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Increased antimicrobial resistance in organisms recovered from otitis media with effusion

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Abstract

Previous studies concerning the microbiology of otitis media with effusion (OME) did not correlate the past use of antimicrobial agents with the recovered organism's antimicrobial susceptibility. A retrospective analysis of cultures obtained from aspirates of 129 children with OME was performed. The study identified the isolated organisms and determined their susceptibility to the most recently administered antimicrobials. Bacterial growth was noted in 58 (45 per cent) patients. Aerobic organisms only were recovered in 37 aspirates (63 per cent of the culture-positive aspirates); anaerobic bacteria in seven (12 per cent); and mixed aerobic and anaerobic bacteria in 14 (24 per cent). A total of 92 bacterial isolates were recovered, accounting for 1.6 isolates per specimen (1.1 aerobes and 0.5 anaerobes). There were a total of 66 aerobic isolates, including *Haemophilus influenzae* non type-b (20 isolates), *Streptococcus pneumoniae* (17), and *Staphylococcus* spp. (seven). Twenty-six anaerobes were recovered, including *Peptostreptococcus* spp. and *Prevotella* spp. (eight each) and *Propionibacterium acnes* (four). Resistance to the antimicrobial used was found in 60 (65 per cent) isolates, recovered from 41 (71 per cent) of the patients. Of the 41 patients in whom resistance was detected, 37 (90 per cent) had been treated within three months of culture and four (10 per cent) had completed treatment more than three months before the cultures were taken ($p<0.01$). The highest rate of recovery of resistant organisms was following trimethoprim-sulfamethoxazole (96 per cent), amoxycillin (71 per cent), and azithromycin (56 per cent). Of the patients treated with amoxycillin, *H influenzae* predominated. *S pneumoniae* was recovered from four of the seven (57 per cent) after trimethoprim-sulfamethoxazole, four of 14 (29 per cent) following amoxycillin, and three of 11 (27 per cent) after azithromycin. The data illustrate the relationship between resistance to the antimicrobials given to children and their recovery from the middle ear of patients with OME.

Key words: Otitis Media with Effusion; Drug Resistance, Microbial

Introduction

Persistence of middle-ear effusion (MEE) in the form of otitis media with effusion (OME) can occur in up to two thirds of children after an episode of acute otitis media (AOM), and takes up to three months to resolve in 90 per cent of patients. Bacteria can be recovered from 21 to 52 per cent of these MEE.[1–5] However, their role in the persistence of the condition is uncertain. The bacteria recovered from the MEE are similar to those isolated from AOM and include *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*.[1–5] The causes of persistence of these organisms is unknown. It is possible that their survival may be due to their resistance to the antimicrobials used.

Previous studies concerning the microbiology of OME did not correlate the past use of antimicrobial agents with the recovered organism's antimicrobial susceptibility.

This study reports our experience in recovery of aerobic and anaerobic bacteria from MEE of children with OME, and determines the susceptibility of the organisms with the patients' most recent antimicrobial therapy. This study includes data from our earlier publication.[6] However, additional patients are included in the analysis.

Patients and methods

One hundred and twenty-nine children (72 males and 57 females) were studied. Their ages ranged from 14 months to 11 years (median age, 3.8 years). Forty-four of the children were younger than two years. Specimens included were only those that were serous or mucoid. No purulent specimens, as determined by Gram stain, were included. Following acute or recurrent otitis media, all of the children underwent placement of tympanic ventilation tubes to correct persistent middle-ear effusion of at least

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three months' duration. The patients' clinical data were obtained by a questionnaire completed by the parents and by review of the patients' records. Bilateral tympanocentesis was performed in 34 patients. Although two ear aspirates were obtained from these 34 patients, only one aspirate per patient (the one containing more organisms) was considered in the final analysis. None had any signs or symptoms of acute infection or had received antimicrobial therapy for at least two weeks prior to sample collection; 54 had received no antimicrobial agents during the three months prior to sample collection. All of the patients had, however, received many courses of antimicrobial therapy within the year prior to surgery. The average number of courses of antimicrobial therapy given in the past year was 3.8/child. The agents used included amoxycillin, amoxycillin-clavulanate, cephalosporins, azithromycin, and trimethoprim-sulfamethoxazole (TMP-SMX). The mean time interval between the last dose of antibiotics and the insertion of tubes was 36.5 ± 11.4 days.

The middle-ear effusions were collected in the operating theatre with the patient under general anaesthesia. The external auditory canal was cleared of cerumen with a blunt curette. The canal was then filled with povidone-iodine solution for three minutes. Removal of this solution was accomplished by irrigation with 50 ml of sterile saline, and the excess saline was absorbed with sterile cotton. The external surface of the tympanic membrane was then swabbed with a sterile cotton applicator, which was immediately streaked on media and incubated for aerobic and anaerobic bacteria.

With a surgical microscope, a myringotomy incision was made in the tympanic membrane. Under direct visualization, the effusion was aspirated through sterile polyethylene tubing attached to a disposable middle-ear fluid collector. Following aspiration, excess air was evacuated from the collecting cup by filling the cup with pre-reduced thioglycolate broth. It was sealed and delivered to the microbiology laboratory, where the specimen was plated on aerobic and anaerobic media within five to 10 minutes of collection.

Sheep blood (five per cent), chocolate, and MacConkey agar plates were inoculated for the isolation of aerobic organisms. The plates were incubated at 37°C aerobically (MacConkey agars) and under five per cent carbon dioxide (blood and chocolate agar) and examined at 24 and 48 hours. For the isolation of anaerobic bacteria, the specimens were plated to a pre-reduced vitamin K_1-enriched brucella blood agar, an anaerobic blood agar plate containing kanamycin and vancomycin, and an anaerobic blood plate containing phenylethyl alcohol and placed into an enriched thioglycolate broth.[7] All media were incubated in anaerobic jars and examined at 48 and 96 hours. The thioglycolate broth was incubated for 14 days. Aerobic and anaerobic bacteria were identified using conventional methods.[7,8] Beta-lactamase activity was determined in all isolates using the chromogenic cephalosporin analog 87/312 methodology.[9]

Minimum inhibitory concentrations (MICs) for aerobic bacteria were determined by the agar dilution method with Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, MD) supplemented with five per cent sheep blood.[10] For anaerobic bacteria the MIC was determined by the agar dilution method using brain heart infusion agar (Difco Laboratories, Detroit).[11] MIC determinations, suspensions with a turbidity equivalent to that of a 0.5 McFarland standard were prepared by suspending growth from blood agar plates in 2 ml of Mueller-Hinton broth (BBL). Suspensions were further diluted 1:10 to obtain a final inoculum of 10^4 CFU/spot. Plates were inoculated with a Steer's replicator and incubated overnight in ambient air at 37°C. Standard quality control strains[10,11] were included in each run. Additionally, MICs of azithromycin were read after an additional 24 hours of incubation. Interpretative criteria were as established by the National Committee for Clinical Laboratory Standards guidelines.[10,11]

Statistical analysis was done using the student *t*-test.

Results

Bacterial growth occurred in 58 of the 129 (45 per cent) patients. Bacterial growth was present in 41 of the 54 (76 per cent) of those not treated within the past three months, and in 17 of the 75 (23 per cent) who were treated within the past three months ($p<0.001$). Aerobic organisms alone were recovered

TABLE I
BACTERIA ISOLATED FROM 58 CULTURE POSITIVE MIDDLE-EAR EFFUSIONS IN PATIENTS WITH OME

	Number of isolates
Aerobic bacteria	
Streptococcus pneumoniae	17
Streptococcus pyogenes	3
Streptococcus group D	1
α-haemolytic streptococcus	4
γ-haemolytic streptococcus	3
Staphylococcus aureus	7 (7)*
Staphylococcus epidermidis	4 (2)
Moraxella catarrhalis	6 (6)
Haemophilus influenzae non type-b	20 (16)
Escherichia coli	1 (1)
Subtotal	66 (32)
Anaerobic bacteria	
Peptostreptococcus spp.	8
Streptococcus constellatus	2
Veillonella alcalescens	2
Propionibacterium acnes	4
Fusobacterium nucleatum	2 (1)
Prevotella melaninogenica	5 (2)
Prevotella intermedia	3 (1)
Subtotal	26 (4)
Total	92 (36)

* = in parentheses, the number of β-lactamase-producing bacteria.

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TABLE II

ORGANISMS ISOLATED FROM EAR ASPIRATES OF 58 CHILDREN WITH OME IN RELATION TO THEIR MOST RECENT ANTIMICROBIAL THERAPY

Preceding antimicrobial	No. patients	*Streptococcus pneumoniae*	*Haemophilus influenzae*	Moraxella catarrhalis	*E coli*	*Streptococcus pyogenes*	Other streptococci	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Anaerobic and aerophilic streptococci*	*Fusobacterium* spp.	*Prevotella* spp.	*P. acnes*	Total: total resistant/number isolates (%)
Amoxycillin	14	2/4	5/6	3/3			1/1	2/2	1/1	0/3	1/1	2/2	0/1	17/24 (71)
Amoxycillin-clauvulanate	6	1/1	0/1					0/1		0/1				1/4 (25)
Trimethoprim-sulfamethoxazole	7	4/4	3/3	1/1		2/2	2/2	1/1	1/1	5/5	1/1	2/3	1/1	23/24 (96)
Cefixime	7	3/3	0/2				0/1	2/2	2/2	0/1		0/1	0/1	7/13 (54)
Cefprozil	6	1/1	1/2	0/1			0/1							2/5 (40)
Cefuroxime axetil	7	0/1	0/2				0/1							0/4 (0)
Azithromycin	11	2/3	3/4	0/1	1/1	0/1	2 (0)	1/1		1/2		2/2	0/1	10/18 (56)
Total	58	13/17	12/20	4/6	1/1	2/3	3/8	6/7	4/4	6/12	2/2	6/8	1/4	60/92 (65)

[a] = resistant isolates/total number of isolates

in 37 aspirates (63 per cent of the culture-positive aspirates), anaerobic bacteria alone in seven (12 per cent), and mixed aerobic and anaerobic bacteria in 14 specimens (24 per cent). Thus, aerobic bacteria were recovered in 88 per cent and anaerobes in 36 per cent of the aspirates with bacterial growth. All pre-operative cultures of the external surface of the tympanic membrane showed no bacterial growth.

A total of 92 bacterial isolates were recovered from the patients (Table I), accounting for 1.6 isolates per specimen (1.1 aerobes and 0.5 anaerobes). Sixty-six isolates were aerobic. The predominant isolates were *H influenzae* non type-b (20 isolates), *S pneumoniae* (17), *Staphylococcus aureus* (seven), *M catarrhalis* (six), and *Streptococcus pyogenes* (three). A total of 26 anaerobic isolates were recovered. The predominant anaerobes were *Peptostreptococcus* spp. and pigmented *Prevotella* spp. (eight each), and *Propionibacterium acnes* (four).

The number of isolates per specimen ranged from one to seven. One isolate per specimen was recovered in 35 instances (60 per cent). The organisms most frequently recovered as a single isolate were, in descending order of frequency, *H influenzae*, (14 times), *S pneumoniae* (eight), *S aureus* (six), and *Prevotella* sp. and *P acnes* (two each).

Resistance to the antimicrobial agents most recently used was observed in 60 of the 92 (65 per cent) isolates (Table II) that were recovered in 41 (71 per cent) of the culture-positive patients. These included 13 of 17 (70 per cent) of *S pneumoniae*, 12 of 20 (60 per cent) *H. influenzae* (all were β-lactamase producers), four of six (67 per cent) of *M catarrhalis* (all β-lactamase producers), two of three (67 per cent) of *S pyogenes*, six of seven (86 per cent) *S aureus* (all β-lactamase producers), six of eight (75 per cent) *Prevotella* spp., two of two (100 per cent) of *Fusobacterium* spp. (all β-lactamase producers), and six of 12 (50 per cent) of anaerobic cocci. The highest rate of recovery of resistant organisms was following TMP-SMX (96 per cent), followed by amoxycillin (71 per cent), azithromycin (56 per cent), and cefixime (54 per cent) (Table II).

A correlation was noted between the presence of antimicrobial resistance and the interval between the recent antimicrobial therapy and tympanocentesis. Of the 41 patients in whom resistance was detected, 37 (90 per cent) had been treated within three months of culture and four (10 per cent) had completed treatment more than three months before the cultures were taken ($p<0.01$). Resistance was present in 18 of the 40 (45 per cent) patients treated within 14–30 days, six of 24 (25 per cent) of those treated within 30–60 days, and four of 21 (19 per cent) of those treated within 60–90 days.

Of the 12 patients who were treated with amoxycillin, *H influenzae* predominated (Table II) *S pneumoniae* was recovered from four of the seven (57 per cent) patients after TMP-SMX, four of 14 (29 per cent) following amoxycillin, three of 11 (27 per cent) after azithromycin. Anaerobic cocci were isolated from five of the seven (71 per cent) treated with TMP-SMX, and three of the 14 (21 per cent) treated with amoxycillin. *Prevotella* spp. was recovered from three of seven (43 per cent) treated with TMP-SMX, two of 14 (14 per cent) treated with amoxycillin, and two of 11 (18 per cent) treated with azithromycin.

Discussion

This study illustrates the relationship between the last antimicrobials given to patients and the recovery of organisms resistant to these antibiotics from the middle ear of patients with otitis media with effusion that failed to improve. A correlation was noted between the presence of antimicrobial resistance and the interval between the most recent antimicrobial therapy and tympanocentesis. The longer the interval, the lesser the chance of recovery of resistant organisms. An association between previous antimicrobial therapy and increased antimicrobial resistance that diminishes over time has been established previously.[12]

Whether the resistance of the organisms in the middle-ear fluid contributed to their persistence in the middle ear, and subsequent development of otitis media with effusion is yet to be determined. However, it is possible that if eradication of these organisms had been achieved, the persistence of ear

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effusion and the subsequent need for tube placement could have been avoided. Some of the antimicrobials used appeared to be less effective in eradication of the infection than others (e.g. TMP-SMX, amoxycillin, azithromycin), while others were not associated in this study with antimicrobial resistance-related failures. These findings confirm previous reports in AOM where such a relationship was found.[13–18] Harrison *et al*. illustrated a higher isolation of *H influenzae* and *S aureus*[12] in patients with recently treated or persistent otitis media compared to untreated AOM. In contrast, Pichichero and Pichichero showed a smaller rate of recovery of organisms from persistent otitis media.[16] Brook and Gober found that 27 of the 43 (63 per cent) isolates recovered from 22 of 34 (71 per cent) children with AOM who failed to respond to antimicrobials were resistant to these agents.[18] The lack of recovery of any organism in about a quarter of the patients may be due to infection due to viruses or atypical organisms.

- **This paper reports a study of 129 children with otitis media persistent for more than three months**
- **All were treated with bilateral grommets and had a sample of the fluid removed from the middle ear cultured**
- **Bacterial growth was found in 45 per cent of patients**
- **65 per cent of isolates showed bacteria that were resistant to the antimicrobial drugs used to treat the acute otitis media that preceded surgery**
- **The authors highlight the potentially important role of antibiotic resistance in the persistence of otitis media with effusion**

This study highlights the potential important role of antibiotic resistant pathogens in the persistence of OME. Further studies utilizing two ear taps[15] could shed more light on this problem, because they can compare bacterial resistance before and after antimicrobial therapy.

The major causes of decreased susceptibility to antimicrobials are the growing resistance of *S pneumoniae* to penicillin and other antimicrobials such as TMP-SMX and macrolides,[19] and the production of β-lactamase by *H influenzae* and *M catarrhalis*.[19] Selection of antimicrobial agents can be improved by knowledge of the resistance pattern of the organisms in the community, and by consideration of the effect of previous antimicrobial therapy[20] or prophylaxis[21] that may select resistant strains. In children with AOM who fail antimicrobial therapy, the selective use of tympanocentesis and aspiration of the MEE for smear, culture and susceptibility studies can be both diagnostic and therapeutic.[22]

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