Study of Some Characteristics of the Bacteria Isolated from Patients with Otitis Media in Babylon Province

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Abstract

One handred swabs were collected from 100 patients with otitis media (OM) (54 females and 46 males) referred to Hilla Teaching Hospital (ENT unit) during the period (from November 2006 through March 2007). The collected samples were investigated for bacterial isolation. The ages of the patients ranged from 7 months to 70 years .

Bacterial cultures were positive in (95%) patients versus (5%) patients revealed negative bacterial culture . The most common type of bacterial isolated were *Moraxella catarrhalis* (20.83%), followed by *Pseudomonas aeruginosa* (20%). Besides, uronic acid was detected in most samples of ear discharge.The virulence factors were detected and found to be capsule, colonization factor antigens, hemolysin, siderophores, and extracellular proteases.

الخلاصة

تم خلال هذا البحث جمع و دراسة ١٠٠ عينه (مسحات اذن وسطى) من ١٠٠ مريض (٥٤ منهم إناث و٤٦ ذكور) مصابين بالتهاب الاذن الوسطى (حسب التشخيص ألسريري الاولي من قبل اختصاصي الأنف والأذن والحنجرة) وممن أحيلوا إلى مستشفى الحلة التعليمي العام (من تشرين الثاني ٢٠٠٦ خلال اذار٢٠٠٧) . تراوحت أعمار المرضى الذين شملتهم الدراسة من ٧ اشهر-٧٠ سنه. كانت نتائج الزرع موجبة ل٩٥ مريض مقابل ٥ نتائج سالبة اتجاه الزرع .الأنواع البكتيرية التي تم عزلها وتشخيصها خلال هذه الدراسة شملت أنواعا بكتيرية موجبة وسالبة لصبغة غرام جاءت في مقدمتها بكتريا *Moraxella catarrahalis* حيث شكلت نسبة ٢٠,٨٣%، جاءت بعدها بكتريا *Pseudomonas aeruginosa* (٢٠%). تضمنت هذه الدراسة الكشف عن حامض اليورونك من افرازات الاذن الوسطى. كما تمّ الكشف عن بعض عوامل الضراوة التي تمتلكهاهذه العزلات البكتيرية لكي تؤهلها للتسبب في التهاب الاذن الوسطى مثل المحفظة و امتلاك عوامل الاستيطان و القابلية على إنتاج الهيمولايسين والسايديروفورات وكذلك القابلية على إنتاج أنزيمات البروتييز الخارجية.

Introduction

Otitis media (OM) means inflammation of the middle ear (ME). It constitutes the most common respiratory tract infection of infancy and early childhood [1]. Otitis media may be acute, or chronic supportive type. Children are mostly affected with acute type, while adults are mostly affected with chronic supportive types [2].

The infection of otitis media might result from viral or bacterial agents; however many complications were reported that may persist in some individuals into adult years [1].

The bacteriologic agents associated with otitis media differ according to the type ; *S.peumoniae* ,non-typable *H. influneza* , and *M. catarrhalis* are most common bacterial of acute OM [3], *P. aeruginosa, S.aureus ,and Proteus spp.* are predominant pathogen of chronic

supportive otitis media, and it was also seen that non-spore forming anarebes such as *Bacteroides* spp can be isolated from exudates obtained from patients with chronic otitis media.

Pathogenic bacteria have the ability to produce several types of virulence factors associated with their pathogencity and major role in the causation of infection in otitis media. The aims of this study were: isolate and identify the bacterial pathogenes associated with otitis media, and to detect uronic acid from ear discharge, and examine some of virulent factors.

Patients and Methods

Patients:

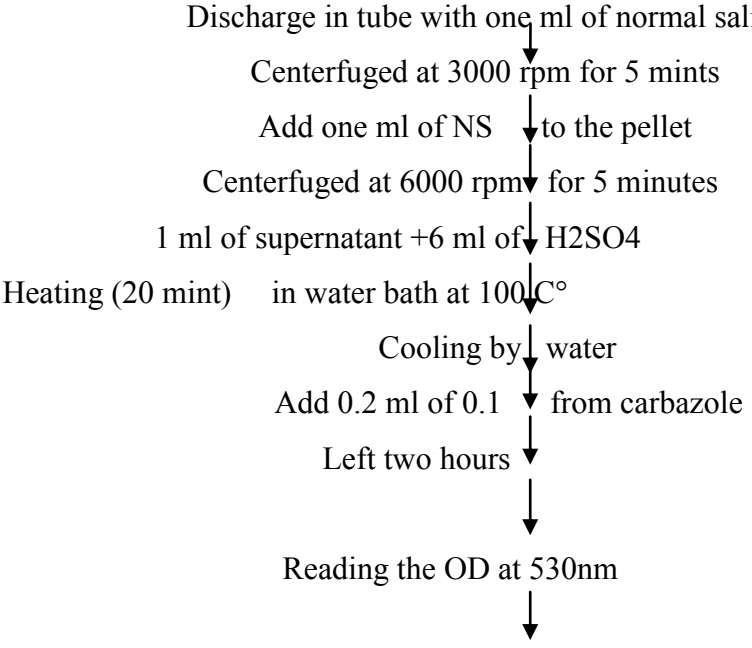
One-handred discharge samples were collected from patients with otitis media (OM) during the period from November 2006 through March 2007 at the unit of ENT in Hilla Teaching Hospital. All patients involved in this study were distributed according to their age, sex, duration of disease, and use of antibiotics. The collection specimens were carried out to [4].

Bacterial Diagnonsis : Bacterial diagnosis at the study of bacteria after incubation the isolates aerobically and anaerobically condition: and virulence factors were studied as described in [5-9]

Extraction of the polysaccharide and detection from colonic acid

The following procedure was designed for the extraction of polysaccharide and detection of uronic acid. Select 22 samples (mucos discharge in otitis media), the steps of methods shown in Figure (1), and the preparation of the concetration of Galactouronic acid (10, 20, 40, 50, 60, 80, 100 mircogram /1ml) as suggested by this study.

Figure1 Stages of extraction of the polysaccharide in discharge of otitis media



Results and Disscussion

Bacterial Isolates from Otitis Media

A total of 100 otitis media swabs were subjected for culturing on different types of culture media, the results revealed that 95 samples gave positive bacterial culture, whereas 5 samples showed no bacterial growth even after 48 hours, which may be due to the consumption of antibiotics by

the patients or the presence of another type of causative agents, that might need a special technique for their detection such as viruses, chlamydia, and mycoplasma.

A total of 120 bacterial isolates were obtained from the 95 samples collected.

Pathogenicity of bacteria in Otitis Media

Sixty eight cases of OM were caused by single bacterial, whereas the mixed bacterial type constitutive (52) is shown in Table (1). The main bacterial types isolated in this study were shown in Table(1) *M.catarrhalis* was the most common bacterial species isolated from ear infections species (20.83%) *M.catarrhalis* infections are encountered more often now than in the past in ear infection, such as in Kufa City [10] reported (1.1)%, in Babylon Governorate [11] reported (3.6)%,and [12] reported (6.48%).

Table 1 Distribution of Bacterial Isolates from Patients with Otitis Media

BACTERIA	SINGLE ISOLATE	MIXED ISOLATES	TOTAL ISOLATES	%
M.CATARRHALIS	14	11	25	20.83
P.AERUGINOSA	16	8	24	20
STERP.PNEUMONIAE	9	14	23	19.66
STAPH.AUREUS	11	10	21	17.5
K. PNEUMONIAE	4	2	6	5
PROTEUS SPP	4	1	5	4.16
ACINETOBACTER SPP	4	1	5	4.16
STAPH.EPIDERMIDIS	4	0	4	3.3
STERP.PYOGENES	1	1	2	1.66
E.COLI	0	2	2	1.66
H.INFLUENZA	0	1	1	0.83
DIPHTHEROIDES SPP	1	0	1	0.83
BACTEROIDS SP	0	1	1	0.83
TOTAL	***68***	***52***	***120***	***100***

This is probably due to bacterium antibiotics resistance properties, and they are commensals of mucosal surfaces of upper respiratory tract. This bacteria occasionally is causative of opportunistic infection

when the patient uses corticosteroid or immune suppressive agents .

Pseudomonas aeruginosa was the second type of bacteria isolated from chronic OM (20%). This result was expected for this organism, due to many reasons, *P.aeruginosa* is the most secondary invaders when the resistance of the middle ear is lowered. The high incidence of *P .aeruginosa* indicates more general antibiotic resistance than in the case with gram positive strains and resistance for phagocytosis and opsonization by producing a large number of extracellular products leading to inhibit the function of the immune system of the cells , this agrees with the result mentioned by [13].

Streptococcus pneumoniae which is considered from the microflora of nasopharynx in healthy adults , had constituted (19.7%), as shown in Table (1), These microorganisms were reported by many researchers such as [14], from the common bacterial species causing acute OM. *S.pneumoniae* was regarded as a pathogen in immunodificient patients , and produced many virulence factors .

The other bacteria that had been isolated in this study was *S.aureus* which represents (17.5%). This frequency may be due to, it may enter the middle ear from external canal as a normal flora and by reflux OM when the tympanic membrane was not intact, *S.aureus* also contains teichoic acid and lipoteichoic acid, capsular material which faciliates the adherence of these bacteria to epithelium. This agrees with the result mentioned by [15] reported that *S.aureus* was the dominant causative agent of CSOM in chlidren older than six years .

Klebsiella pnemoniae , and *E.coli* in 5% ,and 1.66% respectively,as shown in table (1), this may be due to the presence of capsular polysacharide which plays an important role in pathogencity of *K.pnemoniae* in OM infection,

Virulence Factors

Virulance factors for some bacterial isolated from patients with OM were studied. *M. catarrhalis* were tested for their abilities to produce colonization factors antigen by Haemmaglutination Test (HA). The results showed that most isolates of *M. catarrhalis* (80%) were able to produce colonization factors antigen. Most strains of *M. catarrhalis* which are adherent were mediated through fimbriae or pili enhance attachment to host cells and resistance to phagocytosis to provide a way for the bacterium to evade the hosts immune response .

The results in Table (2) reveal that all isolates of *M. catarrhalis* which were isolated from patients with bacterial OM did not possess the polysaccharide capsule. This result is similar to the result observed by [16] who had pointed out that *M. catarrhalis* could not possess the polysaccharide capsule. All *M. catarrhalis* isolates were found not capable of producing the extracellular hemolysin. Only 4 isolates of *M. catarrhalis* were able to produce siderophores.

Furthermore, it was known that the bacteria which were able to produce siderphores had no ability to produce hemolysin, so bacteria need only one mechanism for obtaining iron, to survive and replicate in hosts; microbial pathogens must acquired host iron, this is identical with that result obtained by [17]. The ability of *M. catarrhalis* to produce extracellular proteases was investigated and it was found that only 2 isolates of *M. catarrhalis* were able to produce extracellular proteases. *M.catarrhalis* can produce proteases when reach mucosal surfaces and may often encounter secretory IgA, which could

inhibit their adherence and growth on epithelium. The bacteria that cause disease on these mucosal surface were able to evade the action of secretory antibody by producing IgA proteases, that inactivate IgA antibody and this enzyme play role in colonization of mucosal surface. The bacteria produce several protease which have the capacity to degrade host proteins releasing amino acid as nutrients and may degrade proteins such as IgA which are involved in host defense, and may also be involved in host tissue damage .

The ability of *P. aeruginosa* isolates to produce some virulence factors was investigated and the results are shown in table (2),the isolates were tested for their abilities to produce adherence factors , and it was found that 5 bacterial isolates were able to produce adherence factors , this may be due to the presence of fimbriae, the fimbriae of *P.aeruginosa* will adhere to epithelial cells of the upper respiratory tract and causes OM, this identical to result obtained by [18].

Also, it was found that 60% of *P. aeruginosa* isolates were possessing the polysaccharide capsule which surrounded the bacterial cell,capsular polysaccharide can protect *P. aeruginosa* against the ciliary action of the respiratory tract and complement mediated lysis ,this agree to the result mentioned by [19]. The ability of *P. aeruginosa* to produce hemolysin was investigated and it was found that only 3 isolates of *P. aeruginosa* were able to produce extracellular hemolysin. *P. aeruginosa* produce two hemolysin, it appears to be cytotoxic for most eukaryotic cells, hemolysins conttribute to invasion through their cytotoxic effects on eukaryotic cells.*P. aeruginosa* isolates were also tested for their abilities to siderophores synthesis.The results showed that all isolates of *P. aeruginosa* were able to produce siderophores,and also,it was shown that only 3 isolates of *P. aeruginosa* can produce extracellular proteases. *P. aeruginosa* could produce of large number of extracellular proteases which can cleave IgA which then lead to inhibit the function of the cells of the immune system , thus *P.aeruginosa* is resistant for phagocytosis and opsoization , this agree with that result mentioned by [20].

S. pneumoniae isolates were tested for their abilities to produce colonization factors antigen as shown in table(2),and it was found that all isolates were able to produce colonization factors antigen in the presence human blood (group A) . Further more ,it was found that only 4 isolates from 6 isolates of *S.pneumoniae* 66.6% had capsular polysaccharide ,The capsular polysaccharide is very essential virulence factors through protecting the organisms from complement activation and phagocytosis mediated destruction. Additionally, the detection of hemolysin in blood agar was also studied. It was found that all *S. pneumoniae* isolates produced hemolysin. *S. pneumoniae* produced an Alpha-hemolysin .The same results were obtained by [21]. The function of hemolysin is to provide the microorganism with iron and it makes the bacteria unable to produce any factor for obtaining the iron from environment.Moreover,the ability of *Strep. pneumoniae* to siderophores synthesis was studied, and it was found that all isolates of *S. pneumoniae* could not produce siderophores or iron-chelating factors under low-iron conditions. This result similar with the result obtained by [22]. The ability of *S. pneumoniae* to produce extracellular proteases was investigated and it was found that only one isolates of *S. pneumoniae* were able to produce

extracellular proteases. *S. pneumoniae* can produce immunoglobulin A1(IgA1) proteases, and these proteases are of the serine type which enables *S. pneumoniae* to evade the protective functions of the principal Ig isotype of the upper respiratory tract,these proteases degrade human IgA1. These results were similar to those obtained by [23].

The ability of *Staph.aureus* isolates to produce some virulence factors was shown in table (2). *S. aureus* isolates were tested for their abilities to produce colonization factors antigen , and it was noticed that 4 bacterial isolates could produce colonization factors antigen. Several studies had shown that *S.aureus* strains could adhere to host tissues throgh teichoic acid,this acid was one of the most important component in bacterial cell wall. Moreover, some strains of *S. aureus* 10% had shown to possess the polysaccharide capsule which was an important component in the pathogenesis, and enhances bacterial virulence by modulate *S. aureus* adherence to endothelial surface in vitro, animal studies suggest that it also promotes bacterial colonization and persistence on mucosal surfaces, this agree with result mentioned by [24]. All *S. aureus* isolates had the ability to produce the hemolysin, and the type of hemolysis was Beta-hemolysis *S. aureus* was also tested for it's ability to siderophores synthesis, and the results showed that all isolates of *S. aureus* were not able to produce siderophores. The production of hemolysin would make the bacteria does not need the production of siderophore for obtaining iron because the former would help it to gain the iron. The ability of *S. aureus* to produce extracellular proteases was investigated, and it was found that 2 isolates of *S. aureus* were able to produce extracellular proteases, but other bacterial isolates.

Regarding to *K. pneumoniae* isolates to produce some virulence factors was investigated and the results are shown in table (2). The isolates were tested for their abilities to produce colonization factors antigen, and it was found that 3 of *K.pneumoniae* isolates were able to produce colonization factors antigen due to the positive reaction with human blood cells (groupA). *K. pneumoniae* strains had the ability to possess type 3 pili, which was an important factor in adherence of this type of bacteria to mucous of respiratory tracts. These results was identical with the results obtained by [25]. From the results in table (2), it could be noticed that all *K. pneumoniae* isolates were also possessing the polysaccharide capsule which surround the bacterial cell. This result agrees with the result obtained by [26] who had pointed that the diagnostic feature for *K. pneumoniae* is capsular polysaccharide , and it's essential virulence factor for *K. pneumoniae*.

The results also showed that all *K. pneumoniae* isolates could not produce hemolysin extracellulary when cultured on blood agar, and the type of hemolysis was gamma hemolysis because there was no hemolysis present on blood agar.

Klebsiella pneumoniae isolates were also tested for their abilities to siderophores synthesis.The results showed that all isolates of *K. pneumoniae* were able to produce siderophores, all *Klebsiella* strains have the ability to possess siderophores system.Thus, production siderophores may give access to both sources of iron, resulting in enhanced growth in the host , from the results obtained in this study it was shown that the bacteria can only produce either

hemolysin or siderophores not the both. Also, the ability of *K. pneumoniae* isolates to produce extracellular proteases was investigated and it was found that 3 isolates of *K. pneumoniae* could produce extracellular proteases *K. pneumoniae*. Proteolytic enzymes were very important factors in which the bacteria can degrade protein and then causes penetration to the tissues, this identical with that result obtained by [27].

Table 2 virulence factors detected in bacterial isolates

TYPE OF BACTERIAL ISOLATES	COLONIZATION FACTORS ANTIGEN	CAPSULE	HEAMOLYSIN	SIDROPHORES	EXTRACELLULAR PROTEASES
M. CATARRHALIS	**80%**	**0**	**0**	**40%**	**20%**
P.AERUGINOSA	**50%**	**60%**	**30%**	**100%**	**30%**
S.PNEUMONIAE	**100%**	**66.6%**	**100%**	**0**	**16.6%**
S.AUREUS	**40%**	**10%**	**100%**	**0**	**20%**
K.PNEUMONIAE	***75%***	**100%**	**0**	**100%**	**75%**

Detection of Uronic acid

Uronic acid in 22 samples of ear discharges were selected on the basis of the presence of mucoid discharge and investigated by using colorimetric method (table 3). It was found that uronic acid was detected in most samples of ear effusion taken from *K. pneumoniae* , *S. pneumoniae* and *P.aeruginosa* isolates. This may be attributed to the possession of these bacteria to capsular polysaccharides, which may be secreted extracellulary as a uronic acid. The size of capsules also may have effect on the mount of the uronic acid produced the bacteria. So, the bacteria with small capsule have a little amount of production of this acid . Uronic acid was not detected with the isolates of *M. catarrhalis* when taken the ear sample (infection without discharge) as a controling group. This may be attributed to *M. catarrhalis* had not capsular polysaccharides to produce effusion (we conclude that bacterial isolated which produce $\leq$ 10 Microgram/ml concentration of uronic acid considered less amount of uronic acid and not capable to produce capsule).

Table3 Concentration of uronic acid in isolated bacteria

NO.	*ISOLATES*	PRESENT OF CAPSULES	CONCENTRATION OF URONIC ACID MICROGRAM/ML
1	*STREPTOCOCCUS PNEUMONIAE*	+	22
2		+	29
3		+	15
4		+	28.9
5		+	27.6
6		+	41.5
7		+	31.4
8		+	75
1	*PSEUDOMONAS AERUGINOSA*	+	15
2		-	4.4
3		+	15
4		+	36.6
5		+	56.2
6		+	33
7		-	5.4
1	*KLEBSIELLA PNEUMONIAE*	+	61.2
2		+	18.4
3		+	16.4
1	*STAPHYLOCOCCUS AUREUS*	+	48
2		-	8.5
3		+	17.5
1	*MORAXELLA CATARRHALIS*	-	4.4

Control ≤ 10

Uronic acid can protect the bacteria from secretory IgA produced by mucosal surface of upper respiratory tract infection,therefore uronic acid important in pathogencity of bacteria that causes OM infection and ear discharge. *P.aeruginosa* were mainaly isolated from chronic OM infection , and were characterized by mucoid effusion . This effusion were

may be due to alginate which is the main character for the production of uronic acid, this agree with result mentioned by [28]. *P.aeruginosa* isolated from patients with OM who had a mucoid colony morphology due to overproduction of the alginate, which contribute to the persistence of bacteria in patients this agree with mentioned by [29]. The control of the production of uronic acid by bacteria in ear effusion by a regulator genes, these indicated that genes responsible from uronic acid production found on bacterial chromosome or plasmid, those genes play an important role in increasing the acid production.

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