

Isolation and Identification of Gram Positive Cocci Bacterial Pathogens from Sappurative Otitis Media Infections

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Summary

Otitis Media (OM) is a common disease. It represents a serious problem mainly after winter outbreaks of respiratory tract infections (RTI); its consequences are hearing weakness; or even loss in adults and problems in speech in children. Gram-positive Cocci bacteria are among the causative pathogens. This study concerned with suppurative otitis media caused by gram-positive Cocci bacteria; either as pure culture or mixed with other pathogens. A total of 60 ear swabs were collected from Ear, Nose and Throat Department outpatients in AL-Nuaman hospital in Baghdad and cultured to isolate and identify the most common causative bacterial pathogen among gram positive Cocci. *Staphylococcus aureus* was the most common isolated bacteria (81.6%) representing (53.3%) as pure culture and (28.3%) as mixed. *Streptococcus pneumonia* was the second isolated pathogen (18.3%) representing (8.3%) as pure culture and (10%) as mixed. The mixed culture represented other pathogens like gram-negative bacteria and fungi.

Keywords: Otitis media, gram positive, sappurative, bacterial isolation.

Introduction

Otitis Media is the inflammation of the middle ear resultant from the colonization of pathogenic microorganisms. The most common causative bacteria are (*Hemophilus influenza*, *Streptococcus pneumonia* and *Staphylococcus aureus*) (1). This disease is one of the health care problems due to its complications e.g. brain abscess, meningitis and hearing loss or deafness, especially in children since recurrent OM is the common cause of late speech (2).

Bacterial Otitis Media is more common than other infections resultant from other pathogens. The growing antibiotics resistance showed by many bacterial pathogens also supported by improper use of antibiotics therapy. These circumstances created pathogens with higher virulence and ability for colonization. Hence, most bacterial otitis media are recurrent and mainly called Chronic Otitis Media (COM) (3). This study concerned with the isolation and identification of the most common bacterial pathogen among gram-positive cocci, either as a pure culture or as mixed infections with other pathogens.

Materials and Methods

A total of (104) ear swabs collected from outpatients attending the Dept. of Ear, Nose and Throat in AL-Nuaman Hospital in Baghdad, diagnosed with sappurative bacterial otitis media. Only 60 samples identified as gram-positive cocci infection, from the total 104 samples after primary bacteriological swab culturing and gram stain examinations. The 60 ear swabs samples cultures were taken for further bacteriological tests. All ear swabs were cultured as follow:

On blood agar; (two plates for each swab). This medium was used to support the growth of fastidious bacteria and to differentiate the hemolysis type. It was prepared by adding of 5-10% human blood to the blood agar base, then on MacConkeys' agar; (one plate for each swab). This medium was used for the growth of *enterobacteriaceae* and related gram-negative rods, and lastly on Mannitol salt agar (one plate for each swab). This medium was used for the isolation of staphylococci simultaneous with other tests (4).

All plates were incubated at 37° C for 24 hours only one of the blood agar plates for

each swab was cultured in the presence of CO₂ (microaerophilic conditions) using candle jar (5). Bacterial identification confirmed according to workers (6) by microscopic examination and biochemical tests; catalase test (6), slide and tube methods of coagulase test (7 and 8).

Results and Discussion

Table,1 shows the total isolates and the species of each bacterium isolated.

After bacteriological identification, the most common gram-positive cocci bacterial pathogen was *Staphylococcus aureus*. These bacteria were isolated as pure culture (particular pathogen) from 53.3% and as mixed with other causative agents (poly infections) 28.3% from the total cultured ear swabs 60. The other agents in poly-infections were gram-negative bacteria or fungus.

The diagnosis of *Staphylococcus aureus* was initially done according to the colonies morphology on the culture media and conformed by gram's stain since they were gram-positive cocci, arranged in grape like or in clusters. They were positive in coagulase test; in either slide or tube method, and able to do mannitol fermentation when cultured on mannitol salt agar, the colonies appeared as yellow colonies due to mannitol fermentation in the presence of phenol red indicator (7, 8 and 9). *Staphylococcus aureus* was differentiated from *Staphylococcus epidermidis* which was non hemolytic, coagulase negative and mannitol non fermenter (4). It was clear that bacterial otitis media is most common than other OM infections caused by other pathogens (1 and 11).

Staphylococcus aureus was the most common causative pathogen among gram positive cocci bacteria. This result was consistent with those of (12). They found in this study that *Staphylococcus aureus* caused 45% of OM infections, also (13) found that *Staphylococcus aureus* was the most commonly isolated bacteria from bacterial otitis media infections as pure isolates and mixed with other bacteria (poly infections).

Otitis media is a major health problem in low-income countries as stated by (14). They

also found that *Staphylococcus aureus* bacteria were the predominant pathogen ever isolated from otitis media ear discharges. It represented (42.7%) among all causative bacteria.

Staphylococcus aureus represents the major cause of nosocomial and community-acquired infections because of their autochthonic properties (6). The importance of *Staphylococcus aureus* as a causative pathogen of OM came from the developing antibiotics-resistance of these bacteria and their high ability of colonization (15 and 16).

The second isolated gram-positive cocci bacteria was *Streptococcus pneumoniae*. These bacteria were isolated as pure culture (particular pathogen) from 8.3% and as mixed with other causative agents (polyinfections) in a ratio 10%. These bacteria diagnosed according to their colonies morphology (draught man colonies), and the α -hemolysis on blood agar, the diagnosis conformed by gram's stain; the bacteria appeared as gram-positive diplococci lanceolate in shape. Encapsulated and catalase-negative (4, 5 and 10). As mixed infections or polyinfections, these bacteria were isolated in 38.3% from the total cultured ear swabs 60.

Concerning *Streptococcus pneumoniae*, these bacteria came secondly as causative pathogen in bacterial otitis media infections with a percentage (11%) from total 60 cases. These findings were in agreement with those of (17). They found that *Streptococcus pneumoniae* caused acute bacterial otitis media in 15 patients from total 33.

It is a reality that the bacterial pathogen that causes the respiratory tract infection could cause otitis media by spreading through eustachian tube mucosa like *Streptococcus pneumoniae* (18). The point view of (19) is that otitis media infections resultant from *Streptococcus pneumoniae* are usually chronic exudative discharge cases. While (20 and 21) stated that otitis media infections caused by *S. pneumoniae* in children are mostly develops after respiratory tract infections. Mixed isolates for both *Staphylococcus aureus* and *Streptococcus pneumoniae* can be explained as chronic otitis media infections when the body surface

defenses get low autochthonic bacteria will take their place and induce secondary infection. These findings were supported by the results of (19). They recorded 64 cases of poly infections (38%) of otitis media their total patients 167 of bacterial otitis media. The scientists (22) stated that chronic infections pathogens are usually found mixed. Also, the authors (13) had (12.5%) mixed chronic infections of otitis media from a total cases number 56 patients. And (12) documented that 7 patients (12.5%) from a total number 56 patients had mixed chronic infections of otitis media.

Table,2 illustrates the distribution of the isolated bacteria and their percentages according to the age groups of the patients. The majority of infections were located in the age groups number 3, 4, and 5 for both bacterial species (*Staphylococcus aureus* and *Streptococcus pneumonia*). And if the total number of patients in these groups were taken together; we can find that 84.4% of the pure *Staphylococcus aureus* infections and 64.6% of the *Staphylococcus aureus* mixed growth were located in these categories, in comparable with 80% and 83.3% of the bacteria *Streptococcus pneumonia* as pure and mixed growth. Concerning age's results, the age groups number, 1 & 2 showed no *Streptococcus pneumonia* infections, this finding may indicate that these age groups are

not prone to otitis media infections with this bacteria, and the other age groups were more susceptible to be infected with these microorganisms, in contrast to staphylococcal infections which constituted about 12.6% and 23.5% as pure and mixed in these age groups. Some authors explained the cause of otitis media infections because of RTI when the causative agents succeed to escape through Eustachian tube and cause the infection (23 and 24).

This study concluded that *Staphylococcus aureus* bacteria were the most common bacterial pathogen among gram-positive bacteria that were responsible for bacterial otitis media. These bacteria seemed to be a public health problem as a cause for suppurative bacterial otitis media. They were isolated as pure culture and mixed from 49 otitis media cases (81%). In addition, ages between 21-50 years found more affected with OM than the others.

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Table, 1: Total Isolates and Species of each bacterium isolated.

Species of Bacteria	No.	(%)	Total No.	(%)
<i>Staphylococcus aureus</i> (pure)	32	(53.3)	49	(81.6)
<i>Staphylococcus aureus</i> (mixed)	17	(28.3)		
<i>Streptococcus pneumonia</i> (pure)	5	(8.3)	11	(18.3)
<i>Streptococcus pneumonia</i> (mixed)	6	(10)		
Total	60	100	60	100

Note: mixed = gram negative and Fungus.

Table, 2: Distribution of isolates in relation to patients' ages

E	Age Group	<i>Staphylococcus aureus</i>		<i>S. aureus</i> (mixed)		<i>Streptococcus pneumoniae</i>		<i>S. pneumonia</i> (mixed)		Total No.
		No.	(%)	No.	(%)	No.	(%)	No.	(%)	
1	≤ 10	2	(6.3)	1	(5.9)	—	—	—	—	3
2	11-20	2	(6.3)	3	(17.6)	—	—	1	(16.7)	6
3	21-30	12	(37.5)	3	(17.6)	1	(20)	—	—	16
4	31-40	9	(28.1)	4	(23.5)	2	(40)	3	(50)	18
5	41-50	6	(18.8)	4	(23.5)	1	(20)	2	(33.5)	13
6	51-60	1	(3.1)	2	(11.8)	1	(20)	—	—	4
Total		32	(100)	17	(100)	5	(100)	6	(100)	60

References

1. Tarlow, M. (1998). Otitis media: Pathogenesis and Medical sequelae. Ear-Nose-Throat., 77:3-6.
2. Altemeier, W. (1999). A Trip through the ear in search of deafness. J. Pediatric. Ann., 28(6): 342-344.
3. Robert, J and Ruben, R. (2009). Serous Otitis Media in the 20th and 21st centuries: evolving views and treatments. Cross ref., 129 (4): 343-371.
4. Todar, k. (2005). Text Book of Bacteriology. Chapter: *Staphylococcus aureus*. University of Wisconsin Madison. USA.
5. Atlas, R. (1995). Principles of Microbiology. 1st ed. Mosby comp. USA., PP: 522-541.
6. Albrecht, T. (2008). Medical Microbiology: Guide Textbook. Chapter: Pyogenic bacterial infections Library of Congress pub. USA., P: 255-284.
7. Buxton, A. and Fraser, G. (1977). Animal Microbiology. 1st ed. Blackwell. Oxford. London, PP:143-192
8. Sood, R. (1985). Medical laboratory Technology 1st ed. Type Brothers. India, PP: 367-376.
9. Prescott, L.; Harley, J.; and Klein, D. (1990). Microbiology. Bacterial Taxonomy chapter. WCB. Pub. USA.
10. Atlas, R.; Parks, L. and Brown, A. (1995). Experimental Microbiology: Laboratory Manual. Gram Positive cocci culturing. Mosby comp. USA.
11. Adderson, E. (1998). Preventing Otitis Media: Medical Approaches. J. Ann. 27(2): 101-107.
12. Komoniewska, R.; Pelczar, B.; Nowakowska, M.; Zientara, M. and Rudy, M. (1997). Bacterial flora in chronic middle ear infections in adults. Med. Dosw. Mikrobiol., 49 (1-2): 83-87.
13. Vartianen, and Vartianen, (1996). Effect of aerobic Bacteriology on the clinical presentation and treatment results of chronic suppurative otitis media. J. Laryngol. Otoloy, 110: 315-318.
14. Abera, B. and Biadeglegne, F. (2009). Antimicrobial resistance patterns of *Staphylococcus aureus* and *Proteus spp.* isolated from OM at Bahir Dar Regional Laboratory. Ethiop. Med. J., 47(4): 271-276.
15. Jang, C.; Song, C. and Wang, P. (2004). Topical vancomycin for chronic otitis media with methicillin-resistant *Staphylococcus aureus* otorrhoea. J. Laryngol Otol., 118(8): 645-647.
16. Marzouk, H. (2011). Methicillin-resistant *Staphylococcus aureus* colonization in otitis-prone children. Arch Otolaryngol Head Neck Surg., 137(12): 1217-1222.
17. Andrade, M. (1998). Acute otitis media in children with bronchiolitis. J. Pedia., 101: 617-619.
18. Sheldon, H. (1988). Boyd s introduction to the study of diseases. 10th ed. Lea and Febiger comp. Philadelphia, PP:1367-1382.
19. Mashchenko, A. and Mashkova, T. (2010). Diagnostic criteria for surgical correction of exudative Otitis Media in children. Vestn. Otorinolaringol., (5): 56-57.
20. Gunasekera, H.; O'Connor, T.; Vijayasekaran, S. and Del Mar, C. (2009). Primary care management of otitis media among Australian children. Med. J. Aust., 191(9): 55-59.
21. Tapiainen, T. (2010). Biofilm formation by *Streptococcus pneumoniae* isolates from paediatric patients. vestn. Otorinolaringol, 118 (4): 255-260.
22. Krugman, S.; Katz, S.; Gershon, A. and Wifert, C. (1985). Infectious diseases of children. Otitis Media. Mosby Comp. USA.
23. Ivady, B. and Pasztor, M. (2009). Serotype distribution and antimicrobial susceptibility of streptococcus pneumoniae strain isolated from middle ear fluids of children aged 0-24 months with acute Otitis Media. O. and V. Hetil., 150(20): 935-941.
24. Thistlethwaite, J. (1997). Childhood respiratory infections: Otitis med. J. Update, 23: 94-98.

عزل وتشخيص البكتريا الكروية الممرضة الموجبة لصبغة كرام من التهابات الأذن الوسطى القيحية

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الخلاصة

يعد التهاب الأذن الوسطى Otitis Media مرضا شائعا ويمثل إحدى المشاكل التي تعقب أمراض الجهاز التنفسي في الشتاء. إن عواقب مرض التهاب الأذن الوسطى عديدة، من ضعف السمع أو حتى فقدانه للبالغين إلى مشاكل النطق لدى الأطفال. أن البكتريا الموجبة لصبغة كرام هي احد العوامل الممرضة التي تسبب هذا المرض، حيث عني هذا البحث بالإصابات القيحية التي تسببها هذه البكتريا سواء بشكل إصابة مفردة أو مختلطة مع عوامل ممرضة أخرى. تم جمع 60 عينة (مسحة أذن) من مرضى يراجعون شعبه الأنف والأذن والحنجرة في العيادة الخارجية لمستشفى النعمان العام/ بغداد، وتم زرع تلك العينات (المسحات) وذلك لعزل وتشخيص البكتريا الأكثر شيوعا بين البكتريا الموجبة لصبغة كرام. النتائج: كانت بكتريا *Staphylococcus aureus* هي الأكثر شيوعا وقد عزلت بنسبة (81.6%)، مقسمة الى (53.3%) عزلات نقية و (28.3%) عزلات ممزوجة مع عوامل ممرضة أخرى. أما البكتريا *Streptococcus pneumonia* فقد جاءت بالدرجة الثانية بنسبة عزل (18.3%)، بنسب (8.3%) عزلات نقية و(10%) عزلات ممزوجة مع عوامل ممرضة أخرى إذ كانت العوامل الممرضة الأخرى بكتريا سالبة لصبغة كرام وبعض الفطريات.

الكلمات المفتاحية: التهاب الأذن الوسطى، القيحي، عزل البكتريا، بكتريا موجبة صبغة كرام.