Otology

Notes on the microbiology of cholesteatoma: clinical findings and treatment

Note sulla microbiologia del colesteatoma: rilievi clinici e terapeutici

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SUMMARY

Anomalous proliferation of the cholesteatoma epithelium is caused by extrinsic factors such as toxins or bacterial antigens combined with lytic enzymes, lymphokines and cytokines released from the inflammatory infiltrate. This could explain the close relationship between the aggressiveness of cholesteatoma and repeated bacterial superinfection, therefore it is very important to know the bacteria involved in order to control the regrowth of skin following surgery, reduce the aggressive potential of the cholesteatoma and limit the incidence of complications. This study focused on 70 females and 80 males aged between 15 and 65 years, affected by cholesteatomatous *otitis media*; all underwent bacteriological examination of the auricular secretion. The floral bacteria which proved to play the most important role (60.3%) were the aerobic type and the highest levels were those of *Pseudomonas aeruginosa* (31.1%) followed by *Staphylococcus aureus* (19.1%), *Proteus mirabilis* (7.7%), *Escherichia coli* (1.4%) and *Klebsiella pneumoniae* (1%). Anaerobic floral bacteria were found in a fairly high percentage of cases (38.2%); in particular, anaerobic gram-positive cocci (*Peptococcus* 12.4% and *Peptostreptococcus* in 4.8% of cases), *Bacteroides* (12.4%), *Clostridium* (3.8%), *Fusobacterium* (2.9%) and *Propionobacterium* (1.9%) were isolated. In 3 cases of mycetes (1.4%) only *Aspergillus,* in association with *Pseudomonas* and *Staphylococcus*, was identified. The study showed, then, how effective second generation fluoroquinolones and third generation cephalosporins are (the latter being used in pre-adolescent children), the reason being that these antibiotics work not only on *Pseudomonas* and *Staphylococcus*, but also on the anaerobic bacteria.

KEY WORDS: Chronic otitis • Cholesteatoma • Microbiology • Antibiotics

RIASSUNTO

L'anomala proliferazione del colesteatoma è favorito da fattori estrinseci come tossine od antigeni batterici rilasciati dall'infiltrato infiammatorio in associazione ad enzimi litici, linfochine e citochine. Ciò può spiegare la stretta relazione tra l'aggressività del colesteatoma e le ripetute superinfezioni batteriche, per cui è molto importante conoscere i batteri implicati al fine di controllare la recidiva post-chirurgica, di ridurre il potenziale aggressivo del colesteatoma e di limitare l'incidenza di complicanze. Lo studio prende in esame 70 femmine e 80 maschi di età compresa tra i 15 ed i 65 anni, affetti da colesteatoma e sottoposti ad esame colturale batteriologico della secrezione auricolare. La flora batterica maggiormente rappresentata è quella aerobia: Pseudomonas aeruginosa *(31,1%),* Staphylococcus aureus *(19,1%),* Proteus mirabilis *(7,7%),* Escherichia coli *(1,4%) e* Klebsiella pneumoniae *(1%). Anche la flora batterica anaerobia è stata, comunque, riscontrata in un discreta percentuale di casi (38,3%); in particolare: anaerobic gram-positive cocci (*Peptococcus *12,4% e* Peptostreptococcus *in 4,8),* Bacteroides *(12,4%),* Clostridium *(3,8%),* Fusobacterium *(2,9%) and* Propionobacterium *(1,9%). In tre casi è stato identificato un micete (*Aspergillus*) in associazione con* Pseudomonas *e* Staphylococcus*. Lo studio, inoltre, dimostra l'efficacia terapeutica della seconda generazione dei florochinoloni e della terza generazione di cefalosporine (queste ultime adoperate nei bambini), dal momento che tali antibiotici sono attivi non solo sullo* Pseudomonas *e sullo* Staphylococcus*, ma anche sui batteri anaerobi.*

PAROLE CHIAVE: *Otite cronica • Colesteatoma • Microbiologia • Antibiotici*

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Introduction

In 1964, Gray [1] defined cholesteatoma as "skin in the wrong place"; subsequently, Sade (1993) [2] reported on the "presence of squamous epithelium in the tympanic cavity producing macroscopic amounts of keratin inadequately cleared".

Both definitions refer to the presence of keratinising malpighian epithelium in the middle ear which forms a desquamous keratin lamellar matrix which collects in concentric layers to form the central nucleus of the cholesteatoma.

The matrix is enclosed in a thin layer of connective tissue called the perimatrix [3]. This is separated from adjacent bone by an inflammatory infiltrate which plays a decisive role in the potential spread of the cholesteatoma.

Alterations in the proliferation, differentiation and migration of the keratinocytes is influenced by the activation of fibroblasts in the perimatrix and by the release of cy-

tokines and growth factors from the cells in the inflammatory infiltrate [4 5].

According to one theory, anomalous proliferation of the cholesteatoma epithelium is caused by extrinsic factors such as toxins or bacterial antigens combined with lytic enzymes, lymphokines and cytokines released from the inflammatory infiltrate. This could explain the close relationship between the aggressiveness of cholesteatoma and repeated bacterial superinfection [6-9]. The most frequently identified bacteria in cholesteatoma otitis are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* and anaerobic gram-positive cocci (AGPC) [10]. Various studies on the microbiology have been carried out and what we consider to be the most significant findings are outlined below.

Aygagary et al. [11], in 1981, in studies on 115 patients affected by suppurative otitis, isolated anaerobic bacteria in 10% of cases, aerobic pathogens in 35% and polymicrobial flora in 50%. The most frequent anaerobes were *Bacteroides* and anaerobic gram-positive cocci, while the most frequent aerobes were *Pseudomonas aeruginosa, Staphlococcus Aureus and Proteus*.

Poorery and Iyer [12], in 2002, examined 100 patients and found only aerobic bacteria, of which *Pseudomonas aeruginosa* (35.2%), *Klebsiella pneumoniae* (25.4%) and *Staphylococcus aureus* (14.7%) were the most frequently identified. These percentages were suspect in a sample of 100 subjects.

In studies carried out by Sugita [13], in 1981, on 760 cases, anaerobic bacteria were isolated in 8.2% of cases, among these the most frequent were the anaerobic gram positive cocci (47%) and *Bacteroides* (22%). However, low percentage reported by Sugita seems to derive from the use of inadequate techniques for the growth and development of the anaerobic bacteria [14].

Aim of the study

From a large sample of chronic cholesteatoma otitis patients, the Authors propose to establish which are the principal bacteria implied in superbacterial infection in order to establish a protocol of antibiotic treatment to be used in the pre-operative phase and in the management of possible post-operative complications.

Materials and Methods

Overall 150 patients were included in this study; 70 females and 80 males aged between 15 and 65 (mean age 40 yrs), who were admitted to the Otologic Surgery at the Department of Otorhinolaryngology, at the University "Federico II" of Naples.

All the patients had a history of chronic suppurative otitis dating back to between 3 and 18 years.

Material for analysis was collected otomicroscopically, using a sterile ear tampon with reducing agents for the anaerobes and with a semisolid base for the aerobic bacteria, taking care to avoid contamination of the skin of the external auditory canal. The samples were immediately sent to the microbiology laboratory for the necessary bacteriological processing.

None of the patients examined had received local or systemic antibiotic treatment in the preceding 2 weeks.

The materials gathered were innoculated, for the aerobes and the facultative anaerobes, onto blood agar, Mac Conkey agar and Thayer Martin agar, then incubated for 24 hours at 37° in aerobiosis or under CO_2 at 5%.

For the obligatory anaerobes, a thioglycolated mix and prereduced agar were used (blood agar with hemin and vitamin K1), with incubation in an oxygen-free setting for 48 hours.

For the mycetes, Sabouraud agar was used, incubated at 37° for 48 hours.

Identification of bacteria was carried out according to conventional methods.

The Kirby-Bauer diffusion in agar method was used when testing how sensitive the various isolated organisms were to antibiotics.

The antibiotics tested were: ciprofloxacin, levofloxacin, amikacin, ceftriaxone, cefoperazone, ceftazidime, amoxicillin/clavulanic acid and erythromycin**.**

Results

Out of the 150 samples analysed for aerobic, anaerobic and mycotic germs, 146 (97.3%) tested positive for pathogenic germs. The 4 (2.7%) negative samples were excluded.

Pure cultures were isolated in 86 cases (58.9%) whereas in 60 cases (41.1%) only aerobic bacteria were isolated and in 26 cases (17.8%) only anaerobic bacteria. A polymicrobic flora was isolated in 60 cases (41.1%), in 3 of which (2%) a fungineous growth was identified (Fig. 1).

Overall 209 germs were identified, of which 126 were aerobic (60.3%), 80 anaerobic (38.2%) and 3 mycetes (1.4%) (Fig. 2).

For the aerobics the bacteria isolated were: *Pseudomonas aeruginosa* in 65 cases (31.1%), of which 50 cases (76.9%) were pure cultures, while in the remaining 15 cases (23.1%) *Pseudomonas* was isolated in association with aerobic and anaerobic bacteria.

Staphylococcus aureus was found in 40 cases (19.1%), of which 10 cases (25%) were pure culture and 30 cases (75%) were in association with aerobic and anaerobic bacteria.

Proteus mirabilis was identified in 16 cases (7.7%) always in association with gram negative bacteria.

Escherichia coli was isolated in 3 cases (1.4%) and *Klebsiella pneumoniae* in 2 cases (1%); both were always in association with Pseudomonas.

As far as anaerobic bacteria are concerned, anaerobic

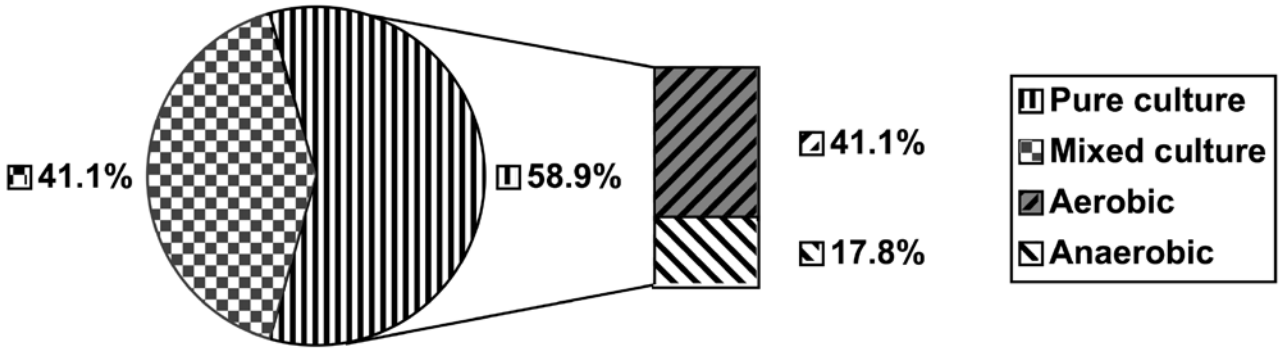

Fig. 1. Percentages of mixed/pure cultures. For pure cultures percentages of aerobic and anaerobic bacteria isolation are reported.

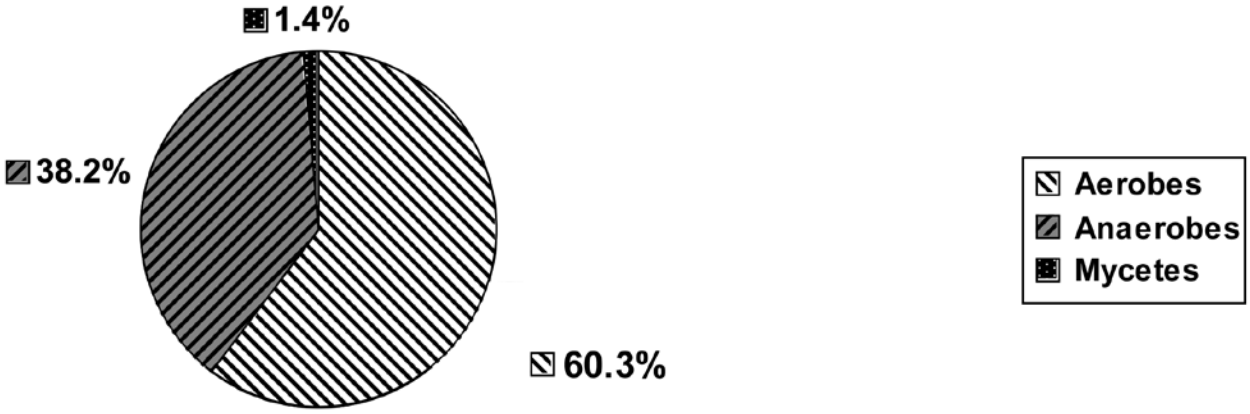

Fig. 2. Percentages of the microbiological species identified: aerobic bacteria, anaerobic bacteria and mycetes.

Table I. Isolated pathogens reported.

Isolated pathogens	No. cases (%)	Pure/mixed culture (%)
Aerobic		
– *Pseudomonas aeruginosa*	65 (31.1)	50 (76.9)/15 (23.1)
– *Staphylococcus aureus*	40 (19.1)	10 (25)/30 (75)
– *Proteus mirabilis*	16 (7.7)	0 (0)/16 (100)
– *Escherichia coli*	3 (1.4)	0 (0)/3 (100)
– *Klebsiella pneumoniae*	2 (1)	0 (0)/2 (100)
Anaerobic		
– *Peptococcus*	26 (12.4)	10 (38.5)/16 (61.5)
– *Peptostreptococcus*	10 (4.8)	4 (40)/6 (60)
– *Bacteroides*	26 (12.4)	8 (30.8)/18 (69.2)
– *Clostridium*	8 (3.8)	2 (25)/6 (75)
– *Fusobacterium*	6 (2.9)	2 (33.3)/4 (66.6)
– *Propionobacterium*	4 (1.9)	0 (0)/4 (100)
Mycetes		
– *Aspergillus niger*	3 (1.4)	0 (0)/3 (100)
Total	209 (100)	

Table II. Sensitivity of the isolated bacteria to the antibiotics tested.

Bacteria identified	No. isolated (%)	CIPR (%)	LEV (%)	AMI (%)	CEFT (%)	CEFO (%)	CEFZ (%)	AM/CL (%)	E (%)
Pseudomonas	65 (31.1)	58	59	61	53	54	56	49	22
Staphylococcus	40 (19.1)	32	33	24	28	30	28	27	18
Proteus	16 (7.7)	16	16	16	16	14	16	9	--
Escherichia	3 (1.4)	2	2	3	3	2	3	3	--
Klebsiella	2 (1)	2	2	2	2	1	2	1	1
AGPC	36 (17.2)	32	34	--	19	21	15	20	10
Bacteroides	26 (12.4)	24	25	--	--	13	--	24	--
Clostridium	8 (3.8)	6	6	--	5	5	3	5	--
Fusobacter	6 (2.9)	6	6	--	6	4	5	6	--
Propionobacterium	4 (1.9)	3	4	--	3	3	3	3	--
Total	206 (100)	181 (87.8)	187 (90.7)	106 (51.4)	135 (65.5)	147 (71.3)	131 (63.5)	147 (71.3)	51 (24.7)

CIPR = Ciprofloxacin; LEV = Levofloxacin; AMI = Amikacin; CEFT = Ceftriaxone; CEFO = Cefoperazone; CEFZ = Ceftazidime; AM/CL = Amoxicillin + Ac. Clavulanic; E = Erythromycin; AGPC = anaerobic gram-positive cocci.

gram positive cocci were isolated in 36 cases (17.2%), (*Peptococcus* in 26 cases (12.4%), of which 10 (38.5%) pure cultures and *Peptostreptococcus* in 10 cases (4.8%), with 4 (40%) pure cultures). The AGPC were followed in order of frequency by *Bacteroides* in 26 cases (12.4%, with 8 (30.8%) pure cultures) and *Clostridium* in 8 cases (3.8%, with 2 (25%) pure cultures), *Fusobacterium* in 6 cases (2.9%, with 2 (33.3%) pure cultures) and *Propionobacterium* in 4 cases (1.9%, always associated with other germs) (Table I).

In the 3 isolated cases of mycetes (1.4%), only *Aspergillus* was identified (always in association with *Pseudomonas* and *Staphylococcus*).

The sensitivity of the antibiotics, tested against all the isolated bacteria, is reported in Table II.

Discussion

The present study shows, in agreement with the literature [10 12 15] that cholesteatomatous otitis media is characterised by the presence of a polymicrobic flora constituted by both aerobic and anaerobic bacteria.

The aerobic species are those most frequently present (60.3% of gems isolated).

The most common aerobic bacteria is *Pseudomonas aeruginosa*, isolated in 31.1% of the cases, followed by *Staphylococcus aureus*, identified in 19.1% of the cases. These data are in agreement with those of Brook reported in 1981 [10], Poorey and Iyer in 2002 [12], Gulati in 1997 [16], and Aslam et al. in 2004 [17], but in disagreement with those of Rama published in 1980 [18], Rao and Reddy in 1994 [19] and Grewal and Shobha in 1996 [20] in which *Staphylococcus aureus* was the most frequently found bacteria.

While the frequency of aerobic bacteria in the literature is fairly uniform, there are discordant results for the anaerobes.

The present study confirms the pathogenic importance of the anaerobic bacteria which were identified in 38.2% of bacteria isolated. However, unlike the data reported by Aygagari et al. [11] and by Karma et al. [21], anaerobic gram-positive cocci (*Peptococcus* and *Peptostreptococcus*) were more frequent than *Bacteroides*, as they were isolated in 17.2% and in 12.4% of cases, respectively.

The importance of this species of bacteria in the microbiology of cholesteatoma is not to be underestimated, in fact, we are convinced that the finding of a negative culture, in the presence of a purulent malodorous secretion, resistant to antibiotic therapy, lead one to suspect a superinfection caused by anaerobic bacteria.

What is more, numerous studies reveal that the anaerobes are often isolated in the course of complications of cholesteatomatosa otitis, like mastoiditis and cerebral abscesses [10 14 22].

Among the different antibiotics tested, ciprofloxacin and levofloxacin seem to be the most effective antibiotics since they are active against *Pseudomonas*, *Staphylococcus* and also against anaerobic bacteria (*Bacteroides*, AGPC and *Clostridium*) (Table II).

These results are in agreement with the studies of Gulati [16], Mirsha et al. [23] and Indudharan et al. [24].

However, one has to take into consideration the fact that the fluoroquinoles must be avoided in children until the age of puberty because of their negative effects on growth and on the development of cartilage and bone tissue; for this reason, a valid alternative may be third generation cephalosporins and amoxicillin/clavulanic acid which, however, have less effect on the anaerobic bacteria and, in particular, on the *Bacteroides* [25 26].

The cephalosporins and the fluoroquinoles are certainly to be preferred to the aminoglycosides (more effective against the pseudomonas) since they are characterised by

minor collateral effects and a wider spectrum of action [27]. Topical treatment, in association with the systemic treatment, is also of important therapeutic value in the treatment of cholesteatoma otitis, but it should be specified that the use of topical antibiotics (pefloxacin, tobramycin) should be preceded by scrupulous local detergent cleaning through aspiration under otomicroscopy and washing with antiseptic substances. For this purpose, we use an acqueous solution of boric or acetic acid at 3% which reduces the pH and has antibacterial properties; this allows a double effect to be obtained: reduction of the bacterial load and removal of the secretions and the epithelial scales thereby allowing better contact for the antibiotic substances in topical use.

In the present study, a mycotic infection was found in 1.4% of *microorganisms* isolated and the mycete was always *Aspergillus*; this type of superinfection is very probably related to the abuse of topical steroids.

Conclusions

Cholesteatomatous otitis media has an insiduous progression, with the tendency to persist and to develop, and is able to determine irreversible sequels and serious extra- and intra-cranial complications; for this reason, the only therapeutic action able to eradicate the condition is surgery, which is always undertaken when possible.

Albeit, medical treatment is to be given since it is able to control skin regrowth, reduce the aggressive potential of the cholesteatoma and limit the incidence of complications.

The most frequently isolated pathogenes during the course of cholesteatomatous otitis are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, AGPCs and *Bacteroides*. Over the last few years, these pathogenic agents have created significant problems of chemo-resistance.

For this reason, the therapeutic approach required hinges on the possibility of isolating the specific aetiological agent through examination of the culture.

In cases where it is possible to carry out isolation of the culture, specific antibiotic treatment can be started.

When, however, it is not possible to perform a bacteriological examination of the auricular secretion, it is advisable to use combined therapy aimed at *Pseudomonas*, *Stafilococcus* and the anaerobic bacteria.

The most effective antibiotics are fluoroquinoles (ciprofloxacin or levofloxacin).

A different approach is used for the treatment of phlogosis, in cholesteatoma, in children. In these cases, it is advisable to use third generation cephalosporins or a combination of amoxicillin and clavulanic acid.

Finally, in mycotic superinfection, it is enough to interrupt the use of topical corticosteroids and start combined antibiotic-antimycotic treatment.

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