armonk, NY, USA). Categorical data was presented as numbers and percentages, while continuous data was displayed as the mean and standard deviation (SD). Appropriate statistical tests were applied to assess significance.

Results

The study included 38 male and 14 female patients aged between 6 months and 5 years. There was a notable connection between recurrent otitis media and factors such as a positive family history of recurrent AOM among parents or siblings, bottle feeding, exposure to smoke, nasal discharge, and snoring (Table 2).

Microbiological examination of ear swabs showed that H. influenzae was the most frequently identified species (38.3%), followed closely by S. pneumoniae at 26.7% (Fig. 3).

The current study revealed that 82.6% of Haemophilus influenzae isolates were non-typeable, whereas only 17.4% were typeable based on PCR results. Results were considered highly significant as the P value was < 0.001. High-level resistance of H. influenzae isolates was observed against ampicillin (78.3%), while high-level sensitivity was seen with Amoxicillin-clavulanate (73.9%) (Table 3) (Wang H-J. et al. [9]).

NTHi was significantly associated with the presence of complications compared to other organisms (78.3%). Complications encountered were in the form of mastoiditis, chronic OM, persistent otorrhea, and recurrence (Table 4).

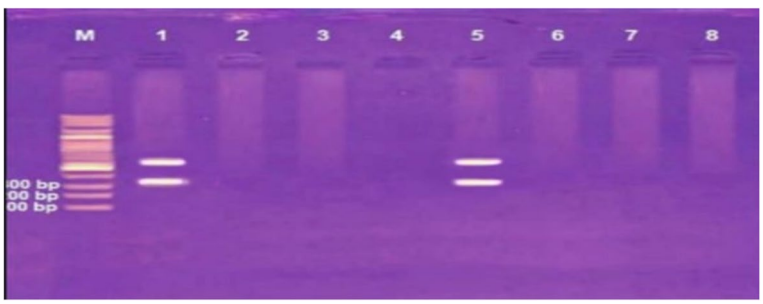

Fig. 2 PCR products bexA and bexB genes H. infleunza: Lane M: marker with 100 bp ladder, lanes 1 and 5: positive for bexA and bexB genes. Other lanes: negative for bexA and bexB genes

Table 2 Association between risk factors and recurrent otitis media

Risk factors	Otitis media patients		OR and Coefficient b	*P* value	OR 95% CL
	No	%			
Young age (Less than 3 years)	38	63.3%	4.45(1.49)	< 0.0001*	(2.23–8.88)
Positive family history of recurrent AOM in parents or siblings	26	43.3%	4.23(1.44)	< 0.0001*	(2.85–6.29)
Bottle feeding	22	36.7%	2.2 (0.79)	0.03*	(1.06–2.94)
Smoke exposure	35	58.3%	3.15(1.15)	0.002*	(1.97–3.54)
Nasal discharge	45	75%	5.73(1.39)	< 0.0001*	(3.47–9.45)
Snoring	20	26.7%	1.91(0.65)	0.02*	(0.98–2.15)

* Statistically significant, *OR* odds ratio, *95% CL* 95% confidence limits

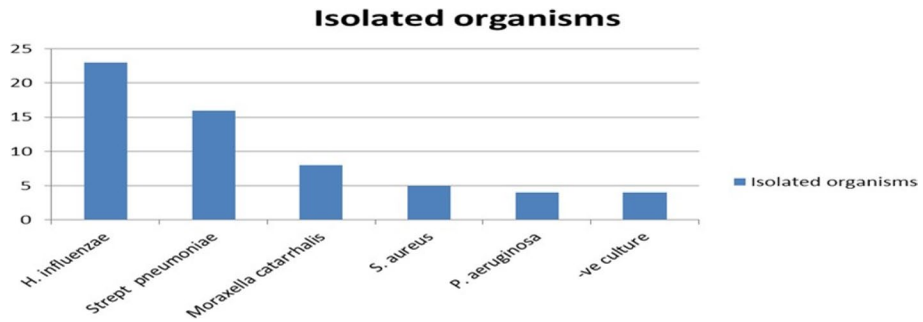

Fig. 3 Distribution of microbiological organisms isolated from ear swab identified by Vitek system. Note: Negative culture could be because of viral infections or infections by atypical organisms like chlamydia and mycoplasma

Table 3 Antibiotic sensitivity patterns isolated Haemophilus influenza by vitek system Wang H-J. et al. [9]

Antibiotic	*Hi* isolates		
	Sensitive	Intermediate	Resistance
Ampicillin	2 8.7%	3 13%	18 78.3%
Ciprofloxacin	12 52.2%	8 34.8%	3 13%
Amoxicillin-clavulanate	17 73.9%	3 13.1%	1 4%
Cefotaxime	8 34.8%	7 30.4%	8 34.8%
Cefuraxime	7 30.4%	8 34.8%	8 34.8%
Erythromycin	9 39.15	5 21.8%	9 39.1%
Imipenem	13 56.6%	6 26.1%	4 17.3%
Chloramphenicol	9 39.2%	7 30.4%	7 30.4%

Table 4 Association between associated complications and isolated organisms identified by Vitek system and PCR

Isolated organisms	Associated complications		
	Positive	Negative	Total
H. influenza	18 78.3%	5 21.7%	23 100%
Strept pneumonia	5 31.2%	11 68.8%	16 100%
Moraxella catarrhalis	2 25%	6 75%	8 100%
S. aureus	1 20%	4 80%	5 100%
P. aeruginosa	1 25%	3 75%	4 100%
-ve culture	2 50%	2 50%	4% 100%

Haemophilus influenza that caused complications was NTHi. Complications encountered were in the form of mastoiditis, chronic OM, persistent otorrhea, and recurrence

Discussion

Acute otitis media (AOM) stands as one of the most commonly diagnosed bacterial infections in children, leading to frequent pediatric visits in both developed and developing nations. Roughly three out of four children experience at least one episode of AOM by the time they reach 3 years of age [1].

AOM is an illness caused by multiple pathogens. Streptococcus pneumoniae (S. pneumoniae) and Non-typeable Haemophilus influenzae (NTHi) are consistently

cited as the primary bacterial agents behind AOM, found in up to 80% of cases. Additionally, other commonly mentioned bacterial pathogens in AOM cases encompass Moraxella catarrhalis (M. catarrhalis) and Streptococcus pyogenes (S. pyogenes) [8].

Recent studies have identified H. influenzae as the prevailing bacterial infection in recovered cases. Specifically, AOM induced by NTHi has been associated with recurrence. In about one-third of instances, recurrent chronic AOM might increase the likelihood of developing chronic suppurative otitis media (CSOM) [3].

Since the introduction of pneumococcal and H. influenzae type b vaccines, there has been an increase in the proportion of non-typeable H. influenzae (NTHi) colonizing the nasopharynx. Currently, the global impact of non-invasive infections caused by NTHi is substantial, encompassing conditions like otitis media, acute bacterial paranasal sinusitis, conjunctivitis, and pneumonia. The rate of antimicrobial resistance in H. influenzae has significantly surged in recent years.

The percentage of H. influenzae susceptible to ampicillin declined from 46% in the period between January and June of 2011 to 39% in the period between January and June of 2018 [10].

Our findings indicate a correlation between younger age and a heightened recurrence rate of OME (P value < 0.0001). This observation aligns with the findings of Riaz N. et al. [4]. This connection could be attributed to anatomical differences in the eustachian tube among younger individuals, characterized by its short, flat structure, acute angle to the horizontal plane, large lumen, and wide isthmus.

The current study showed that the family history of OM was positive in 43.3% of cases. Results were considered significant (s) as the P value was < 0.0001. This agrees with the results of Wijayanti et al. [11] study in Indonesia.

Our study revealed a significant effect of bottle feeding on the increased risk of ROM. This coincides with the results of a meta-analysis by Bowatte et al. [12]. In contrast to these findings, El-Houfey et al. [13] found no correlation between OME and breastfeeding.

Studying the risk factors associated with OM in children, exposure to passive smoking at home was among the first factors investigated. In our patient group, the rate of passive smoking was 58.3%, showing a notable p value (0.002). Similar results were previously documented by Zhang et al. [14]. Conversely, Martines et al. [15] found no statistically significant association between the risk of recurrent otitis media and exposure to smoke.

In our research, we observed a significant association between nasal discharge and snoring with AOM, which aligns with the findings of Kırıs et al. [16] and Baljošević et al. [17]. These studies reported chronic tonsillitis, adenoid hypertrophy, rhinosinusitis, and recurrent upper respiratory tract infections (URTIs) as the most common predisposing factors for OME.

In this study, statistical data for the distribution of microbiological organisms isolated from ear swabs and identified by the Vitek system revealed that H. influenzae constituted the most commonly isolated species (38.3%), followed by S. pneumoniae (26.7%). Other organisms involved were Moraxella catarrhalis (13.3%), Staphylococcus aureus (8.3%), and Pseudomonas aeruginosa (6.7%). Negative cultures were present in 6.7% of cases, possibly due to viral infections or infections by atypical organisms like Chlamydia and Mycoplasma. These results closely align with those observed by Hu et al. [10], who found that in children, Haemophilus influenzae is the most common cause of acute otitis media, conjunctivitis, and acute bacterial paranasal sinusitis.

This aligns with the findings of Sierra et al. [18], who identified H. influenzae in 31 out of 99 (31%) cases and S. pneumoniae in 30 out of 99 (30%) cases as the most frequently isolated bacteria. S. pyogenes was found in only 2 out of 99 (2%) cases, while other bacteria were present in 5 out of 99 (5%) cases. These results are consistent with various studies conducted in Latin America. There is mounting evidence suggesting that H. influenzae, rather than S. pneumoniae, plays a more prevalent role in causing AOM [19]. A previous study conducted in the United States (1995–2003) examined changes in the frequency and pathogens associated with AOM following the introduction of the pneumococcal conjugate vaccine (PCV). This study revealed that among middle ear isolates, H. influenzae was more prevalent than S. pneumoniae, indicating that H. influenzae has become the primary pathogen causing AOM since the introduction of the pneumococcal conjugate vaccine [17].

In our study, 82.6% of Haemophilus influenzae isolates were non-typeable, while only 17.4% were typeable based on PCR results. These results were considered highly significant (Hs) as the P value was < 0.001.

This supports the findings of Sierra et al. [18], indicating that NTHi was involved in 27 out of 31 (87%) AOM episodes in their study. Studies have shown that NTHi contributes to a higher number of recurrent AOM cases compared to S. pneumoniae and S. pyogenes. It is linked to recurring AOM episodes, treatment resistance, and instances of AOM recurring within two weeks after completing antibiotic treatment, making it a challenging pathogen to manage. Our research further corroborated these findings, identifying NTHi as the culprit in 6 out of 7 cases of recurrent AOM.

The current research revealed a significant association between H. influenzae and the presence of complications compared to other microorganisms (78.3%), aligning

closely with findings observed by Cho et al. [20] in Europe.

Our study displayed an elevated level of resistance among H. influenzae isolates to ampicillin (78.3%) and a high degree of sensitivity to Amoxicillin-clavulanate (73.9%). These outcomes closely resembled those observed by Omoding and Bazira [21]. This study, conducted from January to May 2018, focused on clinically healthy children under 5 years old enrolled at the Maternal and Child Health (MCH) Clinic at Mbarara Regional Referral Hospital.

Additionally, Gavrilovici et al. [22] reported that the World Health Organization classified Haemophilus influenzae and Streptococcus pneumoniae as medium priority due to their resistance to ampicillin and penicillin, respectively.

This aligns with Hu et al. [10], who revealed that the rate of antibiotic resistance in H. influenzae was 11% for second- or third-generation cephalosporins, 18% for amoxicillin-clavulanate, and 80% for ampicillin.

This study has some limitations that warrant consideration. The sample size, limited to 60 children from a single center, may not fully represent the broader pediatric population with recurrent otitis media. Exclusion criteria, including age restrictions and antibiotic treatment status, might have omitted relevant cases. Additionally, reliance on subjective data, potential limitations in the sensitivity of the PCR technique used for differentiation, and the scope of antibiotic sensitivity testing could impact the generalizability and comprehensive understanding of the study. Furthermore, the observational nature of the study restricts the establishment of causal relationships between non-typeable Haemophilus influenzae and complications.

Conclusions

The research findings highlighted the significant involvement of NTHi in recurrent acute otitis media (rAOM), often displaying resistance to antimicrobial treatment. The regular immunization of infants with conjugated vaccines targeting H. influenzae type b (Hib) has notably reduced the occurrence of Hib disease. Thus, there is an optimistic outlook for substantial progress in developing effective vaccines against NTHi in the near future. A genome-driven vaccination strategy could potentially unveil more vaccine antigens, providing valuable insights for further development.

Abbreviations

NTHi	Non-typeable Haemophilus influenzae
PCR	Polymerase chain reaction
rAOM	Recurrent acute Otitis Media
AOM	Acute otitis media
LOS	Lipooligosaccharides
OMPs	Outer membrane proteins
PCR	Polymerase chain reaction
SD	Standard deviation
ETG-5	Ear Treatment Gro5 symptom questionnaire
SPSS	Statistical Package for the Social Sciences
DNA	Deoxyribonucleic acid
USA	United States of America
NY	New York
S. pneumoniae	Streptococcus pneumoniae
M. catarrhalis	Moraxella catarrhalis
S. pyogenes	Streptococcus pyogenes
CSOM	Chronic suppurative otitis media
OME	Otitis media with effusion
OM	Otitis media
ROM	Recurrent otitis media
S. pneumoniae	Streptococcus pneumoniae
M. catarrhalis	Moraxella catarrhalis
S. pyogenes	Streptococcus pyogenes
URTIs	Upper respiratory tract infections

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Authors' contributions

The authors confirm their contribution to the paper as follows: AFG and RAE: study idea and design. AMA and MGE: data collection. AMS and EFA: analysis and interpretation of results. EFA and AFG: draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript. The corresponding author confirms that the manuscript has been read and approved for submission by all the named authors. The requirements for authorship as stated have been met, and each author believes that the manuscript represents honest work.

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Availability of data and materials

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

Declarations

Ethics approval and consent to participate

This study was approved by the Benha Faculty of Medicine's ethical committee (REC-FOMBU), Egypt, which approved the study protocol with the approval number MS-17–2-2023. The study was carried out in compliance with the Helsinki Declaration of 1975 and its amendments. A written informed consent form was obtained from all participants' parents or guardians in cases of children below 16 years old.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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