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Main Articles

Bacterial aetiology of non-resolving otitis media in South African children

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Abstract

Little is known of the aetiology, serotypes or susceptibility of the pathogens causing non-resolving otitis media in children receiving care from specialists in private practice in developed or in developing countries. Increased access to antibiotics in the community amongst children receiving such private care in South Africa may be anticipated to lead to levels of resistance similar to those found in countries with similar models of private practice, such as the United States. This study was conducted to determine the aetiology of non-resolving otitis media in South African children receiving private care and to determine the antimicrobial resistance patterns and serotypes of the bacterial isolates.

Middle-ear fluid was cultured from 173 children aged two months to seven years with non-resolving acute otitis media accompanied by persistent pain or fever who were referred to otorhinolaryngologists for drainage of middle-ear fluid within 14 days of the start of symptoms. While 92 per cent of the children had recently received antibiotics and 54 per cent were currently receiving them, bacteria were isolated from 47 children (27 per cent). *Streptococcus pneumoniae* was the most common pathogen (35), followed by *Haemophilus influenzae* (nine), *Staphylococcus aureus* (six), *Moraxella catarrhalis* (two), *Streptococcus pyogenes* (two) and *Pseudomonas aeruginosa* (one). Two isolates were identified in each of eight children. Antimicrobial resistance to one or more antibiotics was found in 33/35 (94 per cent) of the pneumococci isolated, with resistance to penicillin in 86 per cent, resistance to trimethoprim-sulfamethoxazole in 54 per cent and to erythromycin and clindamycin in 69 per cent and 57 per cent, respectively. The pneumococcal serotypes found were 19F (28 per cent), 14 (26 per cent), 23F (23 per cent), 6B (nine per cent), 19A (87 per cent), and four (three per cent). Children with a bacterial pathogen isolated were younger (mean age of 17 months) than children from whom no bacteria were isolated (mean age of 23 months; $p = 0.03$). Isolation of a pneumococcus was also significantly associated with younger age (mean = 16 months *versus* 22 months, $p = 0.03$), the presence of fever (OR = 2.15, $p = 0.049$), and having one or more prior episodes of otitis media within the six months before tympanocentesis (OR = 7.72, $p = 0.03$). Almost all pneumococci isolated from non-resolving acute otitis media in this community are antibiotic-resistant and should be considered especially in young children who have failed previous therapy and who have non-resolving pain or fever.

Key words: Otitis Media; Antibiotics; Treatment Failure; Streptococcus Pneumoniae

Introduction

Otitis media is one of the most common bacterial infections in young children and is responsible for almost one-third of paediatric clinic visits. Acute otitis media typically follows a viral infection produces congestion in the eustachian tube and accumulation of middle-ear fluid.[1] Bacteria proliferate in the middle-ear secretions, resulting in symptomatic otitis media. A bacterial aetiology of the ear infection can be confirmed in 50 to 70 per cent of cases. The most common pathogens are *S pneumoniae* (20–40 per cent), *H influenzae* (10–30 per cent), and *M catarrhalis* (five to 15 per cent). Other streptococci, staphylococci, and Gram negative rods

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have also been isolated.[1] A previous study of tympanocentesis in South Africa conducted in 1987 found a bacterial pathogen in 65 per cent of patients and pneumococci were isolated from 39 per cent of those tested.[2] None of the pneumococci isolated were resistant to penicillin.

The global spread of antibiotic resistance in bacterial pathogens threatens the treatment of many diseases, including otitis media. More than 85 per cent of *M catarrhalis* strains and two to 30 per cent of *H influenzae* isolates worldwide produce beta-lactamase.[3] In South Africa, penicillin resistance was found in 28.9 per cent of invasive pneumococcal isolates submitted to the South African Institute for Medical Research (SAIMR) from children in the public sector between 1995 and 1998[4] and in 45 per cent of nasopharyngeal isolates from children attending private paediatricians in Johannesburg.[5] Such antibiotic resistant organisms can create major problems in the treatment of otitis media, including treatment failures.[6]

The aetiology of non-resolving otitis media and the role of antibiotic resistance in these infections in most countries are unknown. At the time of tympanostomy tube insertion, middle-ear fluid was removed and bacterial culture performed to determine the aetiology of non-resolving otitis media in children and the antimicrobial resistance patterns of the bacterial isolates.

Materials and methods

Children were recruited from 19 otorhinolaryngology (ENT) private practices in Johannesburg and Pretoria. Between July and December 1999 symptomatic children aged two months to seven years with non-resolving acute otitis media accompanied by persistent fever (temperature >37°C) or pain, who were referred to the ENT surgeon for drainage of middle-ear fluid within 14 days of the start of symptoms, and whose parents consented to study participation, were included in the study.

At the time of ear tube placement, middle-ear fluid (MEF) was aspirated from one ear and the fluid placed into cryotubes containing 1 ml of skim milk glucose glycerol broth transport medium.[7] The tubes were then sent on ice to the SAIMR laboratory for analysis. Each specimen was inoculated onto five per cent blood agar with 5 µg/ml gentamicin; bacitracin heated blood agar; dextrose and chloramphenicol agar; MacConkey agar; and five per cent blood agar. Any bacteria isolated were identified by standard methods. Antibiotic susceptibility was determined by disk diffusion using National Committee for Clinical Laboratory Standards (NCCLS) criteria specific to the organism.[7] Minimum inhibitory concentrations (MICs) of appropriate antibiotics were determined using NCCLS methods for all isolates resistant by the disk diffusion method.[8] Serotyping of the haemophilus and pneumococcal strains was carried out using antisera from Murex (Dartford, England) and the Statens Serum Institut, Copenhagen, Denmark, respectively.

Demographic and other patient information including the child's age, sex, exposure to antibiotics or other medications within the previous three months and the number of previous episodes of otitis media were determined by parent interviews or by review of medical records.

Patient and laboratory data were entered and analysed used EpiInfo Version 6.0b.[9] Odds ratio were calculated using 2×2 contingency tables and Chi-square and Fisher exact tests. Means in ages were compared using ANOVA results. For all statistical tests, a p-value of <0.05 indicated statistical significance.

Ethical approval for the study was obtained from the Committee for Studies on Human Subjects of the University of the Witwatersrand.

Results

MEF and demographic information was obtained from 173 children. The median age of the children was 16 months (range two months–84 months) and 54 per cent of the children were male. Inflammation or fluid in the middle ear was present in 83 per cent and 96 per cent of the children, respectively. A history of prior antibiotic use was found in 92 per cent of the children and 54 per cent of the children had received antibiotics within two weeks prior to tympanostomy and culture of MEF. The predominant antibiotics used were penicillins (38 per cent), cephalosporins (39 per cent) and macrolides (14 per cent). Forty-three per cent of the children had one to two episodes of otitis media in the previous six months, 37 per cent had three to four episodes and eight per cent had five to six episodes.

Bacterial pathogens were isolated from 47 (27 per cent) children (Table I). The most common pathogen was *S pneumoniae*, followed by *H influenzae*, *S aureus*, and *M catarrhalis*. More than one organism was isolated from eight children.

Resistance to at least one antibiotic was found in 33 (94 per cent) pneumococci and resistance to at least three different classes of antibiotics (multiple resistance) was found in 12 (34 per cent) of isolates. Penicillin resistance was found in 39 (86 per cent) of the pneumococci, 28 per cent at the high level (Table II). High level resistance to erythromycin and clindamycin was found in 24 (68 per cent) and 20 (57 per cent) of the pneumococci and 14 (40 per cent) had high level resistance to trimethoprim-

TABLE I
BACTERIAL ISOLATES FROM MIDDLE-EAR FLUID OF SOUTH AFRICAN CHILDREN WITH NON-RESOLVING OTITIS MEDIA

Organism	Number	% (n = 47)*
Streptococcus pneumoniae	35	74
Haemophilus influenzae†	9	19
Staphylococcus aureus	6	13
Moraxella catarrhalis	2	4
Streptococcus pyogenes	2	4
Pseudomonas aeruginosa	1	2

*3 children had *S pneumoniae* and *S aureus* isolated, two children had *S pneumoniae* and *H influenzae,* one child had *S penumoniae* and *M catarrhalis,* one child had *H influenzae* and *M catarrhalis* and one child had *P aeruginosa* and *S pyogenes*
†serotypes were type c (2) and untypable (7)

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TABLE II
ANTIMICROBIAL RESISTANCE OF STREPTOCOCCUS PNEUMONIAE MEF ISOLATES (N = 35)

Antibiotic	Intermediate level	High level	MIC	MIC_{50}	MIC_{90}
Penicillin	20 (57)	10 (28)	0.12–4.00	0.5	2.00
Ceftriaxone	8 (20)	–	1.00	0.5	1.00
Erythromycin	–	24 (69)	2–64	64	64
Clindamycin	–	20 (57)	64	64	64
Tmp-smx*	5 (14)	14 (40)	1/19–8/152	4/76	8/152

*trimethoprim-sulfamethoxazole

sulfamethoxazole (tmp-smx). Twenty per cent of the pneumococci were simultaneously resistant to penicillin, erythromycin, and clindamycin and a further 26 per cent showed simultaneous resistance to penicillin, erythromycin, clindamycin, and tmp-smx.

Antibiotic resistance was found in five (56 per cent) of the *H influenzae* strains; one (11 per cent) isolate was resistant to ampicillin, two (22 per cent) were resistant to tmp-smx and two (22 per cent) isolates were resistant to ampicillin and tmp-smx. The ampicillin-resistant strains produced beta-lactamase and all of these *H influenzae* strains were susceptible to co-amoxclav. Ampicillin resistance was found in one *M catarrhalis* isolate that produced beta-lactamase.

Serotypes 19F, 14, and 23F comprised 77 per cent of the pneumococci isolated from MEF (Table III). All of the pneumococcal serotypes found in MEF are contained in or cross-react with serotypes found in the nine-valent pneumococcal conjugate vaccine.

Children from whom any pathogen was isolated were significantly younger than those children for whom no bacteria were found in MEF (mean age = 17 months *vs* 23 months, $p = 0.03$). Isolation of a pneumococcus also was associated with younger age (mean age = 16 months *vs* 22 months, $p = 0.03$) and with having one or more episodes of otitis media within the six-months before tympanocentesis (28 children *vs* one child, OR = 7.72, $p = 0.03$). Children with fever were 2.15 times more likely to have a pneumococcus isolated from MEF than children without fever (23 children *vs* 12 children, $p = 0.049$) at the time of tympanocentesis. Use of a specific antibiotic or class of antibiotics at any time prior to tympanostomy was not significantly associated with isolation of any pathogen or *S pneumoniae* in particular.

Discussion

In our study of non-resolving otitis media bacteria were isolated from 27 per cent of the children, the dominant pathogen being *S. pneumoniae*. The low overall pathogen isolation rate is almost certainly due to the majority of children being on antibiotics at the time of tympanostomy and the MEF being sampled late in the course of their infection. The pneumococcus was also the dominant pathogen in a previous study of acute otitis media in South Africa[2] and a recent study from Chile.[10] Antibiotic resistance to penicillin was found in a very high percentage of the pneumococci isolated from the middle ear in the present study (85 per cent), more so than in strains isolated from acute otitis media in South Africa in the 1980s (0 per cent),[2] or from recent studies of invasive pneumococcal disease in children in South Africa (29 per cent)[4] or nasopharyngeal isolates of pneumococci from children of similar age in South Africa also attending private practitioners (45 per cent).[5] In addition, the penicillin resistance rates found in our study were much higher than the 30 per cent penicillin resistance rates found in a multinational survey of 917 children with acute otitis media.[11] The high prevalence of antibiotic resistance in our study is consistent with the findings of Dagan *et al.*[12] in which the effect of antibiotic use on *S pneumoniae* isolated from both MEF and the nasopharynx was prospectively evaluated. Within one week of initiation of treatment, 47 per cent of the Israeli children had their initially antibiotic-susceptible pneumococcus isolated from MEF replaced with an initially resistant organism carried in the nasopharynx.

TABLE III
SEROTYPES OF STREPTOCOCCUS PNEUMONIAE ISOLATED FROM MIDDLE EAR FLUID

Serotype	Number	% (n = 35)
4*	1	3
6B*	3	9
9V*	1	3
14*	9	26
19A	3	8
19F*	10	28
23F*	8	23

*Serotypes found in the 9-valent pneumococcal conjugate vaccine. Serotype 19A may cross-react with serotype 19F.

The treatment of pneumococcal otitis media is significantly impacted by the presence of antibiotic resistance. Successful treatment of bacterial otitis media depends on penetration of antibiotic into the middle ear in sufficient concentration and for a sufficient length of time to result in sterilization of the middle-ear fluid. In response to increasing antibiotic resistance and increasing numbers of treatment failures, higher dosages of amoxycillin (80–90 mg/kg/day) are now recommended for the empiric treatment of otitis media.[13]

All of the pneumococci isolated in this study were of serotypes either contained in, or cross-reacting with, serotypes in the nonavalent pneumococcal conjugate vaccine. These serotypes are highly prevalent (91 per cent)[4] amongst blood isolates of pneumococci from children in South Africa. The serotype distribution in the current study shows an excess of serotype 14 and serogroups 19 and 23 (26 per cent, 36 per cent and 23 per cent respectively) from non-resolving otitis compared to blood isolates from children (12 per cent, 18 per cent and five per cent, respectively)[4] while serogroup six is under-

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represented in this study compared to the proportion of this serogroup in blood (nine per cent *versus* 33.5 per cent[4]). A recent trial of a heptavalent pneumococcal conjugate vaccine in Finland found a 57 per cent reduction in episodes of vaccine serotype-specific pneumococcal otitis media and a 51 per cent reduction in cases due to serotypes that cross-react with the vaccine serotypes.[14] In another study in California the efficacy of the same vaccine in preventing otitis media increased as the frequency of episodes increased (from a baseline of seven per cent to 22.8 per cent in children with five episodes in the past six months or six episodes in the past year).[15] The increased vaccine efficacy in that study with increasing numbers of previous episodes is consistent with the seven-fold increased risk of isolation of pneumococci in our study from children who had had a prior episode of otitis in the previous six months. Vaccination could, therefore, prevent more than half of the cases of pneumococcal otitis media in South Africa. Concern has been raised, however, that disease due to vaccine serotypes may be replaced with serotypes not currently found in the conjugate vaccines. The trial in Finland found a 33 per cent increase in episodes due to non-vaccine serotypes.[14] Continued monitoring of serotypes of middle-ear isolates following vaccination with conjugate vaccines is needed to adequately assess the role of serotype replacement on pneumococcal otitis media. These replacement serotypes are not usually antibiotic-resistant although clones of antibiotic-resistant pneumococci of non-vaccine serotype causing acute otitis media in Israel have recently been described.[16]

Conclusion

In conclusion, antibiotic-resistant pneumococci appear to be a major cause of otitis media in children who have failed previous therapy and who have non-resolving pain or fever.

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References

1 Ruuskanen O, Heikki T. Otitis media: etiology and diagnosis. *Pediatr Infect Dis J* 1994;**13**:S23–6
2 Claassen AJ, Hough AP. A microbiological study of acute otitis media in Bloemfontein. *S Afr Med J* 1987;**71**:15–6
3 Baquero F, Loza E. Antibiotic resistance of microorganisms involved in ear, nose and throat infections. *Pediatr Infect Dis J* 1994;**13**:S9–14
4 Huebner RE, Wasas AD, Klugman KP. Trends in antimicrobial resistance and serotype distribution of blood and cerebrospinal fluid isolates of *Streptococcus pneumoniae* in South Africa, 1991–1998. *Int J Infect Dis* 2000;**4**:214–8
5 Heubner RE, Wasas AD, Klugman KP, and the Paediatric Study Group. Prevalence of nasopharyngeal antibiotic-resistant pneumococcal carriage in children attending private paediatric practices in Johannesburg. *S Afr Med J* 2000;**90**:1116–21
6 Dagan R, Abramson O, Leibovitz E, Lang R, Goshen S, Greenberg D, *et al*. Impaired bacteriologic response to oral cephalosporins in acute otitis media caused by pneumococci with intermediate resistance to penicillin. *Pediatr Infect Dis J* 1996;**15**:980–5
7 Gibson LF, Khoury JT. Storage and survival of bacterial by ultra-freeze. Letter. *Appl Microbiol* 1986;**3**:127–9
8 National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing: eighth informational supplement (publication M100-S8). Villanova, PA: National Committee for Clinical Laboratory Standards, 1998
9 Dean AG, Dean JA, Coulombier D, Burton AH, Brendel KA, Smith DC, *et al*. EpiInfo, Version 6: a word processing, database, and statistics program for epidemiology on microcomputers. Atlanta, GA, USA: Centers for Disease Control and Prevention, 1994
10 Rosenblut A, Santolaya ME, Gonzalez P, Corbalan V, Avendano LF, Martinez MA, *et al*. Bacterial and viral etiology of acute otitis media in Chilean children. *Pediatr Infect Dis J* 2001;**20**:501–7
11 Jacobs MR, Dagan R, Appelbaum PC, Burch DJ. Prevalence of antimicrobial-resistant pathogens in middle ear fluid: Multinational Study of 917 children with acute otitis media. *Antimicrob Agents Chemother* 1998;**42**:589–95
12 Dagan R, Leibovitz E, Cheletz G, Leiberman A, Porat N. Antibiotic treatment in acute otitis media promotes superinfection with resistant *Streptococcus pneumoniae* carried before initiation of treatment. *J Infect Dis* 2001;**183**:880–6
13 Dowell SF, Butler JC, Giebink GS, Jacobs MR, Jernigan D, Musher DM, *et al*. Acute otitis media: management and surveillance in an era of pneumococcal resistance – a report from the Drug-Resistant *Streptococcus pneumoniae* Therapeutic Working Group. *Pediatr Infect Dis J* 1999;**18**:1–9
14 Eskola J, Kilpi T, Palmu A, Jokinen J, Haapakoski J, Herva E, *et al*. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med* 2001;**344**:403–9
15 Black S, Shinefield H, Fireman B, Lewis E, Ray P, Hansen JR, *et al*. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. *Pediatr Infect Dis J* 2000;**19**:187–95
16 Porat N, Barkai G, Trefler R, Dagan R. New antibiotic-resistant clones of *Streptococcus pneumoniae*, unrelated to the pneumonococcal vaccine serotypes causing acute otitis media in southern Israel. Abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Sept. 2002, p97, Washington DC; American Society of Microbiology Abstract C2-651

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