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Research Article

Pediatric Acute Mastoiditis: Recent Evolutions in Clinical Presentation and Microbiology

De Bruyne N[1*], Dhooge I[1], De Leenheer E[1], Van Driessche V[2], Coorevits L[3] and Van Hoecke H[1]

[1]Department of Otorhinolaryngology, Ghent University Hospital, Ghent, Belgium
[2]Department of Radiology, Ghent University Hospital, Ghent, Belgium
[3]Department of Microbiology, Ghent University Hospital, Ghent, Belgium

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ABSTRACT

Acute mastoiditis is a well-known complication of acute otitis media in children. Last year we noticed some changes: more virulent pathogens, a more fulminant course of the disease and an increased need for surgical treatment in children with acute mastoiditis admitted to our hospital. Recently, other authors have raised the same concerns. Based on the last 12 consecutive cases of pediatric acute mastoiditis admitted to our hospital and a review of recent literature, we discuss a possible change in presentation, complication rate and microbiology in order to raise vigilance to a possibly changing pathology.

Introduction

Acute (coalescent) mastoiditis is a suppurative complication of acute otitis media (AOM) characterized by acute inflammation of the mastoid air cells and destruction of the thin bony septae between mastoid air cells [1]. Virulent bacteria in combination with an inadequate (or immature) immune response and anatomically less resistant barriers – the latter especially present in very young children – are the main risk factors to develop complications from an acute otitis media [2]. Since the introduction of antibiotics and their application for the treatment of AOM in risk patients and unfavorable disease courses, there has been a drastic decline in the incidence of acute otitis media episodes progressing to acute mastoiditis [3]. Although pediatric acute mastoiditis (PAM) has become rare, nowadays occurring in 0.02-0.25% of cases of AOM in children, it still remains a serious and potentially life-threatening condition, requiring prompt diagnosis and adequate treatment [4]. Due to the anatomical location of the mastoid in the temporal bone and close to the central nervous system, acute mastoiditis can progress to intratemporal (subperiosteal abscess, bezold abscess, facial nerve paralysis, labyrinthitis, apical petrositis) and intracranial complications (meningitis, cerebral venous sinus thrombosis, epidural abscess, subdural abscess, cerebellar or brain abscess).

These complications occur in 10-20% of PAM cases [5]. Although the prevalence, morbidity and mortality resulting from PAM in developed countries have steeply dropped since the antibiotic era and surgical developments of mastoidectomy, the proportion of children with acute mastoiditis developing an intratemporal and/or intracranial complication and requiring mastoid surgery does not seem to have decreased and might even have increased the last 10 to 20 years [5, 6]. There has been recent concern regarding a shift in causative agents, a more complicated disease course and an increasing need for surgery in PAM. Therefore, we reviewed all cases of PAM admitted to our hospital in last 2 years for clinical presentation, diagnostic findings (including imaging and microbiology), treatment and outcome. We critically appraised our findings and compared them with epidemiological, clinical and microbiological trends described in the current literature.

[*]Correspondence to: De Bruyne N, Department of Otorhinolaryngology, Ghent University Hospital, Corneel Heymanslaan 10 (1p1), B-9000, Ghent, Belgium; E-mail: naomi.debruyne@gmail.com, naomdbru.DeBruyne@UGent.be

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Materials and Methods

A retrospective analysis of all consecutive cases of PAM in children aged <16 years old treated at Ghent University Hospital, between 1-4-2018 and 31-3-2020 was performed. Cases of mastoiditis that occurred as a complication of an underlying chronic ear condition (chronic otitis media, cholesteatoma) or neoplastic condition (e.g., Langerhans cell histiocytosis) were excluded. Demographic and medical data, including time of onset of disease, antibiotic use before admission, clinical presentation, results of microbiological sampling, the extensiveness of disease and complications, type of surgery and outcome, were collected from the medical patient files. Acute mastoiditis was diagnosed based on the clinical signs of post-auricular erythema, tenderness and/or swelling with or without fluctuation and protrusion of the pinna [7]. Laboratory tests involving C-reactive protein and white blood cell count were performed in all children upon admission and follow up. Upon admission, sterile swab-sticks (flocked swabs in liquid amies medium) were used to collect a specimen of the ear discharge in case of spontaneous tympanic membrane perforation.

For referred patients, culture results were sometimes already available upon admission. In cases of surgical intervention (tympanostomy tube insertion +/- mastoidectomy), microbiological sampling was (also) done during surgery. For swabs, aerobic culture was performed using Tryptic Soy Agar with 5% sheep blood and GC Chocolate Agar (Becton-Dickinson and Company, Franklin Lakes, NJ, USA), which were incubated for two days. Samples obtained during the surgical intervention were cultured aerobically using Tryptic Soy Agar with 5% sheep blood, GC Chocolate Agar and Columbia CNA Agar with 5% sheep blood/MacConkey biplate (Becton-Dickinson), which were incubated for two days, and a thioglycolate USP medium (Oxoid, Hampshire, UK) for enrichment, which was incubated for seven days. Besides, these samples were cultured anaerobically using Schaedler Agar with vitamin K1 and 5% sheep blood (Becton-Dickinson and Company, Franklin Lakes, NJ, USA) and ARIA with horse blood (Oxoid, Hampshire, UK), which were incubated for four days.

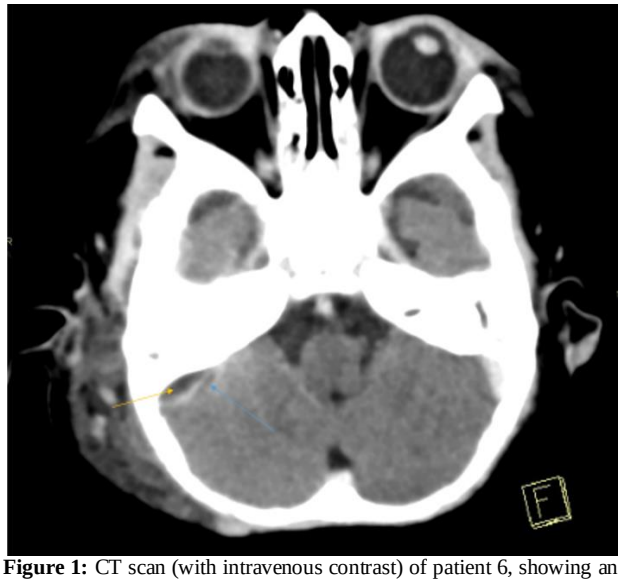

Figure 1: CT scan (with intravenous contrast) of patient 6, showing an epidural abscess and sigmoid sinus thrombosis. Yellow arrow: epidural collection. Blue arrow: sigmoid sinus thrombosis.

CT scan with intravenous (IV) contrast was performed in every patient. All scans were viewed by our head and neck radiologist and showed typical signs of mastoiditis such as cloudy mastoids, osteitis with bony erosions of the mastoid cells and extension with periosteal involvement. MRI was performed in selected cases with suspected intracranial complications but inconclusive CT scan. Complicated mastoiditis was defined as an extension of the infectious process beyond the mastoid system with CT and/or MRI finding of an abscess (subperiosteal, epidural, subdural, cerebellar, brain or bezold's abscess), cerebral venous sinus and/or jugular vein thrombosis, apical petrositis, osteomyelitis beyond the mastoid area, the clinical finding of cranial nerve palsy or meningitis confirmed by lumbar puncture (Figure 1) [8, 9]. Management of children with acute mastoiditis in our center depends on clinical presentation and presence of intratemporal or intracranial complications.

Uncomplicated mastoiditis is treated by tympanostomy tube placement and intravenous wide spectrum antibiotics based on amoxicillin-clavulanic acid. Antibiotic treatment is adjusted if indicated based on culture results. Cases without clinical improvement after 48 hours or showing deterioration of postauricular swelling, fever or lethargy, undergo a mastoidectomy. When intratemporal and/or intracranial complications are present upon presentation, surgery is immediately performed consisting of tympanostomy tube placement (if not already present) and mastoidectomy. Subdural and brain abscesses are treated by the neurosurgeon. In the case of sigmoid sinus thrombosis, the bone over the sigmoid sinus is removed and low molecular weight heparin is started and continued for at least 3 months [10, 11]. Further immunological investigations are performed in case of suspicion for primary immune deficiency (10 alarm signs for primary immune deficiency in children). A review of the English literature in PubMed, Embase and Cochrane library was performed with the search term "pediatric acute mastoiditis" in combination with the search terms "incidence", "microbiology", "complication", "complications", "surgery", "*Fusobacterium necrophorum*", and "*Streptococcus pyogenes*". Selection of recent articles was conducted and a manual search of the bibliographies of the selected articles was done.

Results

Twelve consecutive cases of PAM were included in our study. The mean patient age was 11.5 months (range 3 months - 10 years old), the male to female ratio was 1:1. 6/12 children were younger than 1-year-old, 9/12 were younger than 2-year-old. The majority presented in spring (n=6) and winter (n=3); only 1 patient was admitted in summer and 2 during fall. Nine out of the 12 included patients were referred from other hospitals: some were promptly referred upon (suspicion) of acute mastoiditis, others were transferred because of unfavorable disease course and suspected or proven development of complications. Seven received systemic antibiotic treatment before admission to our hospital. Upon admission, all patients received a CT scan with intravenous contrast if not already performed. As shown in (Table 1), all children presented with a complicated acute mastoiditis. In 11 out of 12 cases, at least one bacterial pathogen was isolated, with identification of *Streptococcus pneumoniae* (n=3), *Streptococcus pyogenes* (n=3, 1 case demonstrating ampicillin resistance), *Fusobacterium necrophorum* (n=3) *Pseudomonas aeruginosa* (n=2), and *Haemophilus influenzae* (n=1).

Table 1: Patient characteristics.

	P/R	M/F	AGE	PAM complication	Causative agent	Type of Antibiotic
1	R	F	10m	Subperiosteal abscess	*S. pyogenes*	Amoxicilline – clavulanic acid
2	R	M	3m	Subperiosteal abscess	*S. pneumoniae*	Amoxicilline – clavulanic acid
3	R	F	10m	Sigmoid sinus thrombosis, transverse sinus thrombosis, internal jugular vein thrombosis, subperiosteal abscess	*F. necrophorum*	Cefotaxim + metronidazole
4	R	M	5 y8m	Subperiosteal abscess	*S. pneumoniae*	Ceftriaxone
5	R	F	10y	Facial nerve paralysis	*Ps. aeruginosa (?)*	Piperaciline-tazobactam
6	R	F	7m	Sigmoid sinus thrombosis, transverse sinus thrombosis, internal jugular vein thrombosis, epidural abscess	*S. pyogenes*	Cefotaxim + clindamycine
7	P	M	3y 6m	Subperiosteal abscess	*S. pyogenes (ampi R)*	Cefotaxim
8	P	F	13m	Sigmoid sinus thrombosis	*F. necrophorum*	Cefotaxim + metronidazole
9	R	M	1y 9m	Subperiosteal abscess	*F. necrophorum*	Ceftriaxone
10	R	M	3m	Subperiosteal abscess	*S. Pneumoniae* *Ps. aeruginosa (?)*	Piperaciline-tazobactam
11	P	M	7m	Subperiosteal abscess	*Geen groei*	Amoxicilline – clavulanic acid
12	R	F	1y 4m	Subperiosteal abscess Sigmois sinus thrombosis	*H. influenzae*	Cefotaxim + metronidazole

P/R: Primary admission; Referral: M/F: Male/Female.

All children were admitted and IV antibiotic treatment – if already available culture-guided – was immediately started or continued. In 5 of our 12 patients antibiotic treatment was altered once culture results were available. Prompt surgical intervention was performed, consisting of a canal wall up mastoidectomy in combination with placement of tympanostomy tubes, if not already present. In the case of sigmoid sinus thrombosis, the sinus was uncovered. In 1 patient (patient 11), subperiosteal abscess drainage via incision was performed. It concerned a very young patient with a poorly developed mastoid cavity. Our patients were hospitalized between 5 days and 23 days for IV antibiotic treatment. After discharge, oral antibiotic treatment was continued for up to 4 weeks and a follow-up consultation was planned after one week. All patients fully recovered and are still in follow-up. In 2 patients, subsequent immunological investigations revealed a primary immune deficiency (IgG2 subclass deficiency).

Discussion

In this study, we present all consecutive cases of PAM as a complication of acute otitis media admitted and treated at Ghent University Hospital over the last two years. Our population consisted of very young children (median age 11.5 months) with a severe disease course. All children presented with intratemporal and/or intracranial complications upon admission and consequently underwent surgical management. Although this case series is limited in number and prone to tertiary center referral bias, the fulminant disease course in all consecutive cases raises concern about a changing trend with a shift in causative agents, a rapid progression to complications and increasing need for surgery. This observation is consistent with literature findings. Whereas the total incidence of PAM seemed to remain stable between 1991 and 2017, with 13.5-16.8/1000000 per year for children under the age of 2 and 4.3-7.1/100000 per year for older children, reports of increased virulence, higher rates of complicated disease upon presentation and consequently more need for mastoid surgery in PAM, especially in young children, are rising [6, 12-17]. Attlmayr *et al.* describe 2 different PAM populations in their tertiary center; the first from 1995-2005 and the second from 2005-2015. The incidence of PAM was similar during both periods; however, the rate in intracranial complications rose from 4.8% in the first study period to 13% during the second part and the need for surgery increased from 23% to 58.7% respectively. Benito *et al.* studied 205 PAM cases between 1996 and 2005 and reported an increase in surgical intervention from 4% in 1996-97 to 33% in 2003-2004 and 70% in 2005.

This study was also performed in a tertiary care center. A rising proportion of children presenting with complicated mastoiditis and consequently requiring surgery was also reported by other tertiary care centers [5, 6, 14, 17-20]. In our population, all children presented with a complicated mastoiditis on admission and all children underwent appropriate surgery, especially the proportion of children (33%) with lateral sinus thrombophlebitis was strikingly high. The higher reporting of complicated disease courses in PAM over the most recent years is very unlikely to be explained by a higher detection rate of complications. A changing microbiology with more virulent disease course has been put forward to explain the recent observations and reports. In accordance with our findings, other case series increasingly detect *S. pyogenes* and *F. necrophorum* as causative agents in PAM [5, 6, 17, 21]. Especially the rise in *F. necrophorum* in acute otitis media and mastoiditis in children is being increasingly documented [21-27]. Gelbart *et al.*

observed a tenfold increase in *F. necrophorum* related PAM between 2011 and 2015. Interestingly, a complicated course of mastoiditis was significantly more frequent among *F. necrophorum* mastoiditis cases versus non-*F. necrophorum* cases and all *F. necrophorum* mastoiditis cases necessitated a surgical intervention versus only 15.6% of non-*F. necrophorum* mastoiditis cases. The rise in the need for surgery is confirmed by other authors [6]. Moreover, pathogens isolated in the operated group demonstrate a higher rate of *F. necrophorum*, suggesting it is the culprit behind the rise in complications and need for surgical intervention in these recent years.

F. necrophorum is a pathogen that was very rarely isolated in PAM in the past; however, it was the causative agent in 3 of our 12 patients. It is an obligatory anaerobic, gram-negative bacterium and is a commensal in the oral cavity after teeth eruption, the female genital tract and alimentary tract [26]. The association between *F. necrophorum* and septic venous thrombosis is well known and is most likely attributed to its ability to cause platelet aggregation [27]. The rise in *F. necrophorum* mastoiditis, especially in very young children, might explain the increased finding of lateral sinus thrombophlebitis during the last years [19, 23]. The increased identification of *F. necrophorum* can only partly be explained by improved and more widespread detection techniques (especially for anaerobes) and a true incidence rise of *F. necrophorum* as an emerging pathogen in (pediatric) acute mastoiditis, especially the last decade, has repeatedly been highlighted [21-25, 28, 29].

A possible explanation for the changing microbiology could be the introduction of the *S. pneumoniae* conjugate vaccine. Since *S. pneumoniae* was one of the most frequent causative agents of PAM, the routine administration of the *S. pneumoniae* conjugate vaccine might provoke a shift in different pathogens. Biesbroek *et al.* showed that after administration of the vaccine, a change of the nasopharyngeal flora in healthy children was observed. They found an increased carriage rate of *F. necrophorum* and other anaerobe pathogens such as *Prevotella* spp. in the upper respiratory tract. Moreover, there is no epidemiological evidence for a rise in complications of PAM after the introduction of PCV7 [20, 30, 31]. Globally the antibiotic consumption remains high. Increasing resistance of bacteria may lead to therapeutic failure and cause a rise in complication rates. This theory is supported by the fact that most patients received antibiotic treatment before admission [5, 6]. In our patients, both *S. pneumoniae* strains were sensitive to ampicillin. Unfortunately, to the best of our knowledge, we do not have numbers on-resistance of *F. necrophorum*. In our patients, one out of two *S. pyogenes* strains showed resistance to ampicillin. Penicillin failure in *S. pyogenes* is dramatically increasing throughout the world, with almost 40% in some regions [26].

Different factors account for this failure, such as alteration of the commensal bacterial microbiota, which can compete for nutrients; protection of *S. pyogenes* by β-lactamase-producing bacteria (*Staphylococcus aureus*, *Haemophilus* spp., *Moraxella catarrhalis*, and anaerobes) that are commonly part of the oral microbiota and coaggregation between *M. catarrhalis* and *S. pyogenes*, which may enhance *S. pyogenes* colonization through the facilitation of its adherence to human epithelial cells [26, 32-34]. Another possible explanation for the rise in complications in PAM might be the implementation of guidelines regarding restricted antibiotic use. There have been reports suggesting that a more restricted antibiotic use in the treatment of AOM might have provoked an increased number of complicated PAM due to suboptimal treatment of otitis media [4]. However, most of our patients received antibiotic treatment before admission, as well as in other series published in literature [5, 6, 17]. Moreover, after the introduction of the guidelines of more restrictive antibiotic use, no change in incidence or rise in complications of PAM was noticed [15, 35].

Limitations to our single center study include being a tertiary care center; therefore, many patients (75% in this population) have been referred from other hospitals. Consequently, our population might reflect an over presentation of children with unfavourable disease courses and children that develop complications. In addition, it concerns a small group of only 12 patients and a short study period of 2 years. The strengths of this case series are that we managed to acquire microbiological data in 11 of our 12 patients; all of them received a CT scan and none were lost to follow-up. To the best of our knowledge, no multi-center prospective studies have been published to evaluate the changing pathophysiology and microbiology of pediatric acute mastoiditis. Moreover, the available studies are descriptive and are not statistically corrected for classification or verification bias. They have also been performed in tertiary care centers and most are single center studies. In future, large and prospective studies are needed to evaluate the true incidence and incidence changes of PAM and its complications, the changes in microbiology and the outcome of conservative and surgical treatment. In addition, these insights will help to identify predictive factors for a complicated disease course and to adapt our diagnostic and management protocol.

Conclusion

PAM is a well-known pathology but might be undergoing some changes requiring adaptation of our management protocol. In our small population of 12 retrospectively collected patients in a tertiary referral center, we suspect a changing trend with a shift in causative agents towards *Streptococcus pyogenes* and *Fusobacterium necrophorum*, a rapid progression to complications and increasing need for surgery. Vigilance is important, especially in children under 2 years of age with a severe course of PAM, as early recognition can lead to improved treatment. Low threshold for CT scan, adequate sampling for culture and a prompt start of antibiotic treatment are of utmost importance. When there is no improvement of the clinical situation over 48 hours or complications occur, surgery is to be performed as soon as possible. More and larger prospective studies are needed to objectivate the incidence of PAM and its complications, microbiology and outcome. Identifying predicting factors for a complicated course of the disease, might help early detection and intervention with adequate antibiotic and surgical treatment.

Conflicts of Interest

None.

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None.

Ethical Approval

The study has been approved by the Ethical Committee of Ghent University Hospital, Belgium (approval number BC-07774).

REFERENCES

1. Mansour S, Magnan J, Nicolas K, Haidar H (2018) Acute Otitis Media and Acute Coalescent Mastoiditis. *Middle Ear Diseases* 85-113. [Crossref]
2. Dhooge IJ, Albers FW, Van Cauwenberge PB (1999) Intratemporal and intracranial complications of acute suppurative otitis media in children: renewed interest. *Int J Pediatr Otorhinolaryngol* 49: S109-S114. [Crossref]
3. Spremo S, Udovčić B (2007) Acute mastoiditis in children: susceptibility factors and management. *Bosn J Basic Med Sci* 7: 127-131. [Crossref]
4. Thompson PL, Gilbert RE, Long PF, Saxena S, Sharland M et al. (2009) Effect of antibiotics for otitis media on mastoiditis in children: a retrospective cohort study using the United kingdom general practice research database. *Pediatrics* 123: 424-430. [Crossref]
5. Gelbart M, Bilavsky E, Chodick G, Raveh E, Levy I et al. (2019) Fusobacterium necrophorum as an Emerging Pathogen of Acute Mastoiditis. *Pediatr Infect Dis J* 38: 12-15. [Crossref]
6. Shavit SS, Raveh E, Levi L, Sokolov M, Ulanovski D (2019) Surgical intervention for acute mastoiditis: 10 years experience in a tertiary children hospital. *Eur Arch Otorhinolaryngol* 276: 3051-3056. [Crossref]
7. van den Aardweg MTA, Rovers MM, de Ru JA, Albers FWJ, Schilder AGM (2008) A systematic review of diagnostic criteria for acute mastoiditis in children. *Otol Neurotol* 29: 751-757. [Crossref]
8. Oestreicher Kedem Y, Raveh E, Kornreich L, Popovtzer A, Buller N et al. (2005) Complications of mastoiditis in children at the onset of a new millennium. *Ann Otol Rhinol Laryngol* 114: 147-152. [Crossref]
9. Vazquez E, Castellote A, Piqueras J, Mauleon S, Creixell S et al. (2003) Imaging of complications of acute mastoiditis in children. *Radiographics* 23: 359-372. [Crossref]
10. Wong BYW, Hickman S, Richards M, Jassar P, Wilson T (2015) Management of paediatric otogenic cerebral venous sinus thrombosis: a systematic review. *Clin Otolaryngol* 40: 704-714. [Crossref]
11. Chalmers E, Ganesen V, Liesner R, Maroo S, Nokes T et al. (2011) Guideline on the investigation, management and prevention of venous thrombosis in children. *Br J Haematol* 154: 196-207. [Crossref]
12. Pang LHY, Barakate MMS, Havas TE (2009) Mastoiditis in a paediatric population: a review of 11 years experience in management. *Int J Pediatr Otorhinolaryngol* 73: 1520-1524. [Crossref]
13. Van Zuijlen DA, Schilder AG, Van Balen FA, Hoes AW (2001) National differences in incidence of acute mastoiditis: relationship to prescribing patterns of antibiotics for acute otitis media? *Pediatr Infect Dis J* 20: 140-144. [Crossref]
14. Attlmayr B, Zaman S, Scott J, Derbyshire SG, Clarke RW et al. (2015) Paediatric acute mastoiditis, then and now: is it more of a problem now? *J Laryngol Otol* 129: 955-959. [Crossref]
15. Anthonsen K, Høstmark K, Hansen S, Andreasen K, Juhlin J et al. (2013) Acute mastoiditis in children: a 10-year retrospective and validated multicenter study. *Pediatr Infect Dis J* 32: 436-440. [Crossref]
16. Kværner KJ, Bentdal Y, Karevold G (2007) Acute mastoiditis in Norway: No evidence for an increase. *Int J Pediatr Otorhinolaryngol* 71: 1579-1583. [Crossref]
17. Benito MB, Gorricho BP (2007) Acute mastoiditis: increase in the incidence and complications. *Int J Pediatr Otorhinolaryngol* 71: 1007-1011. [Crossref]
18. Bilavsky E, Yarden Bilavsky H, Samra Z, Amir J, Nussinovitch M (2009) Clinical, laboratory, and microbiological differences between children with simple or complicated mastoiditis. *Int J Pediatr Otor* 73: 1270-1273.
19. Yarden Bilavsky H, Raveh E, Livni G, Scheuerman O, Amir J et al. (2013) Fusobacterium necrophorum mastoiditis in children - emerging pathogen in an old disease. *Int J Pediatr Otorhinolaryngol* 77: 92-96. [Crossref]
20. Choi SS, Lander L (2011) Pediatric acute mastoiditis in the post-pneumococcal conjugate vaccine era. *Laryngoscope* 121: 1072-1080. [Crossref]
21. Mierzwinski J, Tyra J, Haber K, Drela M, Paczkowski D et al. (2019) Therapeutic approach to pediatric acute mastoiditis - an update. *Braz J Otorhinolaryngol* 85: 724-732. [Crossref]
22. Creemers Schild D, Gronthoud F, Spanjaard L, Visser LG, Brouwere CNM et al. (2014) Fusobacterium necrophorum, an emerging pathogen of otogenic and paranasal infections? *New Microbes New Infect* 2: 52-57. [Crossref]
23. Giridharan W, De S, Osman EZ, Amma L, Hughes J et al. (2004) Complicated otitis media caused by Fusobacterium necrophorum. *J Laryngol Otol* 118: 50-53. [Crossref]
24. Brook I (2015) Fusobacterial head and neck infections in children. *Int J Pediatr Otorhinolaryngol* 79: 953-958. [Crossref]
25. Stergiopoulou T, Walsh TJ (2016) Fusobacterium necrophorum otitis and mastoiditis in infants and young toddlers. *Eur J Clin Microbiol Infect Dis* 35: 735-740. [Crossref]
26. Forrester LJ, Campbell BJ, Berg JN, Barrett JT (1985) Aggregation of platelets by Fusobacterium necrophorum. *J Clin Microbiol* 22: 245-249. [Crossref]
27. Brook I (2013) Penicillin failure in the treatment of streptococcal pharyngo-tonsillitis. *Curr Infect Dis Rep* 15: 232-235. [Crossref]
28. Hagelskjaer LH, Prag J, Malczynski J, Kristensen JH (1998) Incidence and clinical epidemiology of necrobacillosis, including Lemierre's syndrome, in Denmark 1990-1995. *Eur J Clin Microbiol Infect Dis* 17: 561-565. [Crossref]
29. Brazier JS, Hall V, Yusuf E, Duerden BI (2002) Fusobacterium necrophorum infections in England and Wales 1990-2000. *J Med Microbiol* 51: 269-272. [Crossref]
30. Littorin N, Ahl J, Udden F, Resman F, Riesbeck K (2016) Reduction of Streptococcus pneumoniae in upper respiratory tract cultures and a decreased incidence of related acute otitis media following introduction of childhood pneumococcal conjugate vaccines in a Swedish county. *BMC Infect Dis* 16: 407. [Crossref]
31. Biesbroek G, Wang X, Keijser BJF, Eijkemans RMJ, Trzciński K et al. (2014) Seven-valent pneumococcal conjugate vaccine and nasopharyngeal microbiota in healthy children. *Emerg Infect Dis* 20: 201-210. [Crossref]
32. Kaplan EL, Chhatwal GS, Rohde M (2006) Reduced ability of penicillin to eradicate ingested group A streptococci from epithelial cells: clinical and pathogenetic implications. *Clin Infect Dis* 43: 1398-1406. [Crossref]

33. Pichichero ME, Casey JR (2007) Systematic review of factors contributing to penicillin treatment failure in Streptococcus pyogenes pharyngitis. *Otolaryngol Head Neck Surg* 137: 851-857. [Crossref]
34. Schaar V, Uddbäck I, Nordström T, Riesbeck K (2014) Group A streptococci are protected from amoxicillin-mediated killing by vesicles containing β-lactamase derived from Haemophilus influenzae. *J Antimicrob Chemother* 69: 117-120. [Crossref]
35. Groth A, Enoksson F, Hermansson A, Hultcrantz M, Stalfors J et al. (2011) Acute mastoiditis in children in Sweden 1993-2007 - - no increase after new guidelines. *Int J Pediatr Otorhinolaryngol* 75: 1496-1501. [Crossref]