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Occurrence of *Moraxella catarrhalis* isolated from respiratory tract Infection

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Abstract

During the period from November 2011 to February 2012, 96 (69.1 %) isolates of *M. catarrhalis* were isolated from 139 outpatients of both sex (85 male and 54 female) with respiratory tract infection (either Tonsilities , Otitis media , Sinusitis ,or Pneumonia) admitted to or presenting at two hospitals in Al-Najaf governorate. The*M.catarrhalis* appeared to be the most frequent microorganism isolated in this study, which had percentage 75.6% (96) , followed by *Streptococcus pneumonia*, *Staphylococcusaureus*,and *Hemophilusinfluenza*e in 15.7 % (20) , 6.3% (8) ,and 2.4% (3),respectively .*M.catarrhalis* isolates had high frequency of isolate in throat swabs than other samples .In this study , only 14(14.6%) isolates of *M. catarrhalis* were produced sidrophores by growing on M9 medium .In addition, there were 72 (75%) isolates of *M.catarrhalis* appeared to adhere with the epithelial cells and all isolates show resistance to complement.The phenotypic resistance of 40 *Moraxella catarrhalis* isolates to 11 commonly used antimicrobial agents by using Kirby-Bauer disk diffusion method. All isolate of *M. catarrhalis*were appeared to show highest rate of resistance(100%) to Penicillin and Ampicillin .Similarly, the isolate exhibited high rate of resistance to Amoxicillin (95%) and Cefotaxime(72.5%) and mild resistance to Ciprofloxacinand Cephalothin in 62.5% for each, Cloramphinicol (57.5%), 52.5% of isolate showed resistance to Gentamicin and Trimethoprim .Whereas no one of isolates show resistance to (Ampicillin +Cloxacillin) and Tetracyclin. In the present study 15 *M. catarahalis* isolates show MIC ofPenicillin at 512 µg/ml(ie.had highest concentration MIC) while 4 isolate show MIC of Ampicillin at 512 µg/ml.Phenotypic assay was performed to determine the presence of β-lactamase enzyme by using nitrocefin disk . while in genotypic β-lactamase assay, the*bro*-1 gene found in 25 (62.1%) isolates, while*bro*-2 gene was presented only in 3 (7.5%) isolates.

Introduction

*Moraxella catarrhalis*is a human-restricted, encapsulated, gram-negative mucosal pathogen. Further, though previously thought to be a commensal of the upper respiratory tract, the bacterium is now increasingly recognized as a true pathogen of both the upper respiratory tract and the lower respiratory tract of humans. It is the third most common bacterial cause of childhood otitis media (OM) after *Haemophilusinfluenzae*and *Streptococcus pneumoniae*, and it is responsible for up to 20% of cases (Hays,2006 ;Vergison,2008). Next to *H. influenzae*, *M. catarrhalis*is the second most common cause of exacerbations of chronic obstructive pulmonary disease (COPD), estimated to be responsiblefor 10 to 15% of these exacerbations, which accounts for 2 to 4 million episodes in the United States per year (Vries*et al*.,2009).

Rates of *M. catarrhalis*carriage in children and adults differ considerably. About two-thirds of all children are colonized within the first year of life, and it is expected that about half of these children will experience at least one period of OM during this year . In contrast, the rate of carriage in healthy adults is much lower (Fung *et al*.,1992).

*M. catarrhalis*exhibits an almost universal resistance to penicillin related antibiotics, with several studies indicating that world-wide, 90-100% of *M. catarrhalis*isolates produce ß-lactamase (Abe*et al.*,2002). This is astriking statistic when one considers that before 1970 few isolates produced ß-lactamaseenzymes (Catlin,1990Research into *M. catarrhalis*ß-lactamase production has shown that 3 different isotype groups may beidentified, BRO-1, BRO-2 and BRO-3 (Christensen,*et al.*,2010).

*M. catarrhalis*also appears to be able to invade host epithelial cells (Jordan*et al*.,2010), the intracellular survival of pathogens being an important aspect of host immune evasion (Bootsma*et al*.,2000). Moreover, once attached to the host mucosal surfaces, *M. catarrhalis*has the ability to interact and/or compete with the commensal flora and has the apparatus to survive and multiply under challenging nutrient-limiting conditions.Such conditions may result in the formation of microcolonies and biofilms (Christensen *et al*.,2010). Finally, *M. catarrhalis*has the ability to evade and survive host immune responses, a process particularly helped by its ability to withstand the effects of human serum(Bootsma*et al*.,2000). The present study is carried out to achieve the isolation and identification of M.catarrhalis from patient with RTI.

Materials and Methods

Patients and sample collection

This study was carried out in two hospital in in Najaf governorate (Al-Hakeem and Al-Zahra Maternity and Children) .during the period between November 2011 to January 2012.A total of 139 sample (67 throat swabs , 32ear swab ,30 sputum ,10 nose swabs) were collected from out patients suffering from upper respiratory tract infection (pharyngitis ,otitis media ,pneumonia , sinusitis respectively),they included both sex (85 male and 54 female),with different age groups.

Isolation and Identification of bacterial isolates

All samples were cultured on blood agar(Himedia), chocolate agar(Himedia) ,nutrient agar(Himedia), brain heart agar media(Himedia) using standard loop method. The media were incubated in candle jar with Co_2 at 37°C for 24-48hour depending on morphological features of the colonies and microscopically examination with Gram's stain, pure culture on chocolate agar plates were made from each single group of colonies (Gram-negative diplococci with a typical colony appearance). The pure cultures were prepared for biochemical tests to differentiate *M.catarrhalis* from other bacteria.

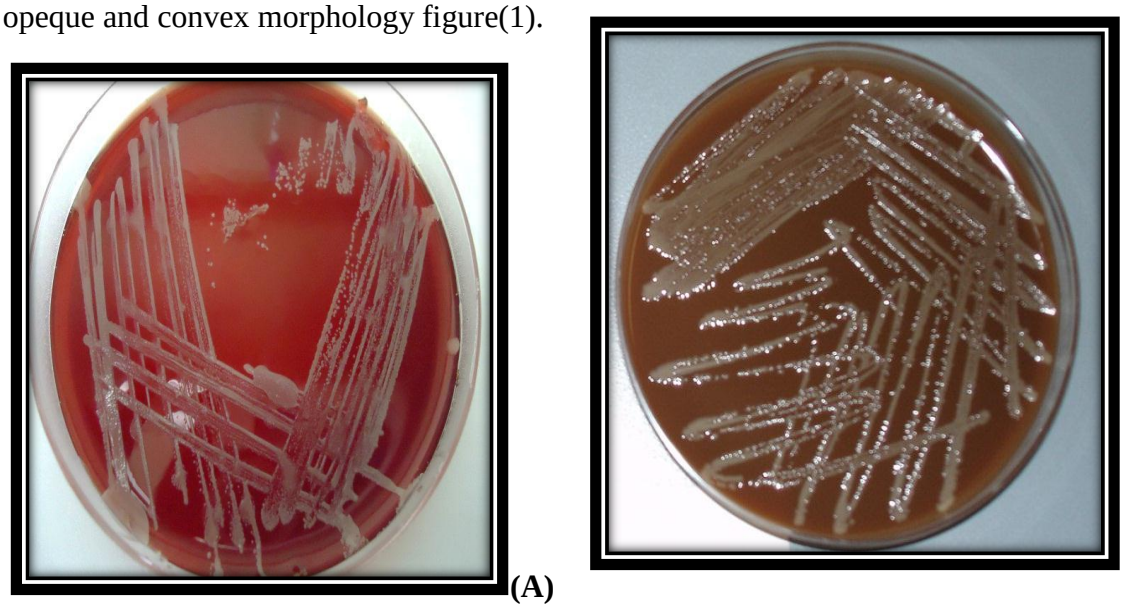
Microscopic properties: Gram's stain was used to examine the isolated bacteria.Biochemical characterization were doing according to (MaccFADIN,2000).Morover ,the biochemical tests with APINH miniaturized diagnostic test were confirmed that all these isolate as *M.catarrhalis* .

Sidrophore detection was performed on M9 media which is prepared as suggested by (Nassif *et al*.,1989). The media was inoculated with single colonies of overnight culture by streaking method incubated for 24hr at 37C°. The appearance of the growth of *M.catarrhalis* on M9 mediaindicated a positive result.Adherence to human oropharyngeal cells test ws doing according to Lomberg *et al.*,(1986). The culture-and spot test (Verduin *et al*.,1995) was used to detection complement resistance of*M.catarrhalis* isolates. All *M .catarrhalis* isolates performed identification to susceptibility testing by modified Kirby-Bauer disk-diffusion method (Bauer *et al*., 1966). The selection of antibiotic was performed according to the guidelines recommended by the Clinical and Laboratory Standard Institute (CLSI, 2011).

Results and Disscusion

Ninty six(69.1 %) isolated of *M. catarrhalis*were isolated from atotal of 139 out patients of both sex (85 male and 54 femal) with RTI . Out of the bacterial isolates of

RTI sampels , the remaining 43 (30.9%) were presented as other bacterial types. All these culture sterile isolates were identified on the basis of Microscopic examination ,colonied morphology and comparison of the biochemical characteristics with standared description in Maccfaddin (2000) and Mims *et al*.,(2008).In microscopic examination (Gram film),the organism was appeared as Gram nagative diplococcus with flattend sides.Colonies of these isolate on blood agar and chocolate agar presented in large , grey , smooth , opeque and convex morphology figure(1).



(A) **(B)**

Figure (1)*M.catarrhalis* on blood agar(A),chocolate agar(B).

The identication of *M.catarrhalis* to the species level involved biochemical testing with production of Oxidase, Catalase ,lack of acid production from the glucose, fructose,lactose, sucrose,and manosereduction of nitrate and non motile .Morover,the biochemical tests withAPINH miniaturized dignostic test were confirmed that all these isolate as *M.catarrhalis* (figure 2)

Figure (2):The Identification Results of*M.catarrhalis* isolateby API NH.

Figure (3) show the incidance of *M.catarrhalis*among other etiological agents isolated from patients with RTI in Najaf governorate, from wich *M.catarrhalis* was appeared the most frequent microorganism isolated in this study, which had frequency of 75.6% (96) , followed by *S. pnumoniae* with 15.7 % (20) ,*Staphylococcus aureus* with 6.3% (8) ,and *Hemophilus influenzae*with 2.4% (3).

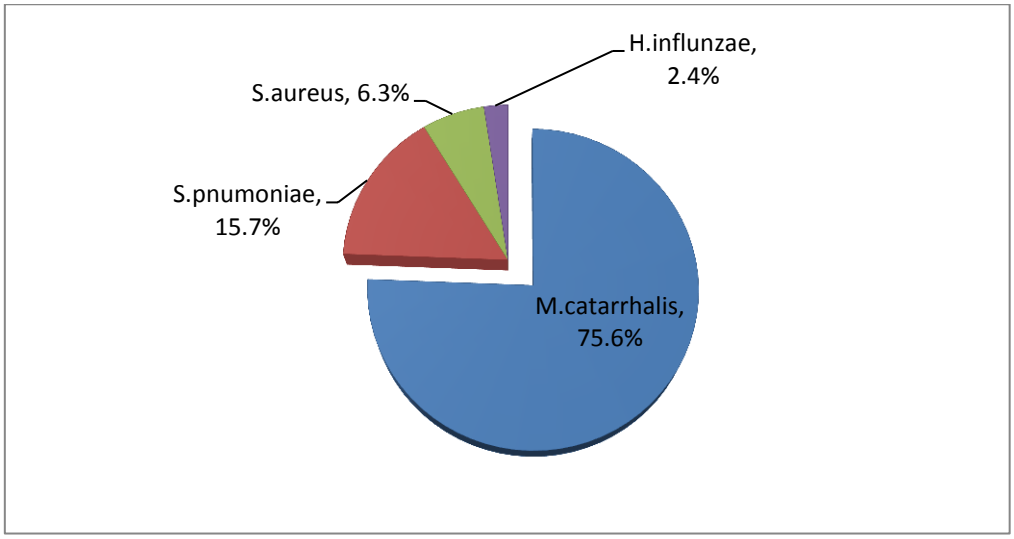


Figure (3) Occurence of *M.ctarrhalis* among other etiological agent in RTI .

M.catarrhalis was found to be the most common isolated respiratory pathogen .It was frequently isolated in pure culture or in combination with *H.influanzae*or *S.pnumonia*e and the seasonal recovary of *M.catarrhalis* was apparent for the November to May period, compared with June to October period (Mc Gillivary *et al*.,2009).

Brockson *et al*. (2012) reported that the three most common bacterial causative agents of OM are *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae* (NTHI) and *Moraxella catarrhalis*, all of which are normal commensal species of the pediatric nasopharynex ,and demonstrated that co-infection with RSV and NTHI predisposed to *M. catarrhalis*-induced ascending experimental OM.Since, Kennedy *et al*.,(2000) showed that In children, viral infection predisposes to bacterial OM by facilitating ascension of select members of the colonizing NP flora into the middle ear space.

The high incidence of *M.catarrhalis* isolated in this study from patient with RTI amonge other bacterial isolates may be atribute to many reasons, **firstly** ,*M.catarrhalis* are the most predomenant pathogen in Nasopherynex of chiled and represent the common case in this study.. Since, there is astrong relationship between age and colonization rates of *M.catarrhalis* (Brockson *et al*.,2012).**Secondly** ,the increase resistance of *M.catarrhalis* to antibiotic especially β-lactam antibiotic that predomenant use in the cure RTI by increase β-lactames enzyme production which my be due to increase predomenant of this bacteria in hospital . and **thirdly**, the average length of stay in hospital is considerably longer for children colonized with *M. catarrhalis* compared to those not colonized and providing evidence that re-colonization with different *M. catarrhalis* types occurs.

Stenfors and Raisanen (2003) isolated *M.catarrhalis* in 16% from exudate of midle ear of patient with CSOM in Finland. Ad-Dahhan (2007) isolated *M.catarrhalis* in 17.4% from patient with URTI in Al-Najaf govenrate. Al-Tememy(2004) found *M.catarrhalis* presented in 6.5% in patients with CSOM in Babyelon city, while Al-Turphy (2000) reffered that *M.catarrhalis* was isolated in 3.6% in patients with OM in Karbala city.In this study *M.catarrhalis* was isolated in 75.6% .

Occurencev of *M. catarrhalis* in clinical specimens:

Table (1) reveals that the high frequent of *M.catarrhalis* isolates was in throat swabs which had afreqency 58.3% , while the percent of isolates in specimens taken from ear, sputum and nose swabs presented in 31.3% , 17.7% and 3.3%, respictively .

Prevelance of*M.catarrhalis* from all specimens in this stady was 69.1% and this result was higher than other previous study(Stenfors and Raisanen,1990,Martinez *et al*.,1999, Ad-Dahhan,2007).

Table (1)Prevalence of *M. catarrhalis* in clinical specimens

Type of specimens	No.of specimens	*M.catarrhalis* isolates	
		No.of isolates	Percentage %
Throat swab	67	56	58.3
Ear swab	32	30	31.3
Sputum	30	17	17.7
Nose swab	10	3	3.3
Total	139	96	69.1

M. catarrhalis may be the single cause of sinusitis, otitis media, tracheitis,bronchitis, pneumonia, and, less commonly, ocular infections in children. where, nasopharyngeal colonization often precedes the development of *M. catarrhalis*-mediated disease(Broides *et al*.,2009). It has been suggested that there is a possible underestimation of isolation rates for *M. catarrhalis*, since the bacterium stops growing in environments with reduced oxygen concentrations, a condition frequently present during sinusitis and otitis media. This would indicate an even greater role for *M. catarrhalis* in the etiology of these infectious diseases(Verduin *et al*.2002).

Several studies reveals that *M. catarrhalis* is the commonest bacteria isolated from specific clinical case.AL-Mazory(2002) isolated *M. catarrhalis* in 13.8% from patients withCOPD as a commonest pathogens,while Boyle *et al*.,(1991) reported that *M. catarrhalis* represent 26% from all sputum of patient with pneumonia.In this study the high percentage(58.3%) of isolates occure in throat swabs .In conterast with Ad-Dahhan(2007) who isolated bacteria in 17.4% from all cases.

Distribution of *M.catarrhalis* acording to the age and sex:

In this study the patients ages categorized into six groups (Table 2). The higher incidence of *M.catarrhalis* isolates were recorded at first age group (1-10)years with 27.1% (26) , followed by the last age group (50- ≥ 60) years and the second age group (11-20) years in 21.9% (21) and 19.8%(19),respectively . The low frequency of isolation was recorded at age group (31-40) years followed by age groups (21-30) years and (41-50) years in apercent 7.3% , 9.4% ,and 14.6% , respectivly .

Table (2) Distribution of *M.ctarrhalis* according to the age

Age (year)	No.of patient	*M.catarhalis isolates*	
		No.	Percentage%
1-10	30	26	27
11-20	26	19	19.8
21-30	18	9	9.4
31-40	17	7	7.3
41-50	20	14	14.6
50-≥ 60	28	21	21.9
Total	139	96	100

In the present study the first age group (1-10) was the most dominance in infection (27%)compared with the age group (31-40) wich recorded lowest percentage of infection (7.3%) .This diffrence may be due to low immunity in this age group(children) .and my be the children during the childhood are most exposed to infection during playing with infected material.Further, the extensive persence of *M.catarrhalis* in the age group (50-≥60)years may be attributed to impaired in immunity system and most elderly are infected with chronic diseases.The result of the currentstudy agrees with somestaudeis (Ad-Dahhan 2007;Vries*et al*.2009) who stated that *M. catarrhalis* was the commonest pathogen in children less than 10 years .

Sidrophores production:

In this study , only 14(14.6%) isolates of *M. catarrhalis* were produced sidrophores by growing on M9 medium containing 200µmol dipyridyl(Fig.4).This result differ from that of Al-Tememy(2004) when she referred that all isolates *of M. catarrhalis* lackes its ability to sidrophore production in her study.

M .catarrhalis expresses specific OMPs in response to iron-limited growth in vitro (Vries *et al*.,2009). Yu and Schryvers(1993)observed *M.catarrhalis* uses iron-saturated human transferrin or lactoferrin as the sole source of iron for growth in the absence of siderophores. These observations suggest that *M. catarrhalis* competes for iron bound to human transferrin and human lactoferrin in a manner similar to that used by *Neisseria* species. *M. catarrhalis* transferrin receptors show a strong preference for iron-saturated transferrin over apotransferrin, and in this regard they differ from *Neisseria* receptors. Labout *et al*.(2011) referred that two of the iron-repressible proteins are OMPs B1 and B2 .

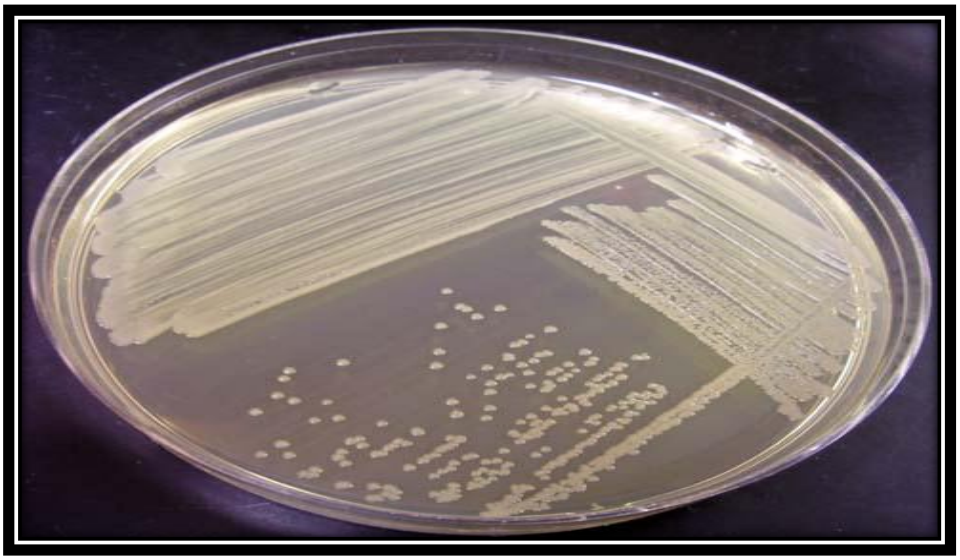


Figure (4)*M.catarrhalis* on M9 medium

Adherance to epithelial cell:

In this study, there were 72 (75%) of *M.catarrhalis* isolate show it ability to adher with epithelial cell.

Attachment to the epithelium of respiratory tract is likely to be an essential step in the pathogenesis of *M. catarrhalis* infection. The general mechanism of cellular adherence of *M. catarrhalis* to host cell surfaces has been studied previously by Rikitomi *et al*. (1991). Hemagglutination in gram-negative bacteria is often associated with expression of pili or nonpilus adhesive proteins that promote attachment to and colonization of host mucosal surfaces. Several studies(Ahmed *et al*.,1992 ;Balder *et al*.,2009 ;and Labout *et al*.,2011) have confirmed that nonfimbriated structure of *M. catarrhalis* are less adheret and therefore can escape phagocytosis but are more invasive, and there was no significant correlation between adherence and the number of fimbriae. Another study found no differences between the source of the isolate (blood or lungs) and hemagglutination (Luke *et al*.,2007).

Complement Resistance:

The complement resistant or sensitive phenotype of the 96 *M. catarrhalis* isolates used in this study had been previously determined using the "culture-and-spot"test by Verduin *et al*.,(1994). This is a rapid and simple test for determining the complement resistance phenotype of *M. catarrhalis,* which exhibits a statistically significant concordance with the serum bactericidal assay and is base on the survival of bacteria on a blood agar plate after the application of a drop of 50% serum.

The pathogenesis of *M. catarrhalis* relies on its capacity to resist the human host defense, including complement . The complement system is very harmful for Gram-negative pathogens, including *M. catarrhalis*, and bacterial complement resistance is one of the most important virulence mechanisms (Nordstrom *et al*.,2005). *M. catarrhalis* has thus developed several efficient strategies to circumvent complement. It has been demonstrated that UspA1 and UspA2 interact and inhibit the alternative pathway of complement by noncovalently binding C3 (Hallstrom*et al*.,2008).

In this study ,all isolates 100%(96/96) were found to be resistant to the effect of complement in human serum. The percentage of complement resistant *M.catarrhalis* isolated appears to be relatively high when compared to some studies (Hol *et al*.,1995)involving healthy children (100% versus 30-60% , Other studies have yielded similar results (Hays, 2003).

Resistance to Antimicrobial Agent:

Table (3) show the phenotypic resistance of 40 *Moraxella catarrhalis* isolates to 11commonly used antimicrobial agents by using Kirby-Bauer disk diffusion method (Bauer *et al*.,1966) .

All isolate exhibited resistance to penicillin and Ampicillin in 100%.vast majority of isolate exhibited high rate of resistance to Amoxicillin 95% and Cefotaxime 72.5% and mild resistance to Ciprofloxacin and Cephalothin in 62.5% for each, to Cloramphinicol in 57.5%, 52.5% of isolate showed resistance to Gentamicin and Trimethoprim at ealse. Whereas all isolates were susceptible to These two antibiotics were the most potent and affective antibiotics against isolate in this study.

Table (4-5) Antibiotic resistance of*Moraxella catarrhalis* isolate to Antibiotic

Type of Antibiotic	symbol	Resistant isolates	
		No. of isolate	Persentage
Ampicillin	AM	40	100
Amoxicillin	AX	38	95
(Ampicillin+cloxacillin)	APC	0	0
Cefotaxime	CTX	29	72.5
Cephalothin	KF	25	62.5
Chloramphenicol	C	23	57.5
Ciprofloxacin	CIP	25	62.5
Gentamycine	GM	21	52.5
Piillincillin	P	40	100
Trimethoprime	TMP	21	52.5
Tetracycline	TE	0	0

In the present study MICs of Pinicillin and Ampicillin for 40 *M.catarrhalis* isolates doing according to CLSI(2011) as showed in the table(4) , in wich 15 *M. catarahalis* isolate show MIC of Pinicillin at 512 µg/ml(i.e.had highest concentration MIC) while only 4 isolate show MIC of Ampicillin at 512 µg/ml.

M catarrhalis isolates that produced BRO-1 enzyme gave the highest MICs (512 µg /ml) to pencillin (15) and Ampicillin (4) than that the isolates produsedBRO-2 enzyme as shown in table (5)

Table (4) MIC of pencillin and Ampicillin for 40 *M. catarrhalis* isolates.

Antimicrobail agent	No. of isolate inhabited at MIC (µg/ml) < 4 8 16 32 64 128 256 512 (Total)
Penicillin	8 1 2 3 2 3 6 15 (40)
Ampicillin	7 5 3 3 6 8 2 4 (38)

Note: 38 are the total number of Ampicillin resistance strain

Table (5) Distribution of Penicillin and Ampicillin MICs among BRO1 and BRO2 producing *M . catarrhalis* isolates .

Antimicrobial agent	**Enzyme type No.of isolates**	**Cumulative No. isolates inhabited at MICs (µg/ml)**
		< 4 8 16 32 64 128 256 512
Penicillin	BRO1 (25)	**0 0 0 0 1 3 6 15**
	BRO2 (3)	**0 0 2 1 0 0 0 0**
Ampicillin	BRO1 (25)	**3 2 0 0 6 8 2 4**
	BRO2 (3)	**0 1 1 1 0 0 0 0**

Schmitz *et al*.(2002) reported that several antimicrobial remained very activeagainst *M.catarrhalis*,including Amoxicillin-clavulanate ((MIC, <0.25 µg/ml), azithromycin (MIC, <0.12 µg/ml), ceftriaxone (MIC, 0.5 µg/ml), and levofloxacin (MIC, <0.03 to 0.06 µg/ml).Isolate producing the Bro-1 enzyme have been shown to be consistently more resistant to ampicillin than strains of the BRO-2 type (Verduin*et al*.,2002). This difference in MICs correlates well with the observation that BRO-1 is produced at a level two to three times higherthan that of BRO-2 (Wallace *et al.*,1990).
In conclusion M.catarrhalis

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