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Bacteriology of chronic sinusitis: the bulla ethmoidalis content*

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SUMMARY

Objective: *To study the microbiology of the bulla ethmoidalis of patients who suffered from chronic sinusitis.*

Design: *A prospective study performed at the Saint-Luc universitary clinic (University of Louvain) from June 1999 to December 2000.*

Material and methods: *Seventy seven patients underwent functional endoscopic endonasal surgery during this period for chronic sinusitis refractory to standard medical treatment. All the patients presented symptoms for more than 3 months. After Povidone-Iodine disinfection, samples were taken into the bulla ethmoidalis after its opening with an endoscopic endonasal control. Samples were transferred to the microbiology laboratory using a thioglycolate medium for aerobic and anaerobic cultures.*

Results: *One hundred forty eight samples were studied. Culture rate was 73,6 %. Thirty nine samples remained sterile. In the 109 culture positive specimens, 135 bacterial isolates were recovered. The main results are: Staphylococcus coagulase negative: 31, Staphylococcus aureus: 22, Streptococcus sp: 20, other Gram positive Cocci: 5, Haemophilus influenzae: 4, non-fermentative Gram negative bacilli: 6, Enterobacteriaceae: 45, anaerobic bacteria: 2.*

Conclusion: *Enterobacteriaceae or enteric gram negative bacilli were frequently encountered in the bulla ethmoidalis of patients suffering from chronic sinusitis. This report suggests that endoscopically guided culture obtained from the ethmoid sinus may accurate our understanding of the microbiology of chronic ethmoidal sinusitis and underline the importance of Enterobacteriaceae in this disease.*

Key words : bacteriology, chronic sinusitis, bulla ethmoidalis

INTRODUCTION

Bacteriology of chronic sinusitis has always been a key point in the management of this disease. In the past, studies were performed by swabbing the nasal cavity with a sterile cotton swab. It helped to know the normal flora of the nasal cavity and the microbiology of acute rhinosinusitis. Then, maxillary chronic sinusitis was studied (Brook et al.,1989; Hartog et al., 1995). The standard method to obtain reliable sinus cultures was the maxillary antral puncture. This method provided much information about the chronic maxillary sinus and differences between the acute phase and the chronic phase were described. However, this technique was invasive, required patient cooperation and was time consuming. Therefore, culture into the middle meatus was studied and the role of middle meatus aspiration culture in the diagnosis of chronic sinusitis was established (Jiang et al.,1993; van Cauwenberghe et al., 1997).

Nasal endoscopy has revolutionized the diagnosis of sinusitis and also the methods of sampling to study the microbiology of sinusitis. Endoscopically guided sinus cultures were performed not only into the middle meatus or in the sphenoethmoid recess but also into the sinuses. Studies appeared with results of the bacteriology of chronic sinusitis with samples from the ethmoid sinus in adults and also in infants (Doyle et al., 1991; Munsk et al., 1991; Jiang et al., 1997). It certainly helped to understand more precisely the role of a bacterial infection in perpetuating the chronic disease in sinusitis.

However, using an endoscopical way of sampling raised some new problems, such as the occurrence of nasal contamination during transnasal sampling. Another problem was to compare the results obtained, with an endoscopic control, by swabbing into the lumen of the sinus or by culturing the sinus mucosa itself.

Therefore many studies are now performed to provide valuable methods of sampling and to emphasize the importance to

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obtain bacteriological results from sinuses only accessible with an endoscopic control.
In this study, we also used an endoscopical way of sampling into the bulla ethmoidalis during functional endoscopic sinus surgery (FESS) to study the bacteriology of ethmoid chronic sinusitis. Since some patients underwent an antral lavage before the ethmoid sampling during FESS, we also compared the results with and without previous antral lavage.

MATERIALS AND METHOD

A prospective study was conducted at the Saint-Luc university clinic between June 1999 and December 2000. Consecutive patients with chronic sinusitis who underwent functional endoscopic sinus surgery were included in this study. The diagnosis of chronic sinusitis was based on clinical, radiographic and endoscopic findings. All the patients had symptoms for more than 3 months and were refractory to standard medical treatment. Patients were excluded if they showed evidence of
- nasal polyposis
- atrophic rhinitis
- immunodepression
- history of FESS
- odontogenic disease
- antibiotherapy within 10 days before the operation
- cystic fibrosis
- patient in intensive care unit
- patient with naso-gastric tube or other indwelling catheter
- extramucosal mycotic sinusitis.

We performed a CT-scan of the sinuses for every patients before surgery. The anterior ethmoidal sinus was affected by a swelling of the mucosa as could be demonstrated on a CT-scan for all patients. When the maxillary sinus was severely affected by chronic sinusitis (stage III-IV), a sinusoscopy under local anesthesia was performed before FESS to place a silicone tube into the maxillary sinus allowing to perform lavages. Lavages were done with physiologic serum (250 ml) and with chloramphenicol + n-acetyl-cystein during 15 to 21 days. After this period, a sinusomanometry was performed and led to FESS when ostium permeability was not resolved after the lavages. Then the tube was removed and surgery was planned within one month.
In those qualified patients, we performed functional endoscopic endonasal sugery under general anesthesia following Stammberger's method. Surgery was performed and specimens were obtained by the same surgeon.
First of all, we used a Povidone-Iodine solution for disinfection of the face and of the nasal vestibule. Then we placed some cotonoids together with vasoconstrictive agents into the nasal fossa for 10 minutes. After their removal, nasal fossa were disinfected by a Povidone-Iodine solution (20 ml in each nasal fossa). Finally, we cleansed the nasal fossa with 20 ml physiologic serum.
FESS was typically performed with a 4 mm, 30° nasal telescope and routinely included an assessment of all visible turbinates and meati. After we incised the vertical portion of the uncinate process, the bulla ethmoidalis became visible. We opened the bulla ethmoidalis using a sterile cutting forceps.

A sterile cotton-tipped swab was introduced into the nasal cavity while retracting the nasal ala with the endoscope. Special care was taken not to touch the nasal vestibule and mucosa before reaching the opened bulla ethmoidalis. Then the sterile cotton-tipped swab was placed into the bulla ethmoidalis and immediately transferred to the microbiology laboratory using a thioglycolate medium.
On arrival at the laboratory, samples were incubated during 24 hours in thioglycolate agar at 35°C. If a growth development was observed after 24 hours a subculture was made on :
- Columbia blood agar (Becton – Dickinson) in capnophilic atmosphere at 35°C ;
- Mueller Hinton blood with Bacitracine and V factors (Becton Dickinson) in capnophilic atmosphere at 35 °C ;
- Wilkins amukin blood (Becton – Dickinson) in anaerobic atmosphere at 35 °C.

Identification of the bacteria isolated from thioglycolate was based on conventional biochemical enzymatical and phenotypical biotyping.
The use of rabbit plasma revealed the presence of free and bound coagulase and allowed the distinction between coagulase negative *Staphylococcus* (CoNS) and coagulase positive *Staphylococcus* (CPS) or *Staphylococcus aureus*.
We used Oxascreen medium (Becton – Dickinson) to investigate Methicillino-resistant *Staphylococcus aureus* (MRSA). We have specified the level of *Streptococcus pneumoniae* to Penicillin with the E-test® System (AB – Biodisk) on Mueller Hinton blood (Blood – Merieux).

RESULTS

Among the 77 patients, 6 underwent unilateral FESS Therefore, the total number of samples from the bulla ethmoidalis was 148. The age of these patients ranged from 19 to 72 years old with an average age of 38,2 years. Of the patients, 36 were male and 41 were female.
In total, 22 patients received antral lavages before FESS, including 2 patients with unilateral disease. Therefore, 42 maxillary sinuses were treated with antral lavages. Of the 148 specimens, 39 remained sterile. Culture rate was therefore 73,6 % (Table 1). There were 82 specimens in which only one bacterium grew, 25 specimens showing evidence of 2 bacteria and 1 specimen with 3 bacteria. In the 109 culture positive specimens, 135 bacterial isolates were recovered (Table 2). Among the Gram positive bacteria, Coagulase Negative *Staphylococcus* (CoNS), Coagulase Positive *Staphylococcus* (CPS) and *Streptococcus* sp. were the most frequently isolated bacteria. Among the Gram negative bacteria, *Enterobacteriaccae* represented the most important encountered family. *Enterobacteriaccae* were found in 45/109 positive cultures

Table 1. Bacteriology of chronic sinusitis. The bulla ethmoidalis content.

Bacteria	Samples after antral lavage (n=42)			Samples without previous antral lavage (n=106)			TOTAL (n=148)		
Aerobic and facultative bacteria	39			94			133		
GRAM (+)	18			60			78		
- *Staph. coag. (-) (CoNS)*		9			22			31	
- *Staph. aureus (CPS)*		3			19			22	
- *Streptococcus pneumoniae*		2			3			5	
- *Streptococcus viridans*		2			12			14	
- *Streptococcus milleri*		0			1			1	
- *Coryneb. pseudodiphteriae*		1			0			1	
- *Enterococcus faecalis*		1			1			2	
- *Bacillus cereus*		0			2			2	
GRAM (–)	21			34			55		
- *Haemophilus influenzae*		4			0			4	
- *Stenotronomas maltophilia*		2			2			4	
- *Acinetobacter baumanii*		2			0			2	
- *Enterobacteriaceae*		13			32			45	
Escherichia coli			2			9			11
Enterobacter aeogenes			1			3			4
Enterobacter cloacae			3			1			4
Klebsellia pneumoniae			2			3			5
Klebsellia oxytoca			0			4			4
Serratia marcescens			0			1			1
Citrobacter diversus			1			4			5
Proteus vulgaris			2			0			2
Proteus mirabilis			2			7			9
Anaerobic bacteria	0			2			2		
- *Peptostreptococcus sp.*		0			1			1	
- *Veilonella sp.*		0			1			1	
TOTAL	39			96			135		

Table 2. Number of isolates per culture and as a percentage of total. n = 148 specimen.

Single organism	82	56 %	
Two organisms	25	16,9 %	positive culture 73,6 %
Three organisms	1	0,7 %	
➨ 135 organisms			

(41,2 %), in 45/148 specimens (30,4 %) and in 45/135 bacteria isolates (33,3 %).

We demonstrated that 27/77 patients (35 %) were infected by an *Enterobacteriaceae* family bacteria (Table 3).

Anaerobic bacteria were present in only 1 patient from whom 2 different species were isolated (Table 4).

Twenty-five paired organisms were cultured from the bulla ethmoidalis (Table 4). CoNS and CPS were respectively paired 7 and 11 times.

Finally, Table 3 gives the percentage of *Enterobacteriaceae*, CoNS and CPS recovered, related to the number of positive culture (n = 109), to the total number of bacteria isolated (n =

Table 3. Bacteria isolates reported to different denominators.

Bacteria isolates (%)	Positive culture n = 109	Total number of bacterial isolated n = 135	Total number of Culture n = 148	Total number of patients n = 77
Enterobacteriaceae	41,2 %	33,3 %	30,4 %	35,0 %
CoNS	28,4 %	22,9 %	20,9 %	27,2 %
CPS	20,1 %	16,2 %	14,8 %	18,1 %

135), to the total number of cultures done (n = 148) and to the total number of patients enrolled in this study (n = 77).
None of the *Staphylococcus* sp. were MRSA and among the *Streptococcus pneumoniae* recovered there wasn't any species showing a low-level penicillin resistance.

Table 4. Bacteria isolates when > one organism. n = 25 paired-organisms.

Enterobacteriaceae – CoNS	1
Enterobacteriaceae – CPS	4
Enterobacteriaceae – *Enterobacteriaceae*	2
Streptococcus viridans – CPS	5
Streptococcus pneumoniae – CPS	2
Streptococcus viridans – CoNS	3
Streptococcus viridans – *Enterobacteriaceae*	3
Bacillus cereus – CoNS	1
Haemophilus influenzae – CoNS	1
Haemophilus influenzae – *Streptococcus pneumoniae*	1
Enterococcus faecalis – CoNS	1
Peptostreptococcus sp. – *Veilonella* sp.	1

DISCUSSION

Many of the studies that have adressed chronic sinusitis are difficult to compare (Verschreagen et al., 1998). The selection of patients (acute vs chronic), ways of sampling (endoscopically guided vs anterior rhinoscopy), site of culture (maxillary vs ethmoid) and method of culture lead to difficulties in the interpretation of the culture results.
Moreover, the bacteria encountered may be expressed in absolute number or may refer to several denominators (total positive culture, total culture, total isolates, total patients). For these reasons, we have tried to report an homogenous cohort of patients undergoing FESS with one site of culture, one method of sampling, one method of culture and one clear denominator.

Bacterial findings of acute sinusitis and of chronic maxillary sinusitis were already studied and have led to a judicious selection of antimicrobial agents (Klossek et al., 1998; Hsu et al., 1998). With the advances of FESS, bacteriology of the ethmoid sinus was getting more interest. Endoscopically guided sinus cultures during surgery was therefore used in this study to explore the microbiology of the bulla ethmoidalis content in chronic sinusitis.

Our results indicate that predominant bacteria found in the bulla ethmoidalis are *Enterobacteriaceae*, CoNS, CPS and *Streptococcus* sp. These bacteria are probably representative of the bacteria encountered in the bulla ethmoidalis content of patients with a history of chronic sinusitis. It could be argued that this study is weakened by the absence of a control group but the microbiology of the bulla ethmoidalis in a normal subject is difficult to explore for many ethical reasons.
Our results lead to some elements of discussion. First of all, we found 31 CoNS (31 isolates in 133 total isolates). CoNS has been isolated in many studies and cultured as the predominant bacteria. However, it is often viewed as a probable contaminant. Jiang et al. found 23 CONS in 121 total isolates in a study published in 1993 and only 3 CONS in 58 total isolates in an other study where he used a cotton-tip passed into a sterile canula to obtain the specimen in the ethmoid cavity (Jiang et al., 1993, 1997). This finding warrants that CONS is a contaminant and that bacteriological studies must reduce the CoNS percentage by using some sophisticated technique of sampling. Nadel et al. (1999) found that CoNS was present in 36 % of the cultures obtained in healthy subjects pointing to the fact that CoNS doesn't play an important role in pathological conditions..

However, some authors postulated that CoNS may become pathogenic, specially in neonates, infants or in patients with nasal tubes in intensive care units (van Cauwenberghe et al., 1997). Moreover, CoNS may produce β-lactamase reporting antibiotic failure (Hartog et al., 1995)). In this condition, CoNS may be considered as a co-pathogen. We think that it is very difficult to avoid CoNS from bacteriological results in chronic sinusitis. We also believe that CoNS which is frequently recovered in paired-isolates (7/25 in this study) doesn't play an important role in chronic sinusitis except in « opportunistic » conditions.
CPS provides the same debate about being a contaminant pathogen or not. However, its role in chronic sinusitis is more important that the CoNS one as CPS is one of the major bacteria found in complicated sinusitis or in pediatric sinusitis (Muntz et al., 1991). We must also underline that CPS was recovered in 11/25 paired isolates. Finally, we hypothesize that CPS frequently colonize the ethmoid cavity after FESS. Therefore, knowing its presence into the bulla ethmoidalis at the time of surgery may lead to an appropriate antibiotic treatment after FESS in order to decrease the risk of such a postoperative CPS infection.

The absence of MRSA may be explained by our exclusion criteria which represent well known conditions for MRSA infection.

Streptococcus sp. also represents a common bacteria in the microbiology of sinusitis. *Streptococcus* sp. certainly plays a less important role in chronic sinusitis than in acute. The emergence of low-level penicillin resistance *streptococcus pneumoniae* represents one of the most exciting features for the infectiologists. It should also be pointed out that susceptibility to antibiotics was only studied for *Streptococcus* sp. and for *Staphylococcus* sp. Antibiotic resistance status for all the recovered bacteria was not the objective of this study.

We further demonstrated that 36,3 % of the patients with a clinical history of chronic ethmoidal sinusitis were colonized with *Enterobacteriaceae* Gram negative bacteria. The *Enterobacteriaceae* are usually found in the gastrointestinal tract and named for this reason enteric Gram negative bacteria. In ENT infection, they were found with a different frequency as a cause of nosocomial sinusitis and otitis, specially in patients with catheters or nasogastric tubes. However, enteric Gram negative bacteria were found to colonize patients with chronic sinusitis without such predisposing factors in the ethmoid sinus. Bolger et al. (1994) reported them as a « new clinical entity ». In chronic ethmoidal sinusitis, 34 % and 43,5 % of the isolated bacteria were enteric Gram negative bacteria, respectively reported by Bolger and Jiang (Bolger et al., 1994; Jiang et al., 1997).

Even earlier, Doyle and Woodham obtained in 19 % of the pediatric patients with chronic sinusitis *Enterobacteriaceae* in their ethmoid biopsies (Doyle et al., 1991).

The presence of *Enterobacteriaceae* may be considered as a pathological condition in the ethmoid sinus arising after failure of conventional antiobiotherapy for chronic sinusitis (Hartog et al., 1995; Rontal et al., 1999).

Our results support findings of other clinical settings and underline the importance of *Enterobacteriaceae* in chronic ethmoidal sinusitis.

We may consider that even if chronic sinusitis is not a pure bacterial disease but rather the result of chronic inflammation, the bacteriology of sinusitis is changing with time. After the acute phase, where *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* are the most frequently cited bacteria, enteric Gram negative bacilli are present in the chronic phase. Whether these bacteria play an important role in the pathogenesis of chronic sinusitis is more debatable since no study with control groups has been reported in the literature. The exact role of a bacterial isolation in chronic sinusitis or even in acute exacerbation of chronic sinusitis is not yet well established. When the inflammation is initiated into the sinusal mucosa, bacterial colonization may be enhanced but the initiating process may be quite different from a bacterial infection.

Concerning the low frequency of anaerobic isolates in this study, we suggested, like Doyle et al. (1991) that the ethmoid sinus may be less susceptible to anaerobes that the maxillary sinus because it is less likely to be obstructed and more exposed to inspired oxygen. Patients with an odontogenic disease, frequently colonolized by anaerobes, were excluded from this study giving an other explanation for this low frequency. Finally, the frequency of anaerobes in chronic sinusitis samples ranges from 0 % to 100 % in the literature and our results are similar to those reported with samples obtained from the bulla ethmoidalis (Jiang et al., 1997).

As previously reported by Bertrand et al. (1993, 1997), maxillary irrigations may be used in chronic sinusitis in an attempt to re-open an maxillary ostium with the hope of avoiding any other surgery on this sinus. Patients included in this report are non-responders to irrigation, confirmed by sinusomanometry, and underwent FESS for both ethmoid and maxillary surgeries. On the total of bacterial isolates, it seems that antral lavage has no effect on the microbiology found in the bulla ethmoidalis of patients suffering from chronic sinusitis, as the culture rate for samples with antral lavage is 92 % and 88 % for samples without previous antral lavage.

REFERENCES

1. Bertrand B, Eloy Ph (1993) Temporary nasosinusal drainage and lavage in chronic maxillary sinusitis: statistical study on 847 maxillary sinuses. Ann. Otorhinolaryngol. 102: 858-862.
2. Bertrand B, Collet S, Betsch C, Rombaux Ph, Eloy Ph (1997) Diagnostic techniques in chronic sinusitis: endoscopy, sinusomanometry. Acta Oto-Rhino-Laryngologica Belgica 51: 259-269.
3. Bolger WE (1994) Gram negative Sinusitis: an emerging clinical entity? American Journal of Rhinology 8: 279-284.
4. Brook I (1989) Bacteriology of chronic maxillary sinusitis in adults. Ann Otol Rhinol Laryngol 98: 426-428.
5. Doyle PW, Woodham JD (1991) Evaluation of the microbiology of chronic ethmoid sinusitis. J. Clin. Microbiol. 29: 2396-2400.
6. Hartog B, Degener JE, Van Benthem PPG, Hordijk GJ (1995) Microbiology of chronic maxillary sinusitis in adults: isolated aerobic and anaerobic bacteria and their susceptibility to twenty antibiotics. Acta Otolaryngol (Stockh) 115: 672-677.
7. Hsu J, Lanza DC, Kennedy DW (1998) Antimicrobial resistance in bacterial chronic sinusitis. American Journal of Rhinology 12: 243-248.
8. Jiang RS, Hsu CY, Leu JF (1993) Comparison of the bacteriologies between the ethmoid and maxillary sinuses in chronic paranasal sinusitis. J. Otolaryngol. Soc. Roc. 28: 308-317.
9. Jiang RS, Hsu CY, Leu JF (1997) Bacteriology of ethmoid sinus in chronic sinusitis. American Journal of Rhinology 11: 133-137.
10. Klossek JM, Dubreuil L, Richet H, Richet B, Beutter P (1998) Bacteriology of chronic purulent secretions in chronic rhinosinusitis. The Journal of Laryngology and Otology 112: 1162-1166.
11. Muntz RH, Lusk R (1991) Bacteriology of the ethmoid bulla in children with chronic sinusitis. Arch. Otolaryngol. Head Neck Surgery 117: 179-181.
12. Nadel DM, Lanza DC, Kennedy DW (1999) Endoscopically guided sinus cultures in normal subjects. American Journal of Rhinology 13: 87-90.
13. Rontal M, Bernstein JM, Rontal E, Anon J (1999) Bacteriologic findings from the nose, ethmoid and bloodstream during endoscopic surgery for chronic rhinosinusitis : implications for antibiotic therapy. American Journal of Rhinology 13: 91-96.
14. Van Cauwenberge P, Ingels K, Bachert C, Wang D (1997) Microbiology of chronic sinusitis. Acta Oto-Rhino-Laryngologica Belgica 51: 239-246.

15. Verschraegen G, Mione S (1998) Difficulties in interpretation of cultures results in sinusitis. Rhinology 36: 55-58.

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