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A prospective, observational, epidemiological evaluation of the aetiology and antimicrobial susceptibility of acute otitis media in Saudi children younger than 5 years of age

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Antibiotic resistance

Abstract *Background:* Information regarding acute otitis media (AOM) aetiology is important for developing effective vaccines. Here, bacterial aetiology and antimicrobial susceptibility of AOM were determined in young Saudi children.

Methods: Children aged 3–60 months with a new episode of AOM, who had not received antibiotics or had received antibiotics for 48–72 h but remained symptomatic, were enrolled in this prospective, observational, epidemiological study in Riyadh. Middle ear fluid (MEF) samples were collected by tympanocentesis or from spontaneous otorrhea, and tested for the presence of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes* and *Moraxella catarrhalis*. Antimicrobial susceptibility of the identified pathogens was assessed using E-tests.

Abbreviations: AOM; acute otitis media; ENT; ear, nose, and throat; EPI; expanded programme on immunisation; MEF; middle ear fluid; NTHi; non-typeable *H. influenzae*

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Results: Between June 2009 and May 2011, 66 children were enrolled. *S. pneumoniae* was detected in 6 episodes and non-typeable *H. influenzae* (NTHi) in 8 episodes. Moreover, *Staphylococcus aureus*, which is an uncommon cause of AOM, was detected in 17 episodes. Pneumococcal serotypes were 7F ($n = 2$), 23F ($n = 2$), 19F ($n = 1$) and 15F ($n = 1$). Susceptibility to cefotaxime was observed in all pneumococcal and *H. influenzae* isolates, to cefuroxime in 4/6 pneumococcal and 8/8 *H. influenzae* isolates, and to penicillin in 5/6 pneumococcal isolates.

Conclusions: *S. pneumoniae* and NTHi were major bacterial contributors for AOM in Saudi children.

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1. Introduction

Acute otitis media (AOM) is one of the most common paediatric bacterial infections, affecting approximately 80% of children by the age of 3 years [1–3]. The main bacteria responsible for AOM in children are *Streptococcus pneumoniae*, *Haemophilus influenzae* and, to a much lesser extent, *Moraxella catarrhalis* and *Streptococcus pyogenes* [4,5]. Although previous studies suggested that approximately 1% of children are diagnosed with AOM in Saudi Arabia, those younger than 4 years of age are more affected than older children, and the incidence of paediatric AOM varies among the different regions and geographic settings. There is limited available information on the prevalence and aetiology of AOM in Saudi Arabia [6,7].

AOM is one of the primary reasons for antibiotic use in children, and inappropriate or extensive use of antibiotics may lead to increasing resistance among pathogens [1]. Multi-drug resistance in *S. pneumoniae* is a major public health concern in many countries across the world [8–10], including Saudi Arabia, where a high and increasing prevalence of antibiotic-resistant pneumococcal isolates is observed [11]. In Saudi Arabia, primary vaccination of infants and young children with pneumococcal conjugate vaccines has been included in the national Expanded Programme on Immunization (EPI) since February 2009. Currently, three pneumococcal conjugate vaccines are available, and the vaccination coverage rates reached 98% of Saudi toddlers younger than 1 year of age in 2010 [12].

The present study was designed to determine the aetiology of AOM in Saudi children, as well as the antimicrobial susceptibility of the identified pathogens and the vaccination status of the children. Up-to-date information regarding AOM aetiology is important for developing and implementing effective vaccines in Saudi Arabia, especially since previous studies suggested that changes in the dis-

tribution of pathogens and pneumococcal serotypes might occur after the introduction of pneumococcal conjugate vaccines [13–17].

2. Methods

2.1. Study design, setting and participants

This prospective, observational, epidemiological study was conducted in a routine clinical setting in Saudi Arabia between June 2009 and May 2011. One primary centre and several satellite centres, belonging to the same primary centre administration and Institution Review Board in Riyadh, were included in this study. Study participants were children between 3 months and 5 years of age, diagnosed as having AOM, and from whom a middle ear fluid (MEF) sample had been obtained by an ear, nose and throat (ENT) specialist. MEF samples were taken by tympanocentesis or by careful sampling of spontaneous otorrhea if perforation had occurred less than 24 h prior to the visit.

Children were eligible if they had at least one sign of otalgia (or its equivalent: irritability), conjunctivitis or fever, and either Paradise's criteria (bulging, diffused or localised inflamed tympanic membranes) [18] or spontaneous otorrhea that had occurred less than 24 h prior to the visit. Moreover, the onset of signs and symptoms of AOM had to occur within 72 h prior to the diagnosis of AOM by a physician. Children were excluded from the study if they were hospitalised during the diagnosis of AOM or during treatment; had otitis externa or otitis media with effusion; had a tympanostomy tube; received systemic antibiotic treatment for a disease other than AOM in the 72 h prior to enrolment; received antimicrobial prophylaxis for recurrent AOM; received antibiotics by the paediatrician or ENT specialist at the enrolment visit prior to the sampling of MEF or spontaneous otorrhea; or had received antibiotics for AOM and were clinically improving.

This study included children with a new episode of AOM who had not yet received antibiotic therapy for the episode (untreated children), and children who had a diagnosis of AOM within 48–72 h prior to study enrolment, had received antibiotic therapy from a physician and remained symptomatic at the time of study entry (children with treatment failure). Children were enrolled by one of the study paediatricians or ENT specialists, and a child could be enrolled for more than one AOM episode if there was a symptom-free interval of at least 30 days between episodes.

The study was conducted according to Good Clinical Practice, the Declaration of Helsinki, and the local rules and regulations of the country [19]. Before enrolment, informed consent was obtained from the parents/guardians of each study participant. No fees for recruiting children were given in this study. All study documents and procedures were approved by the hospital Institution Review Board and research committee, and the Saudi Food and Drug Administration (FDA) was notified of this study as per Saudi regulations.

2.2. Study objectives

The primary objective of this study was to identify pathogens considered likely to be responsible for AOM (*S. pneumoniae*, *H. influenzae*, *S. pyogenes* and *M. catarrhalis*) in MEF samples from Saudi children between 3 months and 5 years of age diagnosed with AOM.

The secondary objectives of this study included the determination of (1) the serotypes of *S. pneumoniae* and *H. influenzae* responsible for AOM in Saudi children; (2) the antimicrobial susceptibility profiles of the isolated bacteria; (3) the proportion of children with treatment failures, recurrent AOM episodes (defined as at least 3 episodes in the last 6 months or at least 4 episodes in the last 12 months) and spontaneous otorrhea; and (4) the proportion of *H. influenzae* isolates identified in children who either received a pneumococcal conjugate vaccine or were unvaccinated against pneumococcal diseases.

2.3. Study procedures

All the children presenting to one of the paediatricians or ENT specialists with AOM were anonymously recorded in a screening logbook and assessed for study eligibility. Demographic information, pneumococcal vaccination status, medical history, care history and general symptoms of the enrolled children were recorded.

After confirmation of the AOM diagnosis by an ENT specialist, a MEF sample was taken by tympanocentesis via passage of a sterile needle through an intact tympanic membrane, or an otorrhea sample was collected after cleaning of the ear canal and via deep aspiration of MEF material through needle insertion in children with perforation of the tympanic membrane that occurred less than 24 h prior to the visit. Throughout the entire study, no serious adverse events related to tympanocentesis were reported, showing that it is a safe procedure. If possible, urine samples were also collected from the enrolled children for antimicrobial activity testing.

2.4. Laboratory procedures

MEF samples were inoculated in chocolate agar (otorrhea samples were inoculated in chocolate agar with bacitracin to avoid mixed flora) and blood agar (with gentamycin) at 35 ± 2 °C in an aerobic atmosphere supplemented with CO₂ [20]. Bacterial identification of the pathogens was made using standard bacteriological procedures: *S. pneumoniae* was identified by Optochin and Bile salt tests; *H. influenzae* identification was based on Gram staining, growth on chocolate agar, failure to grow on trypticase agar with added sheep blood, and nutritional requirement of both hemin (Factor X) and nicotine adenine dinucleotide (Factor V); *S. pyogenes* identification was based on the presence of b-haemolysis, susceptibility to bacitracin and positive co-agglutination; and *M. catarrhalis* identification was based on Gram staining, positive oxidase reaction and characteristic biochemical profiling [20]. *Staphylococcus aureus* identification was based on Gram staining, the presence of coagulation and the use of DNA, and was confirmed using Slides staph plus Kit (Bio Merieux) [20]. Pneumococcal serotypes were identified by Quellung reaction, and *H. influenzae* serotypes were identified using monovalent anti-sera.

In *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* isolates, antimicrobial susceptibility to penicillin, amoxicillin/clavulanate, cefuroxime, cefotaxime, erythromycin, azithromycin, chloramphenicol, tetracycline, levofloxacin and trimethoprim/sulfamethoxazole were tested using E-tests [21]. In *H. influenzae* and *M. catarrhalis* isolates, antimicrobial susceptibility to ampicillin was also assessed and a beta-lactamase test using a chromogenic cephalosporin (Nitrocefim) was performed [20,22].

Antimicrobial activity was evaluated on urine samples, using an antibacterial substance assay, as previously described [23].

2.5. Statistical analysis

Since none of the enrolled children were eliminated in this study, all analyses were performed on the total cohort, which included all children who met all eligibility criteria, complied with the procedures defined in the protocol, had no elimination criteria during the study and for whom results for a MEF sample were available. All analyses were descriptive, and results are presented in terms of frequencies, proportions and 95% confidence intervals (CI).

3. Results

3.1. Clinical and demographic characteristics of enrolled children

Between June 2009 and May 2011, 246 children with AOM were screened and 66 children were enrolled in the study. Two children were enrolled by a paediatrician and 64 children by an ENT specialist. A peak of enrolled children was observed in January 2010 (11 screened and 9 enrolled children), and there was also a peak of screened and enrolled children in January 2011 (55 screened and 13 enrolled children). Of the 66 enrolled children, 62 were classified as untreated children and 4 as children with treatment failures. In the total cohort, all children had experienced a single AOM episode.

In the total cohort, 41% (27/66) of episodes were reported in girls. The mean age of the children was 24 ± 16 months (range 3–58 months) and most of the episodes (53/66 [80%]) were reported in children younger than 36 months of age, with a peak between 12 and 23 months (24/66 [36%]) (Table 1). Eleven episodes (17%) were reported in children who had been vaccinated with a pneumococcal conjugate vaccine, 11 episodes (17%) in unvaccinated children and 44 episodes (67%) in children with an unknown pneumococcal vaccination status. Moreover, 12 episodes (18%) were reported in children who had received at least one dose of *H. influenzae* vaccine. Antibiotic use within the past month was reported in 8% (5/66) of episodes. However, no antimicrobial activity, indicating the presence of antibiotic residuals or metabolites, was detected by the antibacterial substance assay performed on the 7 available urine samples (6 samples from episodes in untreated children and 1 sample from an episode in a child with treatment failure).

None of the AOM episodes were recurrent, according to the definition used in the study,

Table 1 Demographics and clinical history of children experiencing episodes of acute otitis media (total cohort).

Total number of episodes	66
Age (months)	
Mean \pm Standard deviation	24 \pm 16
Range	3–58
Age category	Number (percentage) of episodes
3–11 months	15 (23%)
12–23 months	24 (36%)
24–35 months	14 (21%)
36–47 months	3 (5%)
48–59 months	10 (15%)
Gender	Number (percentage) of episodes
Female	27 (41%)
Male	39 (59%)
Pneumococcal vaccination status	Number (percentage) of episodes
Vaccinated	11 (17%)
Unvaccinated	11 (17%)
Unknown	44 (67%)
Collection methods	Number (percentage) of samples
Otorrhea	23 (31%)
Tympanocentesis	52 (69%)

and only 6/66 (9%) episodes were reported in children with a history of AOM episodes in the past 12 months. The most frequently reported symptom was ear pain (59/66), followed by irritability (29/66), fever (21/66), ear discharge (20/66) and trouble sleeping (20/66). Among the 66 AOM episodes, there were 10 bilateral infections.

3.2. Microbiological aspects of samples

In this study, results were available for 75 MEF samples collected during the 66 AOM episodes; samples from both the left and the right ears were available for 9/10 children with a bilateral infection. Among the 75 MEF samples, 23 were otorrhea samples and 52 were collected by tympanocentesis; 21% (14/66) of episodes and 20% (15/75) of MEF samples were positive for one of the pathogens under investigation. Among these positive episodes, 8/14 were identified in otorrhea samples and 6/14 in samples collected by tympanocentesis. Moreover, 12/14 positive episodes were identified in untreated children and 2/14 in children with treatment failure.

S. pneumoniae was isolated in 7 samples collected during 6 episodes (the same *S. pneumoniae* isolate was detected in 2 samples taken from the same child, who had a bilateral infection), and *H. influenzae* in 8 samples collected during 8 episodes. The 6 *S. pneumoniae* isolates were detected in untreated children, and were identified as serotypes 7F ($n = 2$), 23F ($n = 2$), 19F ($n = 1$) and 15F ($n = 1$). *H. influenzae* isolates were detected in 2/4 children with treatment failure group and in 6/62 untreated children. The 8 *H. influenzae* isolates were non-typeable (NTHi).

All *S. pneumoniae* isolates were detected in children with unknown pneumococcal vaccination status. *H. influenzae* isolates were identified in 4/11 episodes reported in children who had been vaccinated with a pneumococcal conjugate vaccine and in 4/44 episodes reported in children with an unknown vaccination status. No *H. influenzae* isolates were identified in episodes reported in unvaccinated children.

Beside the pathogens under study, other bacteria were identified in 21/66 episodes and 24/75 MEF samples. Among the other bacteria, *S. aureus* was the most common and was detected in 17/21 episodes; 9/17 *S. aureus* isolates were detected in otorrhea samples and 8/17 in samples collected by tympanocentesis.

3.3. Antimicrobial susceptibility

Among the 6 *S. pneumoniae* isolates, all were found to be susceptible to amoxicillin/clavulanate, cefotaxime and levofloxacin; 5, to penicillin, azithromycin and chloramphenicol; 4, to cefuroxime, erythromycin and tetracycline; and 3, to trimethoprim/sulfamethoxazole (Table 2). Two *S. pneumoniae* isolates were resistant to cefuroxime, tetracycline and trimethoprim/sulfamethoxazole, and 1, to erythromycin, azithromycin and chloramphenicol.

Table 2 Antimicrobial susceptibility of *S. pneumoniae* and *H. influenzae* isolated from children with acute otitis media (total cohort).

Characteristics	Categories or parameter	Number of isolates	Percentage of isolates
<i>S. pneumoniae</i> isolates ($N = 6$)			
Penicillin	Susceptible (MIC ≤ 2 $\mu\text{g}/\text{mL}$)	5	83
Amoxicillin/clavulanate	Susceptible (MIC $\leq 2/1$ $\mu\text{g}/\text{mL}$)	6	100
Cefuroxime	Susceptible (MIC ≤ 0.5 $\mu\text{g}/\text{mL}$)	4	67
Cefotaxime	Susceptible (MIC ≤ 1 $\mu\text{g}/\text{mL}$)	6	100
Erythromycin	Susceptible (MIC ≤ 0.25 $\mu\text{g}/\text{mL}$)	4	67
Azithromycin	Susceptible (MIC ≤ 0.5 $\mu\text{g}/\text{mL}$)	5	83
Chloramphenicol	Susceptible (MIC ≤ 4 $\mu\text{g}/\text{mL}$)	5	83
Tetracycline	Susceptible (MIC ≤ 2 $\mu\text{g}/\text{mL}$)	4	67
Levofloxacin	Susceptible (MIC ≤ 2 $\mu\text{g}/\text{mL}$)	6	100
TMP/sulfamethoxazole	Susceptible (MIC $\leq 0.5/9.5$ $\mu\text{g}/\text{mL}$)	3	50
<i>H. influenzae</i> isolates ($N = 8$)			
Penicillin MIC	Mean	16	—
	Standard deviation	14	—
Erythromycin MIC	Mean	4	—
	Standard deviation	2	—
Azithromycin MIC	Mean	3	—
	Standard deviation	1	—
Amoxicillin/clavulanate	Susceptible (MIC $\leq 4/2$ $\mu\text{g}/\text{mL}$)	7	88
Cefuroxime	Susceptible (MIC ≤ 4 $\mu\text{g}/\text{mL}$)	8	100
Cefotaxime	Susceptible (MIC ≤ 2 $\mu\text{g}/\text{mL}$)	8	100
Chloramphenicol	Susceptible (MIC ≤ 2 $\mu\text{g}/\text{mL}$)	7	88
Tetracycline	Susceptible (MIC ≤ 2 $\mu\text{g}/\text{mL}$)	6	75
Levofloxacin	Susceptible (MIC ≤ 2 $\mu\text{g}/\text{mL}$)	8	100
TMP/sulfamethoxazole	Susceptible (MIC $\leq 0.5/9.5$ $\mu\text{g}/\text{mL}$)	0	0
Ampicillin*	Susceptible (MIC ≤ 1 $\mu\text{g}/\text{mL}$)	1	14
Nitrocefin	Negative	2	25
	Positive	6	75

N = total number of *S. pneumoniae* or *H. influenzae* isolates in a given category; MIC = minimum inhibitory concentration; TMP = trimethoprim.

* Results for ampicillin were missing for one *H. influenzae* isolate, which was lost during storage.

Among the 8 *H. influenzae* isolates, all were susceptible to cefuroxime, cefotaxime and levofloxacin; 7, to amoxicillin/clavulanate and chloramphenicol; 6, to tetracycline; and 1, to ampicillin (Table 2). Five *H. influenzae* isolates were resistant to ampicillin; 3, to trimethoprim/sulfamethoxazole; and 1, to amoxicillin/clavulanate and chloramphenicol. The Nitrocefin test (beta-lactamase test) was positive for 6/8 *H. influenzae* isolates. Among the 2 beta-lactamase-negative *H. influenzae* isolates, 1 was not tested against ampicillin and the other was susceptible to ampicillin (minimum inhibitory concentration [MIC] ≤ 1 $\mu\text{g/mL}$).

4. Discussion

To the best of this study's knowledge, this is the first study of AOM aetiology and antimicrobial susceptibility in Saudi children. Interestingly, among the 66 AOM episodes included in this study, none were recurrent and only 4 were reported in children with antimicrobial treatment failure. The observed number of AOM episodes peaked in January 2010 and January 2011; this is consistent with previous observations showing that AOM peaks during the cold months as a consequence of increased viral infections, which is an important predisposition for the development of AOM [24].

In this study, the proportion of *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and *S. pyogenes* isolates (14/66) was lower than what has been observed in other studies [25]. The number of *H. influenzae* isolates was slightly higher than the number of *S. pneumoniae* isolates, and no *M. catarrhalis* or *S. pyogenes* isolates were detected. The serotypes of the pneumococcal isolates were 7F, 23F, 19F and 15F, and all *H. influenzae* isolates were NTHi. Besides the pathogens under study, other bacteria were detected in 21/66 episodes, and the most frequent isolate was *S. aureus* in Saudi children with AOM. Although this bacterium is an uncommon cause of AOM [26], a large proportion of *S. aureus*-positive episodes observed in this study suggests that it might become a more important contributor to AOM following the introduction of pneumococcal vaccines in the routine immunisation schedule. Further studies would be needed to confirm this hypothesis, and the low yield of bacterial cultures in this study does not provide any evidence that the pathogen distribution in Saudi Arabia differs from that observed in other countries.

H. influenzae was identified in 4/11 episodes reported in children who had been vaccinated with a pneumococcal conjugate vaccine and in none of

the unvaccinated children. Although the number of children included in this study was too low and the number of children with an unknown pneumococcal vaccination status too high to draw any relationship between vaccination and pathogen distribution, these results are in line with those of previous studies showing an increase of the proportion of AOM episodes caused by NTHi after the introduction of pneumococcal conjugate vaccines in immunisation programmes [4,27–29].

The first-line treatment for AOM generally recommended by Saudi paediatricians varies, but the most frequently prescribed antibiotics belong to the β -lactam class, and more specifically to the cephalosporin group (personal communication from Khalid A. Al-Mazrou). Based on the breakpoints provided by the Clinical and Laboratory Standards Institute, the 6 *S. pneumoniae* isolates were susceptible to cefotaxime, 5 to penicillin and 4 to cefuroxime, and all *H. influenzae* isolates were susceptible to both cefuroxime and cefotaxime [21].

This study was limited by the small number of enrolled children ($N = 66$) compared with the number of screened children ($N = 246$), which may be due to the facts that tympanocentesis is not practiced routinely in Saudi Arabia and many parents refused the procedure to be done. Other limitations of this study were the small number of isolates with antimicrobial susceptibility results available and the low culture-positivity rates, which may be explained by the diagnostic criteria, by the previous use of antibiotics or by viral aetiology. The use of molecular techniques, such as polymerase chain reaction, and the analysis of nasopharyngeal swabs in addition to the MEF samples could increase the number of positive samples. A further limitation of this study was the high proportion of children with an unknown pneumococcal vaccination status, which may be due to the facts that routine pneumococcal vaccination was recently implemented in Saudi Arabia and parents could not easily recognise the vaccines, or to the fact that children in Saudi Arabia could be vaccinated at any healthcare facility and it was not possible to ask the parents to bring the vaccination card before the visit.

5. Conclusions

In this study, 21% of AOM episodes recorded in Saudi children were positive for either *S. pneumoniae* or NTHi. Although this study was limited by the small sample size, these results confirmed that these pathogens were major bacterial contributors for AOM in young children. Beside these pathogens, *S. aureus*, which is an uncommon cause of AOM,

was detected in 26% of episodes, suggesting that this bacterium might become an important contributor to AOM following the introduction of routine pneumococcal vaccination. Further studies are needed to assess the burden and aetiology of AOM, and the need to introduce additional vaccines in the national immunisation schedule.

Authors' contributions

All authors contributed to the study, were involved in writing and reviewing the paper, and approved the final version. All authors had full access to the data, and the corresponding author had final responsibility for submission of the manuscript.

Role of the funding source

GlaxoSmithKline Biologicals SA was the funding source and was involved in all stages of the study conduct and analysis. GlaxoSmithKline Biologicals SA also took responsibility for all costs associated with the development and publishing of the present manuscript.

Competing interests

K.A.M. has received an institutional grant from GlaxoSmithKline group of companies.

A.M.S. declares no conflict of interest.

W.K. is an employee of GlaxoSmithKline group of companies.

J.Y.P. is an employee of GlaxoSmithKline group of companies.

C.M. is an employee of GlaxoSmithKline group of companies and has stock options.

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