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

Weam Saad Al-Hamadany
Department of Microbiology, College of Veterinary, University of Baghdad, Iraq
akweam@yahoo.com

Accepted on 11/12/2012

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 37º 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 (polypathogenic) 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 (polypathogenic).

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 (polypathogenic) in a ratio 10%. These bacteria diagnosed according to their colonies morphology (draught man colonies), and the α-haemolysis on blood agar, the diagnosis conformed by gram's stain; the bacteria appeared as gram-positive diplococcic lanceolate in shape. Encapsulated and catalase-negative(4, 5 and 10). As mixed infections or polynfections, 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 develop 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%) fromitis 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.

Acknowledgment

Special thanks to Dr. Rajwa H. Al-Rubaei (Department of Biology College of Sciences-Al-Mustansiriya University) and to Dr. Hamid M. Ghani (at the mercy of God). I am grateful to Al-Nuaman hospital ENT department members, and the inner and outer Laboratories staff. I am much obliged to the patient volunteers.

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

<table>
<thead>
<tr>
<th>Species of Bacteria</th>
<th>No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus (pure)</td>
<td>32 (53.3)</td>
<td>49 (81.6)</td>
</tr>
<tr>
<td>Staphylococcus aureus (mixed)</td>
<td>17 (28.3)</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumonia (pure)</td>
<td>5 (8.3)</td>
<td>11 (18.3)</td>
</tr>
<tr>
<td>Streptococcus pneumonia (mixed)</td>
<td>6 (10)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

Note: mixed = gram negative and Fungus.

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

<table>
<thead>
<tr>
<th>E Age Group</th>
<th></th>
<th>Staphylococcus aureus</th>
<th>S. aureus (mixed)</th>
<th>Streptococcus pneumonia</th>
<th>S. pneumonia (mixed)</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>(%)</td>
<td>No.</td>
<td>(%)</td>
<td>No.</td>
<td>(%)</td>
</tr>
<tr>
<td>1 ≤ 10</td>
<td>2</td>
<td>(6.3)</td>
<td>1 (5.9)</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>2 11-20</td>
<td>2</td>
<td>(6.3)</td>
<td>3 (17.6)</td>
<td></td>
<td>1 (16.7)</td>
<td>6</td>
</tr>
<tr>
<td>3 21-30</td>
<td>12</td>
<td>(37.5)</td>
<td>3 (17.6)</td>
<td>1 (20)</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>4 31-40</td>
<td>9</td>
<td>(28.1)</td>
<td>4 (23.5)</td>
<td>2 (40)</td>
<td>3 (50)</td>
<td>18</td>
</tr>
<tr>
<td>5 41-50</td>
<td>6</td>
<td>(18.8)</td>
<td>4 (23.5)</td>
<td>1 (20)</td>
<td>2 (33.5)</td>
<td>13</td>
</tr>
<tr>
<td>6 51-60</td>
<td>1</td>
<td>(3.1)</td>
<td>2 (11.8)</td>
<td>1 (20)</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>(100)</td>
<td>17 (100)</td>
<td>5 (100)</td>
<td>6 (100)</td>
<td>60</td>
</tr>
</tbody>
</table>
Otitis media: Pathogenesis and Medical sequelae. Ear-Nose-Throat., 77:3-6.
عزل وتشخيص البكتريا الكروية الممرضة الموجبة لصبغة كرام من التهابات الأذن الوسطى القيحية

وئام سعادة عبد الحمزة سلمان الحمداني
فرع الإحياء المجهرية، كلية الطب البيطري، جامعة بغداد، العراق

الخلاصة
بعد التهاب الأذن الوسطي Otitis Media مرضاً شائعاً، ويتمثل أحد المشاكل التي تعقب أمراض الجهاز التنفسي في الشتاء. إن عواقب مرض التهاب الأذن الوسطي عديدة، من ضعف السمع أو حتى فقدانه للبالغين إلى مشاكل النطق لدى الأطفال. أن البكتريا الموجبة لصبغة كرام هي أحد 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 بحثي، وتم زرع تلك العينات (المسحات) Staphylococcus aureus، وذلك لعزل وتشخيص البكتريا الأكثر شيوعاً بين البكتريا الموجبة لصبغة كرام. النتائج: كانت البكتريا Staphylococcus aureus هي الأكثر شيوعاً وقد عزلت بنسبة 63.5%، مقسمة إلى 53% عزلات نقيّة و 10.5% عزلات ممزوجة مع Streptococcus pneumoniae عوامل ممرضة أخرى. أما البكتريا أخرى، فقد جاءت بدرجة ثانية نسبة عزل 18.3%، بنسبة 8.3% عزلات نقيّة و 10% عزلات ممزوجة مع عوامل ممرضة أخرى.

الكلمات المفتاحية: التهاب الأذن الوسطي القيحى، عزل البكتريا، بكتريا موجبة صبغة كرام.