Prevalence of Etiologic Bacteria and β-lactamase - Producing Bacteria in Acute and Chronic Maxillary Sinusitis at Phramongkutklao Hospital

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Background: *To date, there is an increasing trend of prescribing antibiotics that cover -lactamase - producing bacteria as the first line drug for sinusitis patients in Thailand. This practice dose not only increases the treatment cost but might also induce resistance to antimicrobial agents.*
Objective: *To determine the prevalence of the pathogenic bacteria in acute and chronic maxillary sinusitis as well as the prevalence of -lactamase - producing bacteria.*
Material and Method: *The maxillary aspiration fluid specimens of 52 acute and chronic sinusitis patients at Phramongkutklao Hospital, Bangkok, Thailand during May 2002-May 2003, were collected. Aerobic and anaerobic cultures were performed and the -lactamase activities of isolated bacteria were determined.*
Results: *Of 58 specimens, 44 (75.9%) and 14 (24.1%) specimens were collected from acute and chronic maxillary sinusitis patients, respectively. Of 44 specimens of acute maxillary sinusitis, the predominant isolates were Haemophilus influenzae (13, 29.5%), Streptococcus pneumoniae (4, 9%), Streptococcus viridan (4, 9%), and Klebsiella pneumoniae (4, 9%). 6 (13.6%) of them were β-lactamase - producing bacteria. Of 14 specimens of chronic maxillary sinusitis, the predominant isolates were Pseudomonas aeruginosa (3, 16.6%), Staphylococcus spp (3, 17.5%) and Streptococcus spp. (2, 11.6%). 1 (7.1%) Haemophilus influenza isolate from this group was β-lactamase - producing bacteria. The percentage of anaerobe was 8.9%, 23.3% for acute and chronic maxillary sinusitis, respectively.*
Conclusion: *The present study was indicated that the common organisms in acute maxillary sinusitis were Haemophilus influenzae and Streptococcus pneumoniae, Streptococcus viridians which were similar to a previous study. But the authors found an increased prevalence of anaerobe bacteria in acute maxillary sinusitis and also found -lactamase- producing bacteria, 21.4% Hemophilus influenzae less than the previous studies (which found -lactamase - producing bacteria around 50% to 70%). The authors found 23.3% anaerobe bacteria in the chronic maxillary sinusitis, less than the previous studies, and the authors also found 8.9% anaerobe in acute maxillary sinusitis*

Keywords: *Acute and chronic maxillary sinusitis, -lactamase - producing bacteria*

J Med Assoc Thai 2005; 88(4): 478-83
Full text. e-Journal: *http://www.medassocthai.org/journal*

Acute sinusitis is an infectious disease commonly complicated from upper respiratory tract infection and caused by virus or bacterial infection. If there is evident of bacterial sinusitis, the treatment of choice is antimicrobial agent. Clinicians usually prescribe antibiotic that covers -lactamase - producing bacteria. These practices increase the cost and may induce resistance to antimicrobial agents.

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Presently, large numbers of patients are being treated for sinusitis at the Department of Otorhinolaryngology, Phramongkutklao Hospital. Various antibiotics are prescribed and used in large amount. Thus, problems of improper antibiotics use and high cost of treatment are eminent. In addition, these may eventually leads to the development of antibiotic-resistance bacteria.

There have been several studies on bacteriology of sinusitis thru the years. Brook et al[1,2] reported in 1981 and 1989 that anaerobes were the most common etiologic agents, which coincided with the study of Erkan et al[3] in 1993 which showed 88% of anaerobes as etiologic agents. In contrary, Ramadan[4] reported in 1994 that sinusitis in adults are caused by aerobes 76.3% and anaerobes 7.6%. By which, the most common aerobes are of *Staphyloccocus spp*. In 1989, Goldenhersh et al[5] reported *Moraxella catarrhalis* and *Streptocoocus spp.* are the most common causes of chronic sinusitis.

In 1984, Wald et al[7] and Friedman et al[8] reported bacterial causes of acute sinusitis which included *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae*. The same as Ahuja et al[17] reported in 1998 bacteriology of acute maxillary sinusitis included *Streptococcus pneumoniae* (30-40%), *Moraxella catarrhalis* (12-20%) and *Haemophilus influenzae* (20-30%). In 1995, Brook et al[6] also reported similar results with addition of bacterial causes of chronic sinusitis which included *Prevotella spp.*, *Fusobacterium spp.* and *Peptostreptococcus*. The present study also reported β-lactamase - producing bacteria in 40% and 77% of acute and chronic sinusitis, respectively. β-lactamase - producing bacteria causing sinusitis included *Haemophilus influenzae* and *Moraxella catarrhalis* while those causing chronic sinusitis included *Staphylococcus aureus*, *Prevotella species*, *Fusobacterium spp.* and *Bacteroides fragilis*.

In Thailand, Prakunhungsit et al[9] reported in 1993 that bacteriology of acute sinusitis included *Haemophilus influenzae* (41.67%) and *Streptococcus spp.* (31.25%) and *anaerobes* (10.42%). In accordance with the subsequent reports by Krongkaew et al[10] in 1999 and Jareoncharsri et al[11] in 2000.

Due to the changing prevalence of bacteriologic agents including β-lactamase -producing bacteria and because no study has been done in Phramongkutklao Hospital which is the main Royal Thai Army Hospital, the authors conducted the present study to determine the bacteriologic causes of acute and chronic sinusitis including β-lactamase - producing bacteria and suggesting guidelines on considering appropriate antibiotics for the treatment.

Material and Method

This was a cross sectional study done in out patients of more than 20 years old, who presented with acute or chronic sinusitis at the Department of Otorhinolaryngology, Phramongkutklao Hospital between May 2002 to May 2003. The inclusion criteria included the patients with signs and symptoms of sinusitis at least 2 majors, or one major plus 2 minors as shown in Table 1[12,15] or Paranasal sinus radiographs showing mucosal thickening of more than 5 mm in one or both sinuses, opacification or air fluid levels or positive CT finding of sinusitis. Exclusion criteria included allergic rhinosinusitis, non-infection sinusitis, dentogenic sinusitis and any contraindication for maxillary antral aspiration e.g. bleeding tendency, immunocompromised host and paranasal sinus tumor and patients taking antibiotics for at least 1 week prior to the study.

The patients were divided into two groups: Group 1 including patients who were symptomatic for less than 4 weeks (acute sinusitis) and Group 2 including patients who had symptoms for more than 8 weeks (chronic sinusitis).

The specimens for bacteriologic study were collected by maxillary antral irrigation. G16-18 needle was used to aspirate fluid from the maxillary sinus via the area below the inferior turbinate by sterile technique[16]. If pus could not be aspirated, 10 cc of normal saline was irrigated and aspirated. Air in the syringe was expelled after specimen collection and the needle tip was sealed with dense rubber and sent to the laboratory within 30 minutes.

The specimens were inoculated on the following culture media: 5% sheep blood agar,

Table 1. Symptoms and Signs of acute/chronic maxillary sinusitis

Major criteria	Minor criteria
- Facial pain	- Cough
- Nasal congestion and obstruction	- Fatigue
- Fever	- Headache
- Purulent nasal discharge	- Dental pain
- Anosmia/Hyposmia	- Ear pressure
- Facial pain/Pressure	- Halitosis

From; Lanza DC, Kennedy DM. Adult Rhinosinusitis: Otolaryngol Head Neck Surg 1997[12]

chocolate agar and Mac Conkey agar. The Mac Conkey agar were incubated at 37°C for 24 hours. While 5% sheep blood agar and chocolate agar were kept under 5% CO_2. All plates were evaluated at 24, 48 and 72 hours to detect aerobes and facultative anaerobes[13].

As an anaerobic study, specimens were inoculated in Wilkins-Chalzen blood agar plates which contained vitamin K, Kewin and selective media such as Egg Yolk agar, Anaerobic Blood agar, Bacteriodes Bile Esculin agar (BBE), Phenylethyl Alcohol agar (PA) and Kanamycin-Vancomycin Laked Blood agar. The inoculated plates were kept in anaerobic jars and evaluated for growth at 48 and 96 hours[13]. The genus and species of bacteria were further identified by biochemical methods.

β-lactamase activity was identified using chromogenic cephalosporin analog 87/312 method by putting the disc Nitrocefin on the colony. The result was interpreted according to the time for each bacteria. The result was interpreted as positive when the disc Nitrocefin turned red[14].

Results

The 58 specimens were collected from 52 patients consisting of 27 males and 25 females, ranging in age from 23-71 years of age (average 39 years). There were 44 (77.67%) specimens from the acute maxillary sinusitis and 14 (23.33%) specimens from the chronic maxillary sinusitis. The result from the bacterial culture showed that 77.3% were positive for acute maxillary sinusitis and 92.9% were chronic maxillary sinusitis. (Table 2, 3)

Cultures from acute sinusitis cases showed *Haemophilus influenzae* in 13 samples (29.5%), *Streptococcus pneumoniae* in 4 samples (9%), *Streptococcus viridans* in 4 samples (9%), *Klebsiella pneumoniae* in 4 samples (9%), *Staphylococcus spp.* in 4 samples (9%) and *Streptococcus gr A* in one sample (2.2%).

Cultures from chronic sinusitis cases yielded anaerobes in 4 samples (23.3%), *Pseudomonas aeruginosa* in 3 samples (16.6%), *Staphylococcus spp.* in 3 samples (16.6%) and *Streptococcus spp.* in 2 samples (11.6%).

Anaerobic culture in specimens from acute and chronic maxillary sinusitis showed 8.9% and 23.3% positive yields, respectively. The isolated anaerobes included *Prevotella spp.*, *Peptostreptococcus anaerobius* and *Prophyromonas gingivalis* (Table 4).

β-lactamase - producing bacteria, included 3 *Haemophilus influenzae* (21.4% of all *Haemophilus influenzae*), 1 *Moraxella catarrhalis* produced β-lactamase (Table 4).

Discussion

By comparing the results in the present study with previous studies both local and abroad, it was found that bacteria causing acute sinusitis included *Haemophilus influenzae* 29.5%, *Streptococcus spp.* 20.2% (*S. pneumoniae* 9.0%) similar to studies done in 1988, 1999 and 2001[9-11]. But differing from studies from abroad reported in 1984 and 1996[6-8] with the addition of *M. catarrhalis*, which was not included in their studies.

As for bacteriology of chronic sinusitis, anaerobes were found in 23.3% which is 3 times higher compared to that of acute sinusitis which was only 8.9%. This coincides with the increasing incidence reported in 1981, 1989, 1994 and 2000[1-3,11]. However, in the present study, the authors found a lower percentage of anaerobes of 23.3% compared to the other reports of 39-60%. These may have resulted from difficulty in anaerobic cultures which may decrease the growth yield.

Table 2. The percentage of the bacteria isolated from the maxillary sinusitis

Culture	Percentage of bacteria culture in the specimens	
	Acute maxillary sinusitis	Chronic maxillary sinusitis
No growth	10 (22.7%)	1 (7.1%)
Pure organism	26 (56.8%)	9 (64.2%)
2 organisms	7 (15.9%)	4 (28.5%)
3 organisms	1 (6.5%)	-
Total	44 (100%)	14 (100%)

Table 3. Total bacterial both aerobic and anaerobic isolated from 58 specimens

Culture Result		Percentage of bacterial culture	
		Acute maxillary sinusitis	Chronic maxillary sinusitis
Pure organism	aerobic	25 (56.8%)	8 (57.1%)
	anaerobic	1 (2.2%)	1 (7.1%)
Mix organism	aerobic	6 (13.6%)	1 (7.1%)
	aerobic and anaerobic	2 (4.5%)	3 (21.4%)
	anaerobic	-	-
Total		34 (77.2%)	13 (92.8%)

Table 4. The detail of aerobic and anaerobic bacteria isolated from 58 specimens

Organism	Acute	Chronic
Gram-positive coccobacilli		
Haemophilus influenzae	13 (29.5%)	1 (5.8%)
- *-lactamase positive*	3 (6.8%)	-
- *-lactamase negative*	10 (22.7%)	1 (5.8%)
Gram-positive cocci		
Streptococcus	9 (10.5%)	2 (11.6%)
- *Streptococcus pneumoniae*	4 (9.0%)	-
- *Streptococcus group A*	1 (2.2%)	-
- *Viridans group of Streptococcus*	4 (9.0%)	1 (5.8%)
- *Streptococcs non group A,B,C*	-	1 (5.8%)
Staphylococcus	4 (9.0%)	3 (17.5%)
- *coagulase- positive Staphylococcus*	2 (4.5%)	1 (5.8%)
- *coagulase- negative Staphylococcus*	2 (4.5%)	2 (11.7%)
Corynebacterium	1 (2.2%)	-
Gram-negative coccobacilli		
Moraxella catarrhalis		
- *-lactamase positive*	1 (2.2%)	-
Gram-negative cocci		
Neisseria spp.	1 (2.2%)	-
Gram-negative bacilli		
Citrobacter spp.	2 (4.5%)	-
Acinetobacter spp.	2 (4.5%)	-
Serratia marcescens	-	1 (5.8%)
Gram-negative rod		
Klebsiella		
- *Klebsiella pneumoniae*	4 (9.0%)	1 (5.8%)
- *Klebsiella species*	2 (4.5%)	-
Enterobacter species	1 (2.2%)	1 (5.8%)
Pseudomonas		
- *Pseudomonas spp.*	-	1 (5.8%)
- *Pseudomonas aeruginosa*	-	3 (16.6%)
Anaerobe	4 (8.9%)	4 (23.3%)
Prevotella	2 (4.5%)	2 (11.7%)
Peptostreptococcus anaerobius	1 (2.2%)	-
Prophyomonas gingivalis	-	1 (5.8%)
Gram-negative bacilli	1 (2.2%)	1 (5.8%)

There are mixed organisms isolated from one specimen

It is widely known that β-lactamase- producing bacteria are major causes of problems in the treatment of sinusitis nowadays. Because these bacteria are resistant to penicillin, which is the first group of antibiotics used in treatment, it has been found that β-lactamase production is commonly found in *Haemophilus influenzae, Moraxella catarrhalis.* From the present study, it was found that 21.4% of *Haemophilus influenzae* produced β-lactamase, which is less than in previous studies that found 40-70%[6,7,9,11]. While 100% of *Moraxella catarrhalis* yielded β-lactamase similar to other studies.

The antimicrobial susceptibility showed that 78.6% *Haemophilus influenzae,* 100% Viridans group of *Streptococcus* and *Streptococcus spp.* were sensitive to ampicillin and penicillin but 50.0% *Streptococcus pneumoniae* showed resistance (Table 5, 6).

Table 5. Susceptibility test of Haemophilus influenzae

Susceptibility test of Haemophilus		% Sensitive
Ampicillin	11/14	78.6
Amoxicillin/clavulanic	14/14	100.0
Ampicillin/sulbactam	3/3	100.0
Cefotaxime	6/6	100.0
Ceftriaxone	10/10	100.0
Ofloxacin	4/4	100.0
Ciprofloxacin	4/4	100.0
Chloramphenicol	11/13	84.6
Cotrimoxazole	10/14	71.4
Tetracycline	10/14	71.4

Table 6. Susceptibility test of Streptococcus

Susceptibility test of Streptococcus			
	S. pneumoniae (4)	*Strep. Viridans* (5)	*Streptococcus spp.* (2)
Penicillin	50%	100%	100%
Ofloxacin	100%	-	100%
Chloramphenicol	100%	50%	100%
Tetracycline	75%	100%	-
Erythromycin	100%	100%	50%
Vancomycin	100%	100%	100%

Conspicuously, the bacteriologic profile of sinusitis has been changing through the years as well as the antimicrobial susceptibility. Likewise, the growing number of β-lactamase - producing bacteria has been recognized. For the present study, the physicians should be alert to changing antimicrobial treatment in sinusitis, so they can prescribe the appropriate antimicrobial therapy for patients who suffer from acute and chronic sinusitis by prescribing a simple antibacterial agent such as high dose amoxicillin instead of using the second generation cephalosporin or amoxicillin plus clavulanic acid which is expected to lower the cost of the treatment.

Conclusion

The present study shows that prevalence of bacteria causing acute sinusitis at the Department of Otorhinolaryngology, Phramongkutklao Hospital is similar to other studies wherein the prevalence of *Haemophilus influenzae*, *Streptococcus spp.* and β-lactamase - producing bacteria are low, especially *Haemophilus influenzae* at 21.4%. The prevalence of anaerobes in chronic sinusitis is increased to 23.3%.

Acknowledgment

The authors wish to thank the medical staff of the Department of Otolaryngology and Microbiology of Phramongkutklao Hospital and College of Medicine. This research was granted by the Phramongkutklao Hospital Foundation.

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การศึกษาความชุกของเชื้อแบคทีเรียก่อโรคและแบคทีเรียที่สร้างเอนไซต์ β-lactamase ในผู้ป่วยโพรงอากาศข้างจมูกอักเสบเฉียบพลันและเรื้อรัง ในโรงพยาบาลพระมงกุฎเกล้า

กรีฑา ม่วงทอง, อภิชาต สุวาส, ยุพา ผลโภค, สุรเดช จารุจินดา, สุดาลักษณ์ ฉันทรัชดา, ราม รังสินธุ์

วัตถุประสงค์*: โพรงอากาศข้างจมูกอักเสบที่เกิดจากเชื้อแบคทีเรีย พบได้บ่อยที่เกิดตามมาหลังทางเดินหายใจส่วนต้นอักเสบ การวิจัยนี้มีจุดประสงค์เพื่อศึกษาถึง ชนิดของเชื้อแบคทีเรียใน โพรงอากาศข้างจมูกอักเสบ เฉียบพลันและเรื้อรัง ทั้งเชื้อ aerobe และ anaerobe และรวมทั้งหาอุบัติการณ์ของเชื้อแบคทีเรียที่สร้างเอ็มไซต์ β-lactamase*

วัสดุและวิธีการ*: ศึกษาในผู้ป่วยโพรงอากาศข้างจมูกอักเสบเฉียบพลันและเรื้อรัง ใน รพ.พระมงกุฎเกล้า ตั้งแต่ พ.ค. 45 ถึง พ.ค. 46 โดยศึกษาจากของเหลวที่ดูดเก็บมาจาก Maxillary sinus จำนวน 52 คน และนำไปเพาะเชื้อเพื่อศึกษาถึงชนิดของเชื้อแบคทีเรีย ทั้งเชื้อ aerobe และ anaerobe และศึกษาถึงความชุกของเชื้อที่สร้างเอนไซต์ b-lactamase*

ผลการวิจัย*: จากโพรงอากาศข้างจมูกอักเสบเฉียบพลัน 44 ตัวอย่าง และเรื้อรัง 14 ตัวอย่าง พบเชื้อแบคทีเรียในโพรงอากาศข้างจมูกอักเสบเฉียบพลัน ได้แก่ Haemophilus influenza ร้อยละ 29.5, Streptococcus pneumoniae ร้อยละ9, Streptococcus viridan ร้อยละ 9 และ, Klebsiella pneumoniae.ร้อยละ 9 และ เชื้อแบคทีเรียในโพรงอากาศข้างจมูกอักเสบแบบเรื้อรัง ได้แก่ Pseudomonas aeruginosa ร้อยละ 16.6, Staphylococcus sppร้อยละ 17.5 และ Streptococcus spp ร้อยละ 11.6% การศึกษานี้พบ เชื้อแบคทีเรียแบบ anaerobe จากโพรงอากาศข้างจมูกอักเสบแบบเฉียบพลันและเรื้อรัง คิดเป็นร้อยละ 8.9%, 23.3% ตามลำดับ โดยชนิดของเชื้อที่พบเป็นกลุ่ม Prevotella sp, Peptostreptococcus anaerobius และ Prophyromonas gingivalis. พบเชื้อ H. influenzae ที่สร้างเอนไซต์ b-lactamase ร้อยละ 21.4 ขณะที่ Moraxella catarrhalis และ Staphylococcus coagulase positive ทั้งหมดสร้างเอนไซต์ b-lactamase*

สรุปการวิจัย*: การศึกษาวิจัยครั้งนี้ ได้ศึกษาถึงเชื้อแบคทีเรียจาก ผู้ป่วยโพรงอากาศข้างจมูกอักเสบเฉียบพลันและเรื้อรังที่เข้ารับการรักษา ใน รพ.พระมงกุฎเกล้า พบว่าเชื้อแบคทีเรียมีความคล้ายคลึงกับเชื้อแบคทีเรียที่ศึกษาวิจัยอื่นทั้งในประเทศ และต่างประเทศ แต่การศึกษานี้พบว่า เชื้อแบคทีเรีย H. influenzae ที่พบในโพรงอากาศข้างจมูกอักเสบเฉียบพลัน สร้างเอนไซต์ b-lactamase เพียงร้อยละ 21.4 น้อยกว่าการศึกษาวิจัยอื่นที่พบถึงร้อยละ 40-70 และพบว่าเชื้อแบคทีเรียแบบ anaerobe จากโพรงอากาศข้างจมูกอักเสบเรื้อรัง คิดเป็นร้อยละ 23.3 มีอุบัติการณ์ต่ำกว่าเมื่อเปรียบเทียบกับการศึกษาวิจัยอื่น*