

Bacterial and Mycotic Organisms Etiology of Chronic Otitis Media with Mastoiditis at Srinagarind Hospital

Wanlop Kaewkes
Suthee Kraitrakool*
Unchalee Tattawasatr
Nareas Waropastrakool

Department of Microbiology and
* Department of Otolaryngology,
Faculty of Medicine
Khon Kaen University

แบคทีเรียและเชื้อราที่เป็นสาเหตุของโรคหูน้ำหนวก และโพรงกระดูก มาสตอยด์อักเสบเรื้อรังในโรงพยาบาลศรีนครินทร์

วัลลภ แก้วเกษ ภ.บ., พ.บ., วท.ม.(จุลชีววิทยา) สุทธิ ไกรตระกูล พ.บ., ว.ว.(โสต ศอ นาสิก
และลาอริ่งจิววิทยา)* อัญชลี ตัดตะวะศาสตร์ ภ.บ., วท.ม.(จุลชีววิทยา)
นเรศ วโรภาสตระกูล วท.ม.(จุลชีววิทยา)

ภาควิชาจุลชีววิทยาและ

* ภาควิชาโสต ศอ นาสิกและลาอริ่งจิววิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

การเพาะเชื้อจากสิ่งส่งตรวจ จากหนองในโพรงกระดูกมาสตอยด์ของผู้ป่วย
ด้วยโรคหูน้ำหนวกและโพรงกระดูกมาสตอยด์อักเสบเรื้อรังที่ต้องผ่าตัดด้วยวิธี radical
mastoidectomy ซึ่งมีอายุระหว่าง 7 ปี ถึง 52 ปี จำนวน 50 คน ที่โรงพยาบาล
ศรีนครินทร์ มหาวิทยาลัยขอนแก่น พบว่าเพาะเชื้อแบคทีเรียขึ้น 45 ราย คิดเป็น
ร้อยละ 90 แต่ไม่พบเชื้อราในการศึกษาครั้งนี้ ในจำนวนนี้พบเชื้อแบคทีเรียชนิดแอโรบัสต์
อย่างเดียวยังร้อยละ 53.33 แบคทีเรียชนิดแอนแอโรบัสต์อย่างเดียวยังร้อยละ 8.89 และ
เป็น mixed infection ร้อยละ 37.78 เมื่อแยกชนิดของแบคทีเรียพบว่าแบคทีเรีย
แอโรบัสต์ที่พบบ่อยเป็นกลุ่มแบคทีเรียกรัมลบรูปแท่ง ที่พบบ่อยที่สุดคือ *Proteus mirabilis*
พบร้อยละ 30.0 รองลงมาเป็น *Pseudomonas aeruginosa* ร้อยละ 21.42, *Providencia*
rettgeri ร้อยละ 4.29, *Klebsiella species* ร้อยละ 1.43, *Enterobacter species* ร้อยละ 1.43
และ *Acinetobacter calcoaceticus var anitratus* ร้อยละ 1.43 แบคทีเรียกรัมบวกรูปกลมที่
พบได้แก่ enterococci ร้อยละ 7.14, *Staphylococcus aureus* ร้อยละ 1.43 และ alpha strepto-
cocci ร้อยละ 1.43 แบคทีเรียในกลุ่มแอนแอโรบัสต์ที่พบบ่อยที่สุดคือ *Peptostreptococcus*
species ร้อยละ 14.29 รองลงมาเป็น *Clostridium species* ร้อยละ 4.29, *Bacteroides*
melaninogenicus ร้อยละ 4.29, *Bacteroides species* ร้อยละ 2.86, *B. fragilis* ร้อยละ 1.43,
Fusobacterium species ร้อยละ 1.43 และ *Peptococcus species* ร้อยละ 1.43

ผลการทดสอบความไวของยาปฏิชีวนะต่อแบคทีเรียที่เป็นสาเหตุของโรคหู
น้ำหนวกและโพรงกระดูกมาสตอยด์อักเสบเรื้อรังของ *P. mirabilis* และ *Ps. aeruginosa*
ซึ่งพบได้บ่อย พบว่าไวต่อยา gentamicin ร้อยละ 94.74 และ 100 ตามลำดับ *P. mirabilis*
ยังไวต่อ trimethoprim/sulfamethoxazole ร้อยละ 88.24 และไวต่อ cephalothin ร้อยละ
80 สำหรับ *Ps. aeruginosa* ไวต่อ tobramycin ร้อยละ 100 และไวต่อ polymyxin ร้อยละ 90

Fifty patients, 7 to 52 years of age, with chronic otitis media with mastoiditis and treated with radical mastoidectomy, at Srinagarind Hospital, Khon Kaen University were studied. The pus swabs from mastoid antrums were cultured and identified for bacterial and fungal infection. Forty five specimens (90%) were found the bacterial growth on the cultures but no growth appeared on fungal cultivation. The bacteria isolated were aerobes, anaerobes and mixed organisms in the percentage of 53.33, 8.89 and 37.78, respectively. The gram negative bacilli found in aerobic group were *Proteus mirabilis* (30.0%), *Pseudomonas aeruginosa* (21.42%), *Providencia rettgeri* (4.29%), *Klebsiella species* (1.43%), *Enterobacter species* (1.43%), *Acinetobacter calcoaceticus var anitratu*(1.43%), and the gram positive cocci were enterococci (7.14%), *Staphylococcus aureus* (1.43%) and alpha streptococci (1.43%). The bacteria found in anaerobic group were *Peptostreptococcus species* (14.29%), *Clostridium species* (4.29%), *Bacteroides melaninogenicus* (4.29%), *Bacteroides species* (2.86%), *B. fragilis* (1.43%), *Fusobacterium species* (1.43%) and *Peptococcus species* (1.43%)

The predominant bacteria, *Proteus mirabilis* and *Pseudomonas aeruginosa* isolates, were susceptible to gentamicin in the percentage of 94.74 and 100, respectively. *P. mirabilis* isolates were also sensitive to trimethoprim/sulfamethoxazole (88.24%) and cephalothin (80%), and *Ps. aeruginosa* isolates were also susceptible to tobramycin (100%) and polymyxin (90%).

INTRODUCTION

Chronic otitis media with mastoiditis is commonly found in Srinagarind Hospital, Khon Kaen University. A residual active state is indicated by continued inflammation with exudation of otitis media⁽¹⁾. The irreversible destruction of mucosa lining in middle ear and mastoid antrums are occurred. The reepithelization of the demolished mucosa and epithelial lining takes place by epithelial ingrowth in the middle ear or mastoid, then a cholesteatoma elabo-

rates and the constant regeneration of epithelium evokes the cholesteatoma to grow. In active chronic mastoiditis, the mucopurulent secretion from the mucous membrane often shows a foul odor due to the accountable bacteria, the underlying bone necrosis, and/or the cholesteatoma.

The acute flare up of chronic mastoiditis associated with cholesteatoma is usually found in the majority of complications from mastoiditis. Infections and resultant complications ramble by direct extension more frequently than by retrograde thrombophlebitis. The persistent occurrence of these complications is elucidated by poor vascularity and lack of antibiotic penetration. Crabtree⁽¹⁾ explained that the acute exacerbation of mastoiditis in chronic otitis media was almost always associated with cholesteatoma. Venezia et al⁽²⁾ reported six of fifteen chronic mastoiditis patients associated with cholesteatoma, four of them developed venous sinus thrombosis and one found intracerebellar abscess too. Moreover, Seigler et al⁽³⁾ reported a case of bacterial meningitis complicated with chronic mastoiditis.

The objectives of this study are to find out the sorts of bacterial and fungal organisms which are the etiology of chronic otitis media with mastoiditis in Srinagarind Hospital and to investigate the patterns of antibiotic sensitivity of the bacteria identified.

MATERIALS AND METHODS

Fifty patients, 7 to 52 years of age, attended in Srinagarind Hospital, Khon Kaen University during April 1983 to April 1987 with the diagnosis as chronic otitis media with mastoiditis and received radical mastoidectomy were recruited in the study. The pus swabs from mastoid antrums were collected in Stuart transport and in thioglycolate medium. The swabs in Stuart transport medium were microscopically examined for

Gram stain and acid fast stain (Kinyoun stain), and cultured on blood, Mac Conkey (Difco), chocolate and Sabouraud dextrose agar (Difco). The aerobic gram negative bacilli from cultures were isolated and identified by the method of Lennette⁽⁴⁾ and the aerobic gram positive cocci were identified according to the colonial morphology and biochemical properties⁽⁴⁾. The pus swabs in thioglycolate media were incubated at 37 °C for 18-24 hours and subsequently cultured on blood agar with vitamin K under anaerobic condition at 37 °C for 48 hours. The anaerobic bacteria were identified according to the method of Holeman⁽⁵⁾. Drug sensitivity test was performed by Kirby-Bauer's method⁽⁶⁾. The gram negative bacilli were tested against Ampicillin, Chloramphenicol, Colistin, Gentamicin, Kanamycin, Tetracycline, Trimethoprim/sulfamethoxazole and Cephalothin. The nonfermentative gram negative bacilli were tested against Kanamycin, Gentamicin, Amikacin, Tobramycin, Carbenicillin and Polymyxin. The gram positive cocci were tested against Cephalothin, Chloramphenicol, Ampicillin, Erythromycin and Penicillin.

Yeast identification was based on sugar fermentation, assimilation test, morphology of chlamydospore on Cornmeal agar and germ tube morphology in plasma broth⁽⁷⁾. Mold isolates were studied by slide culture technique, mounted with lactophenol cotton blue and identified according to Mc Ginnis's method⁽⁸⁾.

RESULTS

The identifications of bacteria in pus from mastoid antrums of fifty chronic otitis media with mastoiditis patients were done and presented in Table 1. Forty five specimens (90%) showed bacterial growth. The bacteria found in cultures were aerobes, anaerobes and mixed organisms in the percentage of 55.33, 8.89 and 37.78, respec-

Table 1 Bacteria isolated from chronic otitis media with mastoiditis patients

organisms	Number of isolates	percentage
Aerobic bacteria		
<i>Proteus mirabilis</i>	21	30.00
<i>Providencia rettgeri</i>	3	4.29
<i>Klebsiella species</i>	1	1.43
<i>Enterobacter species</i>	1	1.43
<i>Pseudomonas aeruginosa</i>	15	21.42
<i>Acinetobacter calcoaceticus</i> var <i>anitratus</i>	1	1.43
<i>Staphylococcus aureus</i>	1	1.43
<i>Streptococci</i>	1	1.43
<i>Enterococci</i>	5	7.14
Anaerobic bacteria		
<i>Peptococcus species</i>	1	1.43
<i>Peptostreptococcus species</i>	10	14.29
<i>Bacteroides melaninogenicus</i>	3	4.29
<i>Bacteroides species</i>	2	2.86
<i>B. fragilis</i>	1	1.43
<i>Clostridium species</i>	3	4.29
<i>Fusobacterium species</i>	1	1.43
Total	70	100

tively. In the aerobic group, the gram negative bacilli isolated were *Proteus mirabilis* (30.0%), *Pseudomonas aeruginosa* (21.42%), *Providencia rettgeri* (4.29%), *Klebsiella species* (1.43%), *Enterobacter species* (1.43%), *Acinetobacter calcoaceticus* var *anitratus* (1.43%), and the gram positive cocci were enterococci (7.14%), *Staphylococcus aureus* (1.43%) and alpha streptococci (1.43%). The anaerobic bacteria found in the cultures were *Peptostreptococcus species* (14.29%), *Bacteroides melaninogenicus* (4.29%), *Bacteroides species* (2.86%), *B. fragilis* (1.43%), *Fuso-*

bacterium species (1.43%) and *Peptococcus species* (1.43%). The culture of *Mycobacterium tuberculosis* and fungi showed no growth of these organisms.

The drug sensitivity test of the predominant bacteria, *Proteus mirabilis*, *Providencia rettgeri*, *Pseudomonas aeruginosa* and enterococci, was tabulated in Table 2. These organisms were susceptible to gentamicin in the percentage of 94.74, 100, 100 and

100, respectively. Moreover, they also got high rate sensitivity to the others tested drugs; *P. mirabilis* isolates were susceptible to trimethoprim/sulfamethoxazole (88.24%) and cephalothin (80%); *Ps. aeruginosa* isolates were susceptible to tobramycin (100%) and polymyxin (90%); and enterococci isolates were susceptible to penicillin (100%), chloramphenicol (100%) and ampicillin (75%).

Table 2 The susceptibility pattern of the bacteria identified from chronic otitis media with mastoiditis patients.

Organisms isolates	No. of isolates	Ampicillin		Chloramphenicol	colistin	Gentamicin	Kanamycin	Tetracycline	Trimethoprim/ Sulfamethoxazole		Amikacin	Carbeni- cillin		Polymyxin mycin	Tobra- mycin	Erythro mycin	Penicillin	
		60*	80						20	25								
<i>Proteus mirabilis</i>	21	60*	80	63.16 (21.05**)	0	94.74	66.67 (16.67)	0	88.24									
<i>Providencia rettgeri</i>	3	(33.33)		66.67 (33.33)		100	100	33.33	100									
<i>Pseudomonas aeruginosa</i>	15					100	28.57			20 (20)	25	90 (10)	100					
Enterococci	5	75 (25)		100		100										100	100	

* percent sensitive

** percent intermediate sensitive

DISCUSSION

The bacteria recovered in chronic mastoiditis varies considerably, the predominant organisms are usually gram negative bacilli⁽⁹⁾. Friedman⁽¹⁰⁾ found the bacteria of chronic otitis media in 1,700 patients as follows: *Staphylococcus aureus* (31.7%), *S. aureus*, penicillin resistant (12.9%), *Bacillus proteus* (25.4%), *Pseudomonas aeruginosa* (12.8%), mixed (8.4%), *Escherichia coli* (8.1%), *Streptococcus pyogenes* (7.0%), *Streptococcus viridans* and *Streptococcus pneumoniae* (4.6%) and no growth (10.6%).

Brock⁽¹¹⁾ notified the bacteria in patients with chronic otitis media and found aerobes, anaerobes and mixed organisms in the percentage of 48.5, 13.2 and 38, respectively. The predominant aerobes

were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus species*, *Klebsiella pneumoniae* and *Hemophilus influenzae*. The anaerobic groups were gram positive cocci, *Bacteroides species* and *Clostridium species*. In this study, the bacteria found in culture were aerobes, anaerobes and mixed organisms in the percentage of 55.33, 8.89 and 37.78, respectively. The predominant aerobes were *Proteus mirabilis* and *Pseudomonas aeruginosa*, and the predominant anaerobes were *Peptostreptococcus species*.

In the study of Harker and Koontz⁽¹²⁾, Over fifty percent of the patients possessed both aerobic and anaerobic bacteria in the cholesteatoma. *Pseudomonas aeruginosa* was the most common organism in aerobic

culture. *Proteus species*, *Escherichia coli*, Klebsiella-Enterobacter-Serratia and alpha hemolytic streptococcus were also exhibited. Of the anaerobic organisms identified, *Bacteroides species* were the most frequently engaged succeeded by varieties of anaerobic staphylococcus or streptococcus.

In this study, *Mycobacterium tuberculosis* and fungi were not isolated. Samual and Fernandes⁽¹³⁾ reported twenty three cases of tuberculous mastoiditis, nine of which were complicated by facial palsy.

The drug sensitivity test of the isolated bacteria from chronic otitis media with mastoiditis patients are different from place to place. The Division of Microbiology, Department of Pathology Ramathibodi Hospital⁽¹⁴⁾ reported that (i) *Proteus mirabilis* isolates were susceptible to gentamicin (80%), cephalothin (69%), kanamycin (47%), trimethoprim/sulfamethoxazole (37%), ampicillin (34%) and chloramphenicol (32%) and that (ii) *Pseudomonas aeruginosa* isolates were susceptible to amikacin (80%), tobramycin (62%), gentamicin (61%) and carbenicillin (56%). Gherunpong et al⁽¹⁵⁾ reported the *in vitro* susceptibility of 107 clinical isolates of *Pseudomonas aeruginosa* to four aminoglycosides by microdilution broth method. The susceptibility patterns were gentamicin (40.1%), tobramycin (44.8%), netilmicin (59.8%) and amikacin (84.1%).

ACKNOWLEDGEMENTS

The financial support from Khon Kaen University is acknowledged. Authors would like to thank Dr. Pramote Tongkrajai and Dr. Nuanchan Chittasophon for their suggestions. Our thanks are also extended to Miss Plernpis Sirisajjang for laboratory assistance.

REFERENCES

1. Crabtree JA. Acute otitis and mastoiditis. In: English GM, ed. Otolaryngology. volume 1. Philadelphia : Harper & Row Publisher, 1986:4-10.
2. Venezio FR, Naidich TP, Shulman ST. Complications of mastoiditis with special emphasis on venous sinus thrombosis. J Pediatr 1982;101(4) : 509-13.
3. Siegler D, Faiers MC, Willis AT. Bacteroides meningitis complicating chronic mastoiditis. Postgrad Med J 1982;58 (683) : 560-1.
4. Lennette EH, Spaulding EH, Traut JP. Manual of Clinical Microbiology. 2nd ed. Washington DC. : American Society for Microbiology, 1977.
5. Holeman LV., Moore WEC. Anaerobic Laboratory manual. Virginia Polytechnic Institute, 1972.
6. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized sample disk method. Am J Clin Patho 1966; 45:493.
7. Ajello L, Georg LK, Kaplan W, Kaufman C. Laboratory Manual for Medical Mycology. USPHS publ No.944. Washington DC:U.S. Government Printing Office, 1980.
8. Mc Ginnis MR. Laboratory Handbook of Medical Mycology. New York : Academic Press, 1980.
9. Paparella MM, Shumrick DA. Otolaryngology. 2nd ed, Volume 2, W.B. Saunder Company, 1980:1473.
10. Friedman I. Pathology of otitis media (III) with particular reference to bone changes. J Laryngol 1957a;71 : 313-20.
11. Brock I. Chronic otitis media in children. Am J Dis Child 1980; 134:564-6.
12. Harker LA, Koontz FP. The bacteriology of cholesteatoma. In : Mc Cabe BF, Sadi J, Abrason MI, eds. Cholesteatoma, Birmingham : Aesculapius, 1977:264.
13. Samual J, Fernandes CM. Tuberculous mastoiditis, Ann Otol Rhinol Laryngol 1986;95 (3Pt1) : 264-6.
14. Division of Microbiology, Department of Pathology, Ramathibodi Hospital. Antimicrobial susceptibility of clinical isolates at Ramathibodi Hospital Jan-Dec 1985. Rama Med J 1986; 9(1):39.
15. Gherunpong V, Wisuthsareewong W, Laelaporn A. *In vitro* susceptibility of *Pseudomonas aeruginosa* to gentamicin, tobramycin, netilmicin and amikacin. Congress on bacterial and parasitic drug resistance. Bangkok, Thailand, 1986:99.