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Isolation and Detection of *Moraxella catarrhalis* from children Infected with Acute Otitis Media in Al-Kadhemiya Pediatric Hospital

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Abstract

Objectives: A study was carried out to assess isolation and detection of *Moraxella catarrhalis*(*M. catarrhalis*) isolates from 100 specimens received from Children at 1-3 years of age suffered with Acute Otitis Media (AOM) in Al-Kadhemiya Pediatric Hospital.

Methods: The identity of isolates was confirmed by staining method (Gram stain) , culturing, biochemical test(catalase, citrate utilization, indole production, urease production, motility, methyl red, Voges-Proskauer and DNase production) and Sensitivity test.

Results: from 100 samples, 8 isolates of *M. catarrhalis* were collected. The biochemical activities especially the DNase production, motility, methyl red and catalase were fixed as features of it.

All the isolates were resistant to Ampicillin (AMP), Penicillin (PEN) and Amoxicillin (AMX) because it has the ability to produce ß-lactamase. While the isolates were susceptible to Erythromycin (ERY),Tetracycline (TET) and Chloramphenicol (CLH).

Conclusion: This study showed that *M. catarrhalis* is one of the main bacterial agents that causing Acute Otitis Media (AOM). The production of DNase enzyme was the characteristic feature of it from others bacterial causing agents of AOM. Also most strains of *M. Catarrhalis* produce ß-lactamase, which make it resistant to many antibiotics like Ampicillin, Penicillin and Amoxicillin.

Keywords: *Moraxella catarrhalis*, Acute Otitis Media (AOM), ß-lactamase production.

Introduction

Otitis media with effusion (OME) is one of the most common childhood diseases and in several otological problems it is consider as main cause of it (1). OME is defined as the presence of fluid in the middle ear with absence of clinical signs or symptoms that related to acute ear infection (2). In children, acute otitis media is the most common bacterial infection and the prevalence of it is approximately 20% also the peak incidence of it occurs at the first year of age with 70% experiencing at least one episode by age 3 years (3).

Loss of the hear is the main feature of OME(4). In the first 3 years of age, the infection leads to a negative effect on the development of language (5). Although the etiology of OME is

still unclear, bacterial and viral infections have an important role in its pathogenesis(6). The bacterial agents consider as the main cause of 22–52% of OME cases(7). After *Streptococcus pneumoniae* and non typeable *Haemophilus influenza*, *Moraxella catarrhalis* is the third leading cause of otitis media(8). *Streptococcus pneumoniae and Moraxella catarrhalis* which act as the predominant bacterial pathogens of OME can isolate by culture (9). *M. catarrhalis* is associated with 10-15%of acute otitis media cases. This bacteria is frequently found as a commensal of the upper respiratory tract (10)

Currently the taxonomic position of the genus *Moraxella* is as follows:

Family *Moraxellaceae*

Order *Pseudomonadales*

Class *Gammaproteobacteria*

Phylum *Proteobacteria*

Moraxella catarrhalis is a Gram-negative non-motile capsule-less aerobic diplococcus. Metabolically it can be characterized by the lack of fermentation of glucose, lactose, sucrose and maltose; it is able to carry out the positive oxidase, catalase and DNase (detected using DNase test agar with methyl green) reactions. Also can reduce nitrate and nitrite; performs hydrolysis of tributyrin (an isomeric glyceryl ester of butyric acid) and does not utilise 5% sucrose to form polysaccharide (11).

On rich standard laboratory media like brain heart infusion broth/agar or tryptic soy digest media *M. catarrhalis* grows well although supplements like boiled blood, vitamin and amino acid enrichment (chocolate agar) will result in better growth. *M. catarrhalis* is grown at 35-370C in the atmosphere of 3-7% CO2, but it can tolerate a wider range of temperatures (20-420 ºC) and ambient air. *M. catarrhalis* forms 2-4 mm big grey or non-pigmented, smooth, convex and opaque colonies on the solid media that can be pushed undamaged on the surface of agar with a loop (“hockey puck” effect)(12).

Materials and Methods

Collection of Sample

Non duplicate middle-ear swabbed samples were collected under aseptic conditions from100 children (50 Male and 50 Female) in age of 1-3 years suffer from acute otitis media in Al-Kadhemiya Pediatric Hospital.

Isolation and growth of bacteria

According to Verduin *et.,al* (13), primary isolation of bacteria, the culture Medias for detection of *Moraxella catarrhalis* were as follows:

The middle-ear swabbed samples were cultured aerobically on Blood agar (BA), Chocolate agar (CA) and MacConkey agar (MCA). The powdered medium was mixed with distilled water and steamed to dissolve the agar. The mixture was then sterilized in an autoclave at 121° C and subsequently allowed to cool to about 45° C. About 15 to 20 mls of the sterilized molten agar medium were poured into sterile Petridishes and left undisturbed until the agar were set. Blood agar was made by mixing sterilized molten nutrient agar at about 45°C- 50°C with 2mls of blood before pouring into the plates, while Chocolate agar was made by heating blood agar to 70°C - 80°C until it became chocolate brown in color. The middle-ear swabbed samples were inoculated into broth cultures for 4-6 hrs and later inoculated on to plates of Blood agar (BA), Chocolate agar (CA) and MacConkey agar(MCA). The plates were incubated aerobically at 37° C for 24 hours. After overnight incubation, the colonies on the positive plates were sub-cultured onto nutrient agar slants and routine conventional laboratory techniques including Gram staining, catalase, citrate utilization, indole production, urease production, motility, methyl red, Voges-proskauer and DNase production tests (14).

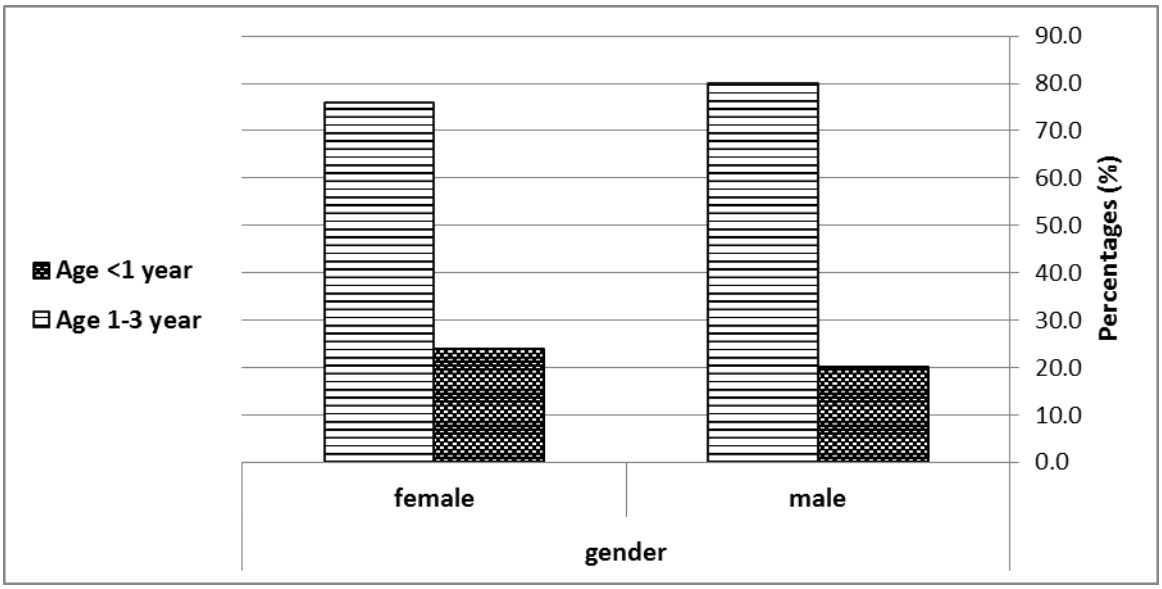
Antibiotic Sensitivity Testing

The antibiotic sensitivity of *Moraxella catarrhalis* isolated from the acute otitis media samples was performed by disk diffusion method (DDM) on Muller-Hinton agar plates as described by the National Committee for Clinical Laboratory Standards(15). 0.1 ml of the bacterial isolate was seeded into each of the Petridishes containing Mueller-Hinton agar and were allowed to stand for 30 min to enable the inoculated organisms to pre-diffuse. The commercially available discs containing the following antibiotics**:** Ampicillin (AMP)10 µg, Penicillin (PEN) 10 µg, Amoxicillin (AMX) 10µg, Erythromycin (ERY) 15 µg, Tetracycline (TET) 30µg and Chloramphenicol (CLH) 30 µg were aseptically placed at reasonable equidistance on the surfaces of the Muller-Hinton agar plates with a sterile forceps and were incubated for 18 - 24hrs at 37°C.Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimeters (16).

Results

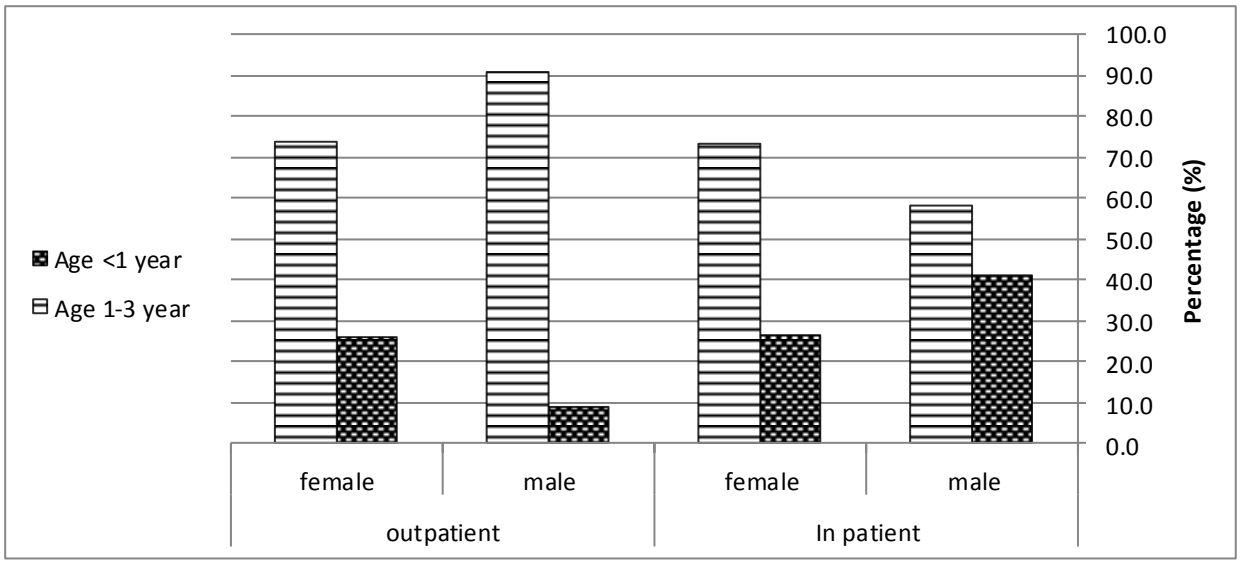
In the presented study, the patients cases in which the samples were collected distributed according to three directions;

First direction based on age and gender; equal number of cases were taken from each gender (males and females) as 50 cases for each one, but the distribution differ according to the age, generally the cases were taken from two group, first less than 1 year (<1year) and second was between 1 to 3years (1-3years). The occurrence of the infection was in high incidence in age between 1-3 years (80% males) and (76% females) than that at <1year(20% males) and (24% females) ($P \geq 0.05$) as shown in figure(I).



Fig(I): distribution of patients according to the Age and the Gender.

Second direction based on the visiting of patients to the hospital, as inpatients and outpatients, the cases of AOM in outpatients were higher (90.9% males) and (73.9%females) in age between 1-3 years than the inpatients (58.2% males) and (73.3% females) at the same age, while the results in age less than 1year were reversed, that mean the percentage of inpatients (41.2% males) and (26.7% females) was highest than the outpatients(9.1% males) and (26.1% females) as shown in figure (II).



Fig(II): distribution of cases according to the visiting to the hospital.

Third direction demonstrated that the occurrence of AOM in collected samples if it primary or secondary infection. The incidence of the AOM in collected cases was higher as the secondary infection in age of <1years (24.2% males)and (28% females) than the primary infection (11.8% males) and (20% females). In age at 1-3years the percentage of incidence was increased as primary infection (88.2% males) and (80% females) than the secondary infection (75.8% males) and (72% females), the results showed in figure (III).

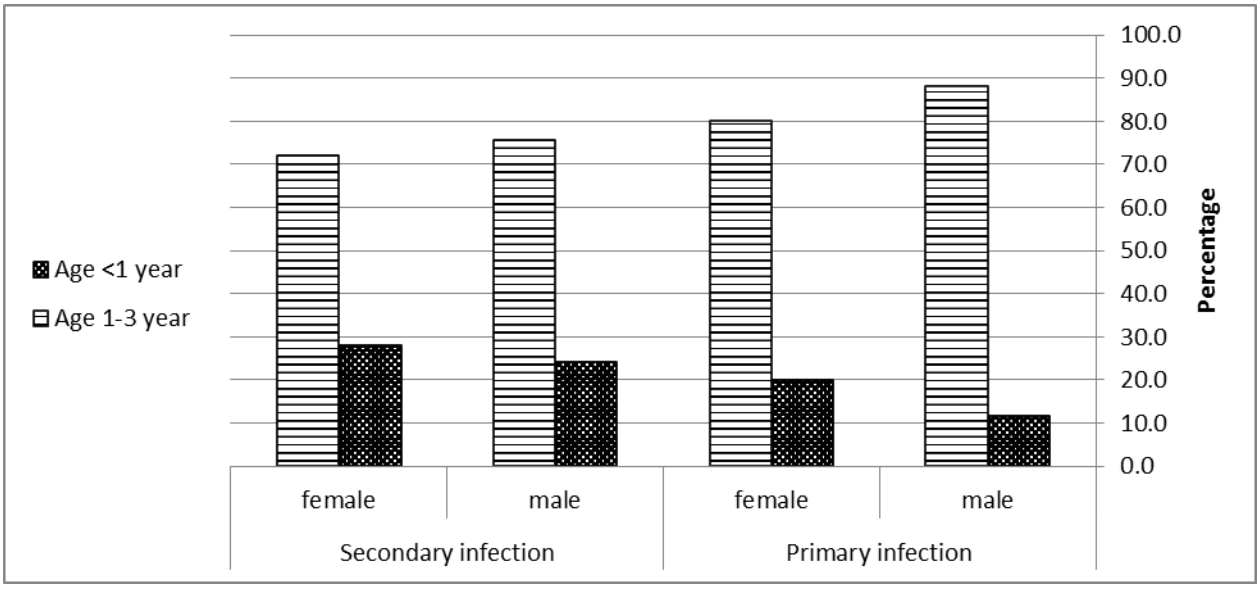
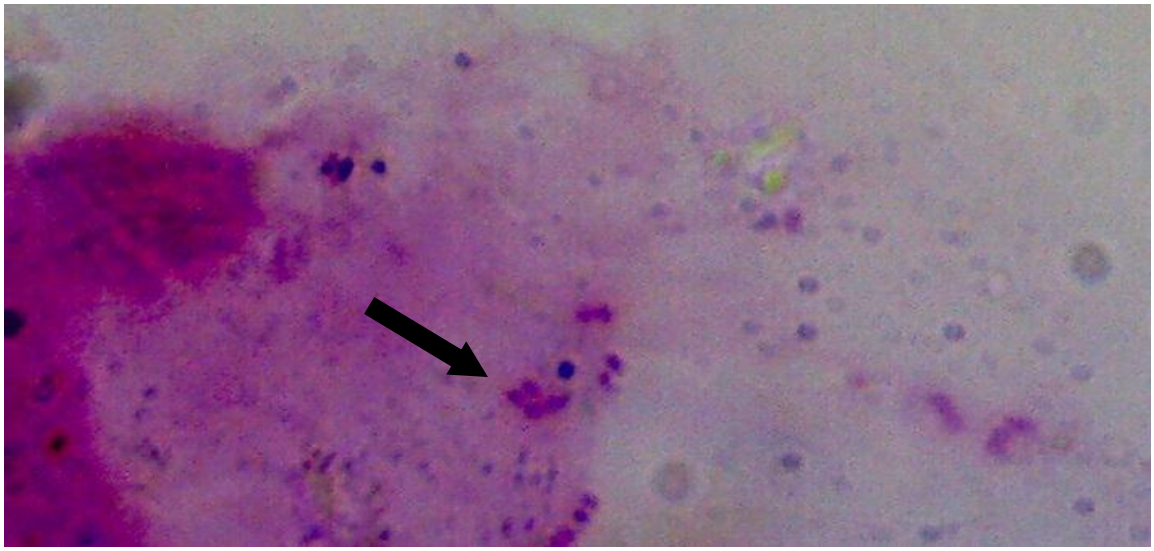


Fig (III): tested cases according to the occurrence of AOM

Isolation and growth of bacteria

From the 100 samples which collected, 8 isolates of *M. catarrhalis* were collected after the culture methods and using of biochemical tests.



Fig(IV): appearance of *M. catarrhalis* by gram stain, the bacteria is gram negative diploccoci

After culture of collected samples, biochemical tests were done to diagnose and isolate the pathogenic bacteria ,the results were established in table (I)

Table(I): results of testing the pathogenic isolates by biochemical tests .

Pathogenic Isolates	Gram Reaction	Catalase	Citrate	Indole	Methyl Red	Vogas Prokauer	Motility	DNase production
M. catarrhalis	-	+	-	-	-	-	-	+
S. pneumonia	+	-	-	-	-	-	-	-
H. influenza	+	+	-	-	-	-	-	-
S. aureus	+	+	-	-	-	-	-	+
S. pyogenes	+	-	-	-	-	-	-	-
E. coli	-	-	-	+	+	-	+	+
K. pneumoniae	-	-	+	-	-	+	-	+

The pathogenic isolates from the collected samples were contained gram positive and gram negative bacteria, results as shown in table (II).

Table(II): Result of pathogenic bacterial isolates from the collected samples

Pathogen	No. (100)		
	Male(50)	Female(50)	Total(100)
Moraxella catar	3 (6%)	5 (10%)	8%
Streptococcus pneun	10(20%)	11(22%)	21%
Haemophilus influ	12(24%)	15(30%)	27%
Staphylococcus a	7(14%)	9(18%)	16%
Streptococcus pyog	2(4%)	4(8%)	6%
Escherichi	1(2%)	4(8%)	5%
Klebsiella pneum	2(4%)	2(4%)	4%
Total	37(42.5%)	50(57.5%)	87

Sensitivity test

The sensitivity test results of the *M. catarrhalis* were fixed in table(III).

Table (III) Antibiotic Sensitivity Pattern of *M. catarrhalis* Isolates

Antibiotic / Isolates	AMP.			PEN.			AMX.			ERY.			TET.			CHL.		
	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I
I	+	-	-	+	-	-	+	-	-	-	+	-	-	+	-	+	-	-
II	+	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	+	-
III	+	-	-	+	-	-	+	-	-	-	-	+	-	+	-	-	+	-
IV	+	-	-	+	-	-	+	-	-	-	-	+	-	+	-	-	+	-
V	+	-	-	+	-	-	+	-	-	-	-	+	-	+	-	-	+	-
VI	+	-	-	+	-	-	+	-	-	-	-	+	-	+	-	-	+	-
VII	+	-	-	-	+	-	+	-	-	-	-	+	-	+	-	-	-	+
VIII	-	-	+	-	-	+	-	+	-	-	-	+	-	-	+	-	-	+
Total	7	0	1	6	1	1	7	1	0	0	2	6	0	7	1	1	5	2

***R (Resistance): therapy will not be effective when administrated.**

****S (Susceptible): effective therapy when administrated.**

*****I (Intermediate): therapy may fail at normal concentration but will be effective at high concentration**

Discussion

Isolation of the bacteria

The results of the isolation was 8 isolates of *M. catarrhalis* from 100 samples collected from the patients($P \leq 0.05$), this result was agreed with Yamanaka*et., al*(2008), who established that percentage of M. catarrhalis is 10-15% of the bacterial causing agents which considered according to Gok *et,.al* the main cause of 22–52% of OME.

Biochemical tests results

Identification of *M. catarrhalis* in our study done by many measures, one of it one by undergo the collected samples to series of biochemical tests. Results of the biochemical tests were agree with Peiris et., al (1993) (17), who reported the biochemical activities of *M. catarrhalis* specially the production DNase enzyme that differentiate it from *Streptococcus pneumonia* and *Haemophilus influenza* which considered the causes of AOM. Results of Sensitivity tests *M.catarrhalis* strains seemed to be resistant to ampicillin, penicillin and amoxicillin, which makes it inappropriate choices of antibiotic against *M. catarrhalis*. These results similar to those of Woodhead (2011) (18), who suggested that the resistance of *M. catarrhalis* to the above antibiotics because of the β-lactamase production, which is resistant to ampicillin, penicillin and amoxicillin. In the other hand, the susceptibility of the isolates to erythromycin, tetracycline and chloramphenicol resemble to results of Felmingham, and Gruneberg (2000) (19), who established that all strains of *M. catarrhalis* were susceptible to erythromycin, tetracycline and chloramphenicol.

Conclusion

Acute otitis media considered the most common bacterial infection treated with antibiotics in children. The scientific researches fixed that *M. catarrhalis* is the second or third most common pathogen to cause acute otitis media together with *Streptococcus pneumoniae* and non typable *H. influenza* (20). For the correct microbiologic diagnosis of bacterial AOM, tympanocentesis and culture of middle ear fluid is required. Tympanocentesis is not routinely performed and the rate of *M. catarrhalis* AOM may thus be underrated. Compared to *S. pneumoniae* and *H. influenzae*, *M. catarrhalis* causes a relatively mild course of AOM (21). *S. pneumoniae* AOM are clinically more severe than those caused by *H. influenzae* and

M. catarrhalis and are more often associated with high fever, tympanic membrane bulging and redness and severe otalgia (22). A lower spontaneous tympanic membrane perforation rate and no case of mastoiditis in children younger than 5 years of age with *M. catarrhalis* AOM was observed (23).

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