R E S U L T S

* From the outpatient clinic of the E.N.T. department in Benha Faculty of Medicine, three hundred children (0-12 years) of varying age and sex, with a diagnosis of acute otitis media, were chosen to be the subject of this study. Patients who received antibiotics within the previous week of their visit or who had had an episode of otitis media during the previous month, were excluded from the study.

* Bacteriological study of the middle ear fluid of these children, searching for B. catarrhalis, was done. Also serological examination for antibodies (agglutinins) to B. catarrhalis was done both in the acute and convalescent stage of the disease. The results of the serological study were correlated with the results of isolation of B. catarrhalis as an evidence for its pathogenic role in such cases. Furthermore, all the isolated strains of B. catarrhalis were tested for beta-lactamase production and for their in vitro susceptibility to selected antimicrobial agents using both the disc diffusion method and broth tube dilution method.

TABLE 4.1 shows age and sex of all the 300 children with AOM in the study.

- The ages of the 300 children included in this study varied from 3 months to 12 years:
    * 41 children (13.7%) were below one year of age.
    * 87 children (29%) were between the age of one and two years.
    * 39 children (13%) were between the age of two and three years.
    * 36 children (12%) were between the age of three and four years.
    * 34 children (11.3%) were between the age of four and five years.
    * 15 children (5%) were between the age of five and six years.
    * 18 children (6%) were between the age of six and seven years.
    * 12 children (4%) were between the age of seven and eight years.
    * 18 children (6%) were between the age of eight and twelve years.
- As regards sex, 162 of children (54%) were males while 138 were females (46%).

Table 4.1:

Age and sex of all 300 children with acute otitis media in the study.

AGE			SEX			
Groups	Number of cases	%	Female		Male	
			No.	%	No.	%
0-12 m* (0-1 y*)	41	13.7%	19	46.3%	22	53.6%
13-24 m (1-2 y)	87	29%	42	48.3%	45	51.7%
25-36 m (2²3 y)	39	13%	17	43.6%	22	56.5%
37-48 m (3-4 y)	36	12%	16	44.5%	20	55.5%
49-60 m (4-5 y)	34	11.3%	15	40.1%	19	55.9%
61-72 m (5-6 y)	15	5%	6	40%	9	60%
73-84 m (6-7 y)	18	6%	9	50%	9	50%
85-96 m (7-8 y)	12	4%	6	50%	6	50%
97-144 m (8-12 y)	18	6%	8	44.5%	10	55.5%
TOTAL	300	100%	138	46%	162	54%

* m = month.

* y = years

TABLE 4.2 shows other data for all 300 children with AOM in the study.

- Unilateral AOM was recorded in 246 cases (82%) while a bilateral disease was recorded in 54 cases (18%).
- Of all the cases 75 patients (25%) had one or more previous attack(s) of AOM. The rest of patients had never had AOM before.
- History of a preceding or associated disease(s) was reported in 255 cases (85%) while no history of a preceding or associated disease(s) was reported in 45 cases (15%).
- Perforated ear drum was recorded in 279 cases (93%), while myringotomy was done for 21 patients (7%) only.
- Mild otalgia was reported in 17 cases (5.7%), moderate otalgia was reported in 137 cases (45.6%), while severe pain was reported in 146 cases (48.7%).
- Fever $\leqslant$ 38°C was recorded in 147 cases (49%), while fever > 38°C was recorded in 159 cases (51%).
- Positive bacterial culture was recorded in 240 cases (80%), while no growth was recorded in 60 cases (20%).

Table 4.2:

Other data for all 300 children with acute otitis media in the study.

Data	No. of patients	%
* Unilateral AOM*	246	82%
* Bilateral AOM	54	18%
* Previous attack(s) of AOM	75	25%
* History of preceding or associated disease(s):		
. +ve history	255	85%
. -ve history	45	15%
* Ear drum:		
. Perforated	279	93%
. Myringotomy	21	7%
* Otalgia:		
. Mild	17	5.7%
. Moderate	137	45.6%
. Severe	146	48.7%
* Temperature:		
. $\leq$ 38°C	147	49%
. > 38°C	153	51%
* Bacteriological finding:		
. Positive cultures	240	80%
. No growth	60	20%

* AOM : acute otitis media.

TABLE 4.3 and TABLE 4.4 show bacteriological finding in middle ear discharge obtained from 300 children with AOM in the study.

** Streptococcus pyogenes was found in 22% of patients.

** Staphylococcus aureus was found in 17% of patients.

** Streptococcus pneumoniae was found in 13% of patients.

** Haemophilus influenzae was found in 6% of patients.

** Branhamella catarrhalis was found in 6% of patients.

** Staphylococcus epidermidis was found in 4% of patients.

** Diphtheroid was found in 4% of patients.

** Gram negative bacilli were found in 17% of patients:

- Proteus species in 5.7%.
- E. coli in 4.3%.
- Pseudomonas aeruginosa in 4%.
- Klebsiella in 3%.

** No growth was found in 20% of patients.

Table 4.3:

Bcteriological finding in middle ear discharge obtained from 300 children with acute otitis media in the study.

ORGANISM	No.of patients = No.of cultures			% of all patients
	Pure culture	Mixed culture	Total	
. Strept. pyogenes	54	12 (a)*	66	22%
. Staph. aureus	41	10 (b)*	51	17%
. Strept. pneumoniae	30	9 (c)*	39	13%
. Haemophilus influenzae	14	4 (d)*	18	6%
. Branhamella catarrhalis	12	6 (e)*	18	6%
. Staph. epidermidis	8	4	12	4%
. Diphtheroid	3	9	12	4%
. Gram negative bacilli:				
. Proteus	17	-	17	5.7%
. E. coli	13	-	13	4.3%
. Pseudomonas	12	-	12	4%
. Klebsiella	9	-	9	3%
* Total number of positive cultures			240 (f)*	80%(f)*
No growth	-	-	60	20%
* Total in study			300 (f)*	100%(f)*

(a) Strept. pyogenes was found in mixed culture with:

- Staph. aureus in 4 cases.
- Branhamella catarrhalis in 2 cases.
- Staph.epidermidis in 3 cases.
- Diphtheroid in 3 cases.

(b) Staph. aureus was found in mixed culture with:

- Strept. pyogenes in 4 cases.
- Branhamella catarrhalis in 2 cases.
- Staph. epidermidis in one case.
- Diphtheroid in 3 cases.

(c) Strept. pneumoniae was found in mixed culture with:

- Haemophilus influenzae in 4 cases.
- Branhamella catarrhalis in 2 cases.
- Diphtheroid in 3 cases.

(d) Haemophilus influenzae was found in mixed culture with:

- Strep. pneumoniae in 4 cases.

(e) Branhamella catarrhalis was found in mixed culture with:

- Strept. pyogenes in 2 cases.
- Staph. aureus in 2 cases.
- Strept. pneumoniae in 2 cases.

(f) This number is less than the sum of the preceding totals because there were 27 patients with more than one organism in their middle ear fluid cultures.

Table 4.4:
Bacteriological finding in middle ear discharge of 300 children with acute otitis media in relation to age.

ORGANISM	Culture	Age groups									Total	
		0-1 y	1-2 y	2-3 y	3-4 y	4-5 y	5-6 y	6-7 y	7-8 y	8-12 y	No.	%
Strept. pyogenes	Pure Mixed	8 1	14 4	7 3	5 1	5 -	3 2	4 1	3 -	5 -	66	22%
Staph.aureus	Pure Mixed	6 1	10 2	4 1	3 1	4 2	3 1	4 2	3 -	4 -	51	17%
Strept. pneumoniae	Pure Mixed	5 3	11 1	4 1	4 -	3 1	- 2	1 1	1 -	1 -	39	13%
Haemophilus influenzae	Pure Mixed	3 3	4 -	3 -	2 -	2 -	- 1	- -	- -	- -	18	6%
Branhamella catarrhalis	Pure Mixed	2 -	3 1	3 1	3 -	1 2	- 1	- 1	- -	- -	18	6%
Staph.epidermidis	Pure Mixed	2 -	4 1	- 1	2 -	- -	- 1	- 1	- -	- -	12	4%
Diphtheroid	Pure Mixed	1 2	2 3	- 1	- 2	- 1	- -	- -	- -	- -	12	4%
Gram-negative bacilli	Pure Mixed	2 -	15 -	7 -	9 -	8 -	2 -	3 -	2 -	3 -	51	17%
No growth	-	7	18	7	6	8	3	3	3	5	60	20%
Number of the patients		41	87	39	36	34	15	18	12	18	300*	100%*

* This number is less than the sum of the preceding total because there were 27 patients with more than one organism.

I. RESULTS OF ISOLATION OF BRANHAMELLA CATARRHALIS FROM MIDDLE EAR FLUID SAMPLES:

* Eighteen (18) strains of B. catarrhalis were isolated from middle ear fluid samples of 300 children with AOM (6%). Twelve (12) strains were isolated in pure cultures, while 6 strains were isolated in mixed cultures with: Strept. pyogenes, Staph. aureus and Strept. pneumoniae in 2 cases each (**Table 4.3, Table 4.4** and **Table 4.5**).

* All isolated strains of B. catarrhalis were identified according to the criteria of identification stated by **Doern & Morse (1980)**. Plate (1) shows a microscopic film of B. catarrhalis in culture stained by Gram' stain. Plate (2) and (3) show colonies of B. catarrhalis on a plate of a blood agar after 24 hours and 48 hours incubation respectively.

Table 4.5:

Clinical and bacteriological finding of the 18 children with acute otitis media from whom B. catarrhalis was isolated.

No.	Age	Sex	Unilateral OR Bilateral	Previous attack(s)	Preceding or associated disease(s)	Symptoms & Signs				Bacteriological finding	Beta lactamase
						Otalgia	Temp.	Ear Drum	Ear discharge		
1	11 m	M	Unilateral	+ (1)	Chest infection	Moderate	38	Perforated	Mucopurulent-whitish	Pure :B.catarrhalis	+ ve
2	24 m	F	"	+ (2)	Common cold + sinusitis	Moderate	37.9	Perforated	Mucopurulent-whitish	Pure :B.catarrhalis	+ ve
3	83 m	M	"	-	Chest infection	Moderate	38	Perforated	Mucopurulent-whitish	Mixed:B.catarrhalis + S.pneumoniae	- ve
4	36 m	F	"	-	Chest infection + pharyngitis	Moderate	38	Perforated	Mucopurulent-whitish	Pure :B.catarrhalis	+ ve
5	38 m	M	"	-	Common cold	Mild	37.8	Perforated	Mucopurulent-whitish	Pure :B.catarrhalis	- ve
6	49 m	M	"	-	Pharyngitis	Mild	37.6	Perforated	Mucopurulent-yellowish	Mixed:B.catarrhalis + S.aureus	- ve
7	10 m	F	"	-	Common cold + cleft palate	Moderate	37.8	Myringotomy	Mucopurulent-whitish	Pure :B.catarrhalis	+ ve
8	40 m	F	"	+ (1)	Chest infection + pharyngitis	Moderate	38	Perforated	Mucopurulent-whitish	Pure :B.catarrhalis	+ ve
9	36 m	M	"	-	Chest infection	Moderate	38	Perforated	Mucopurulent-yellowish	Mixed:B.catarrhalis + S.pyogenes	- ve
10	30 m	M	"	+ (1)	Common cold + adenoid	Mild	37.6	Perforated	Mucopurulent-whitish	Pure :B.catarrhalis	+ ve
11	56 m	F	"	-	Chest infection	Mild	38	Perforated	Mucopurulent-whitish	Pure :B.catarrhalis	+ ve
12	61 m	M	"	+ (1)	Common cold	Moderate	38	Perforated	Mucopurulent-whitish	Mixed:B.catarrhalis + S.pneumoniae	- ve
13	21 m	M	"	-	Pharyngitis	Mild	37.6	Perforated	Mucopurulent-whitish	Pure :B.catarrhalis	+ ve
14	28 m	F	"	-	Common cold	Mild	37.8	Perforated	Mucopurulent-whitish	Pure :B.catarrhalis	+ ve
15	48 m	M	"	+ (4)	Common cold + adenoid	Moderate	37.8	Myringotomy	Mucopurulent-whitish	Pure :B.catarrhalis	+ ve
16	60 m	F	"	-	Common cold	Mild	37.7	Perforated	Mucopurulent-yellowish	Mixed:B.catarrhalis + S.aureus	- ve
17	19 m	M	"	-	Chest infection	Moderate	38	Perforated	Mucopurulent-whitish	Pure :B.catarrhalis	+ ve
18	21 m	F	"	-	Common cold	Moderate	37.8	Perforated	Mucopurulent-yellowish	Mixed:B.catarrhalis + S.pyogenes	- ve

Plate (1):

Microscopic picture of B. catarrhalis in culture. Gram' stain showing Gram-negative diplococci.

Plate (2):
Colonies of B. catarrhalis on a blood agar plate after 24 hours incubation.

Plate (3):
Colonies of B. catarrhalis on a blood agar plate after 48 hours incubation.

* **TABLE 4.6** shows the frequency of isolation of B. catarrhalis from middle ear fluid of the 18 children with AOM in relation to age.

  The majority of B. catarrhalis strains were isolated from children below 5 years old. In older children, AOM with B. catarrhalis seemed to be rarer.

* **TABLE 4.7** shows the frequency of isolation of B. catarrhalis from middle ear fluid of the 18 children with AOM in relation to sex.

  Eight strains were isolated from female patients (44.4%), while 10 strains were isolated from male patients (55.6%).

* **TABLE 4.8** shows other data for the 18 children with AOM from whom B. catarrhalis was isolated:

  - All cases (100%) were unilaterally affected.
  - Of all the cases 6 patients (33.3%) had one or more previous attack(s) of AOM. The rest of patients had never had AOM before.
  - History of a preceding or associated disease(s) was reported in 100% of cases. The incidence of such disease(s) was illustrated in **Table 4.9**.
  - Perforated ear drum was recorded in 16 patients (88.9%), while myringotomy was done for 2 patients

(11.1%). One of them had a cleft palate and a common cold attack, while the other had adenoid and also a common cold attack with a history of four previous attacks of AOM (**Table 4.5**).

- Ear discharge was mucopurulent and whitish in 14 cases (77.8%). In the rest 4 cases (22.2%), the discharge was mucopurulent and yellowish. In such 4 cases, B. catarrhalis was isolated in mixed culture with strept. pyogenes in 2 cases and staph. aureus in the other 2 cases (**Table 4.5**).
- Mild otalgia was reported in 7 cases (38.9%), moderate otalgia was reported in 11 cases (61.1%), while severe otalgia was not reported in any case.
- Fever $\leqslant$ 38°C was recorded in all the 18 cases (100%). None of the patients had fever above 38°C.
- B. catarrhalis was isolated in pure culture in 12 patients (66.7%), while it was isolated in mixed culture in 6 patients (33.3%).

Table 4.6:

Isolation of Branhamella catarrhalis from middle ear fluid of the 18 children with acute otitis media in relation to age.

Age (in years)	No. of B. catarrhalis isolates			
	Pure	Mixed	Total	%
0-1 y	2	-	2	11%
1-2 y	3	1	4	22.2%
2-3 y	3	1	4	22.2%
3-4 y	3	-	3	16.7%
4-5 y	1	2	3	16.7%
5-6 y	-	1	1	5.6%
6-7 y	-	1	1	5.6%
7-8 y	-	-	-	-
8-12 y	-	-	-	-
TOTAL	12	6	18	100%

Table 4.7:

Isolation of Branhamella catarrhalis from middle ear fluid of the 18 children with acute otitis media in relation to sex.

Age (in years)	No. of B. catarrhalis isolates	In Female		In Male	
		No.	%	No.	%
0-1 y	2	1	50%	1	50%
1-2 y	4	2	50%	2	50%
2-3 y	4	2	50%	2	50%
3-4 y	3	1	33.3%	2	66.7%
4-5 y	3	2	66.7%	1	33.3%
5-6 y	1	-	-	1	100%
6-7 y	1	-	-	1	100%
7-8 y	-	-	-	-	-
8-12 y	-	-	-	-	-
TOTAL	18	8	44.4%	10	55.6%

Table 4.8:

Other data for the 18 children with acute otitis media from whom Branhamella catarrhalis was isolated.

Data	No. of patients	%
* Unilateral AOM	18	100%
* Bilateral AOM	-	0%
* Previous attack(s)	6	33.3%
* History of preceding or associated disease(s):		
. +ve history	18	100%
. -ve history	-	0%
* Ear drum:		
. Perforated	16	88.9%
. Myringotomy	2	11.1%
* Ear discharge:		
. Mucopurulent-whitish	14	77.8%
. Mucopurulent-yellowish	4	22.2%
* Otalgia:		
. Mild	7	38.9%
. Moderate	11	61.1%
. Severe	-	0%
* Temperature:		
. < 38°C	18	100%
. > 38°C	-	0%
* Bacteriological finding:		
. Pure culture	12	66.7%
. Mixed culture	6	33.3%

Table 4.9:

Incidence of preceding or associated disease(s) in the 18 children with acute otitis media from whom Branhamella catarrhalis was isolated.

Disease(s)	No. of patients	%
* Chest infection only	5	27.8%
* Pharyngitis only	2	11%
* Chest infection + pharyngitis	2	11%
* Common cold only	5	25.8%
* Common cold + adenoid	2	11%
* Common cold + sinusitis	1	5.6%
* Common cold + cleft palate	1	5.6%
TOTAL	18	100%

* **TABLE 4.10 and FIGURE 4.1** show seasonal isolation of B. catarrhalis strains from 18 children with AOM in the study.

The majority of B. catarrhalis strains were isolated during the winter months (10 strains : 55.6%). The rate of isolation declined during spring (2 strains only : 11.1%). No isolates was obtained during summer. During autumn the rate of isolation rised again (6 strains : 33.3%). From the 10 strains isolated during winter, 7 were beta-lactamase - positive and 3 were beta-lactamase - negative. From the 2 strains isolated during spring, one was beta-lactamase - positive and the other one was beta-lactamase - negative. From the 6 strains isolated in autumn, 3 were beta-lactamase positive and 3 were beta-lactamase-negative.

Table 4.10:

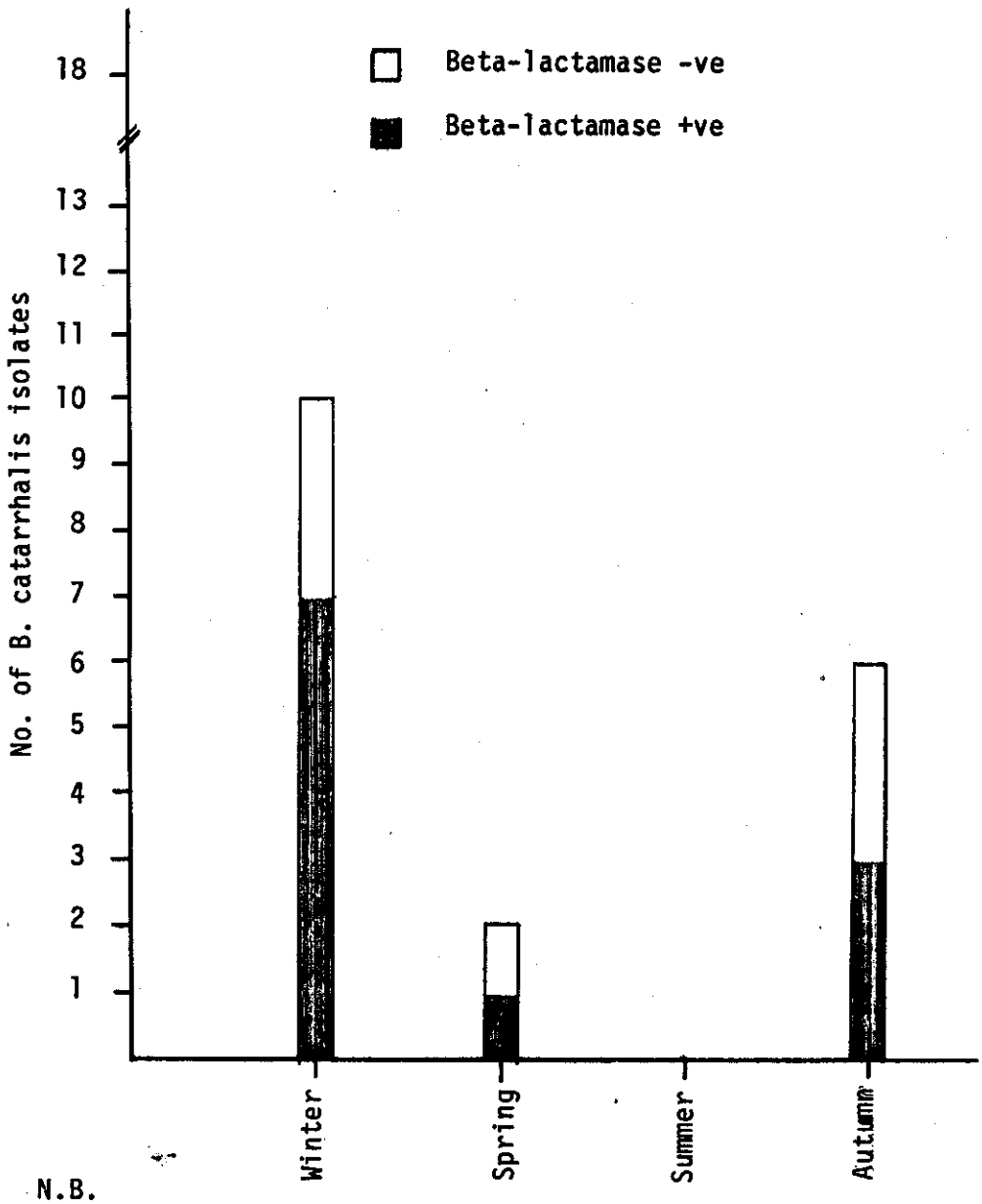
Seasonal isolation of Branhamella catarrhalis strains from 18 children with acute otitis media in the study.

Season	No. of B. catarrhalis isolates			%
	Beta lactamase +ve	Beta lactamase -ve	Total	
Summer	-	-	-	0
Autumn	3	3	6	33.3%
Winter	7	3	10	55.6%
Spring	1	1	2	11.1%
TOTAL	11	7	18	100%

N.B.:

* Summer : June, July and August.
* Autumn : September, October and November.
* Winter : December, January and February.
* Spring : March, April and May.

Fig. 4.1:
Seasonal isolation of <u>Brahmamella catarrhalis</u> strains from 18 children with acute otitis media in the study.



N.B.

* Winter : December, January and February.
* Spring : March, April and May.
* Summer : June, July and August.
* Autumn : September, October and November.

II. RESULTS OF SEROLOGICAL EXAMINATION FOR ANTIBODIES (AGGLUTININS) TO BRANHAMELLA CATARRHALIS IN SERUM:

* **TABLE 4.11 and FIGURE 4.2** give the titers of antibodies (agglutinins) to B. catarrhalis in the acute and convalescent-phase sera of the main study group (14 children with AOM from whose middle ear fluid, B. catarrhalis had been isolated either alone "in 10 children" or with other pathogen "in 4 children").

** Reciprocal titers up to 160 were recorded in the acute-phase sera.

** In 2 children younger than one year (10 and 11 months old), antibodies to B. catarrhalis were undetectable in the acute-phase sera but reached reciprocal titers of 40 and 20 in the covalescent-phase sera respectively.

** In older children (21 to 33 months old), the antibodies to B. catarrhalis were detected in the acute-phase sera. An increase in the titer between the acute and convlescent-phase sera was observed in the 10 children from whose middle ear fluid, B. catarrhalis was isolated in pure culture, while no change in the titer was observed in the 4 children from whom B. catrrhalis was isolated together with other

pathogen (with Staph. aureus in 2 cases, Strept. pyogenes in one case and with Strept. pneumoniae in one case).

* **TABLE 4.12 and FIGURE 4.3** give the titers of antibodies (agglutinins) to B. catarrhalis in the acute-phase and convalescent-phase sera of the control group (14 children with AOM, with matched age and sex as the main study group, and from whose middle ear fluid, bacteria other than B. catarrhalis were isolated).

  ** Antibodies to B. catarrhalis were recorded in 9 of these 14 children and more often in older children (36 months up to 83 months old). There were, however, no changes in the antibody titer between the acute and convalescent-phase sera, and the overall levels were lower (all reciprocals $\leqslant$ 80) than in the children who had otitis media and from whose middle ear fluid B. catarrhalis was isolated.

  ** Antibodies to B. catarrhalis were undetectable both in the acute and convalescent-phase sera in 5 children of the control group and this was more often in the young children (< 36 months old).

Table 4.11:

Titres of antibodies (agglutinins) to Branhamella catarrhalis in the acute phase and convalescent-phase sera of the main study group (Group 1).

Data of the main study group				Branhamella antibody titer	
No.	Age in month	Sex	Bacteriological findings in middle ear discharge	in acute phase	in conv.* phase
1	10 m	F	Pure : B. catarrhalis	-	40
2	11 m	M	Pure : B. catarrhalis	-	20
3	21 m	M	Pure : B. catarrhalis	20	80
4	24 m	F	Pure : B. catarrhalis	40	80
5	28 m	F	Pure : B. catarrhalis	40	160
6	30 m	M	Pure : B. catarrhalis	40	80
7	36 m	M	Mixed: B. catarrhalis + S. pyogenes	20	20
8	38 m	M	Pure : B. catarrhalis	80	160
9	40 m	F	Pure : B. catarrhalis	80	160
10	48 m	M	Pure : B. catarrhalis	160	640
11	49 m	M	Mixed: B. catarrhalis + Staph. aureus	40	40
12	56 m	F	Pure : B. catarrhalis	80	320
13	60 m	F	Mixed: B. catarrhalis + Staph. aureus	40	40
14	83 m	M	Mixed: B. catarrhalis + pneumococci	80	80

* conv. = convalescent

Fig. 4.2:
Titers of antibodies (agglutinins) to Branhamella catarrhalis in the acute-phase and convalescent-phase sera of the main study group (Group 1).

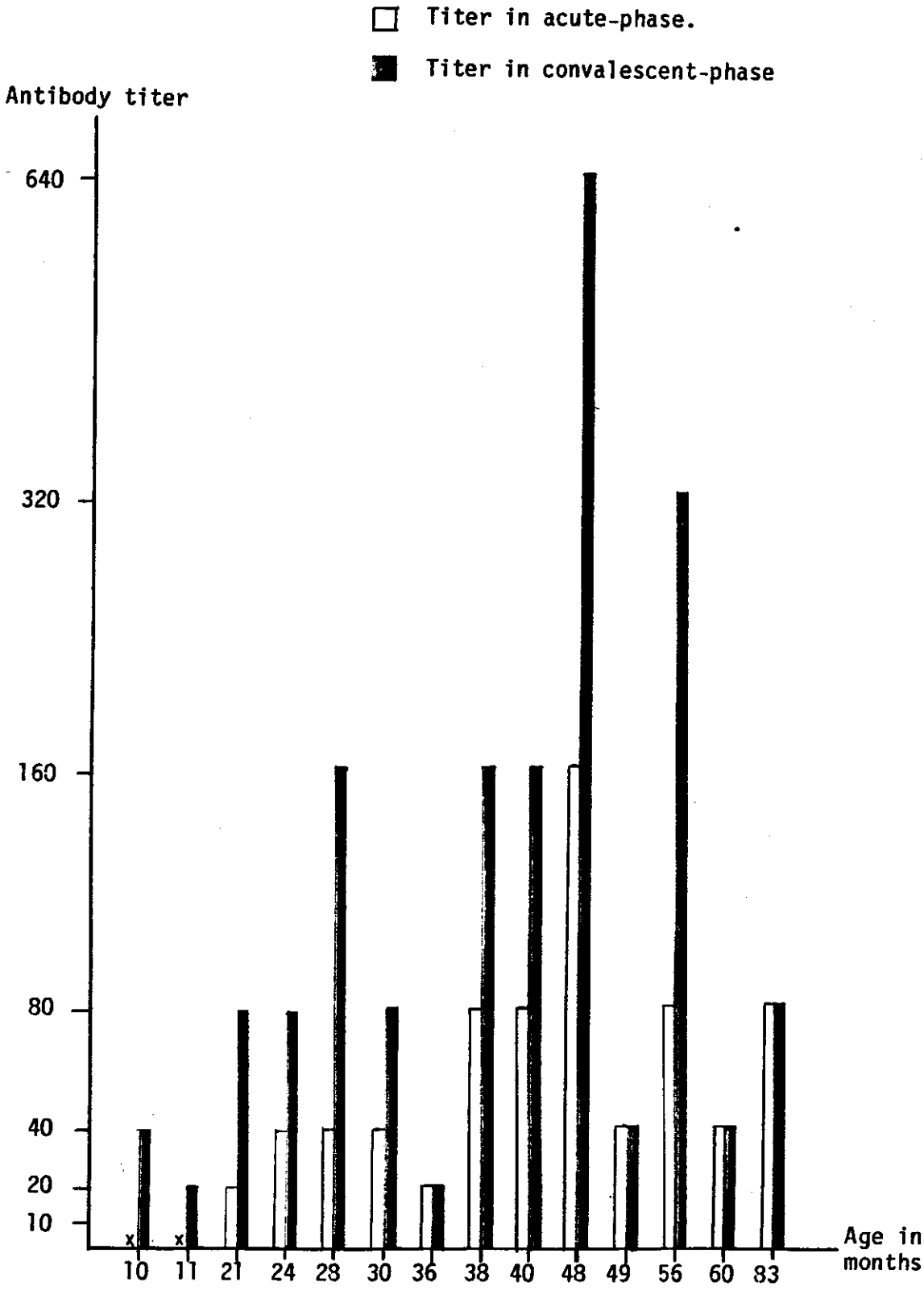


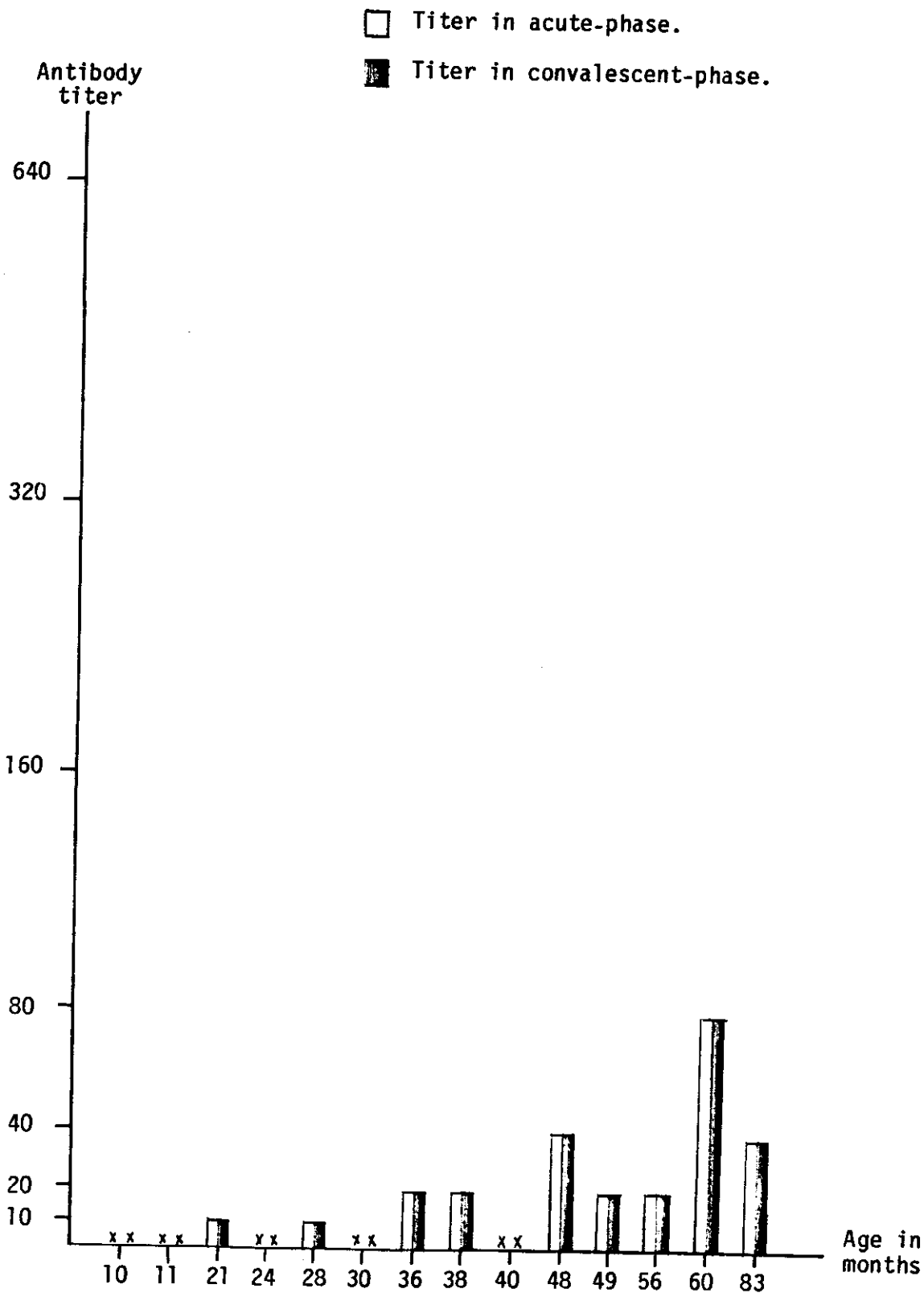
Table 4.12:

Titers of antibodies (agglutinins) to Branhamella catarrhalis in the acute phase and convalescent-phase sera of the control group (Group 2).

Data of the control group				Branhamella antibody titer	
No.	Age in month	Sex	Bacteriological findings in middle ear discharge	in acute phase	in conv.* phase
1	10 m	F	Pure : Strept. pyogenes	-	-
2	11 m	M	Pure : H. influenzae	-	-
3	21 m	M	Pure : G. -ve bacilli (E. coli)	10	10
4	24 m	F	Pure : Staph. aureus	-	-
5	28 m	F	Pure : Strept. pyogenes	10	10
6	30 m	M	Pure : Strept. pneumoniae	-	-
7	36 m	M	Pure : Strept. pneumoniae	20	20
8	38 m	M	Pure : H. influenzae	20	20
9	40 m	F	Pure : Staph. aureus	-	-
10	48 m	M	Pure : Strept. pneumoniae	40	40
11	49 m	M	Pure : Staph. aures	20	20
12	56 m	F	Pure : Strept. pyogenes	20	20
13	60 m	F	Pure : Strept. pneumoniae	80	80
14	83 m	M	Pure : Staph. aureus	40	40

* conv. = convalescent

Fig. 4.3:
Titers of antibodies (agglutinins) to Branhamella catarrhalis in the acute-phase and convalescent-phase sera of the control group (Group 2).



III. RESULTS OF ANTIBIOTIC SUSCEPTIBILITY OF THE ISOLATED STRAINS OF BRANHAMELLA CATARRHALIS:

* **TABLE 4.13** shows the results of beta-lactamase testing of the 18 B. catarrhalis strains isolated from 300 children with AOM in the study.
  Out of the 18 isolates, 11 strains (61.1%) were beta lactamase-positive, while the other 7 strains (38.9%) were beta-lactamase-negative.

* **TABLE 4.14** shows the results of the in vitro antibiotic susceptibility testing (Disc diffusion method) of the 18 B. catarrhalis strains isolated from 300 children with AOM in the study.

  * Eight strains were resistant to penicillin G, while 6 strains were susceptible and 4 strains showed intermediate sensitivity.

  * Five strains were resistant to ampicillin, while 9 strains were susceptible and 4 strains showed intermediate sensitivity.

  * Nine strains were resistant to amoxicillin, while 7 strains were susceptible and 2 strains showed intermediate sensitivity.

  * Nearly all B.catarrhalis strains were susceptible to amoxicillin-clavulanate, erythromycin, TMP-SMX, cephradine and cefotaxime.

* **TABLE 4.15** shows the relation between beta-lactamase production by the isolated strains of B. catarrhalis and the sensitivity to the antimicrobial agents included in the study.

    * All strains which do not produce beta-lactamase (7 strains) were susceptible to all the antimicrobial agents included in the study. The only exception was one strain (strain No. 6) which showed intermediate sensitivity to penicillin G.

    * All strains which produce beta-lactamase (11 strains) were more or less resistant to penicillin G, ampicillin and amoxicillin. Out of these 11 beta-lactamase positive strains:

    - Eight strains were resistant to penicillin G, while 3 strains (strains No. 4, 8 & 14) showed intermediate sensitivity.

    - Five strains were resistant to ampicillin, 4 strains (strains No. 4, 8, 11 & 14) showed intermediate sensitivity, while 2 strains (strains No. 2 & 15) were susceptible.

    - Nine strains were resistant to amoxicillin, while 2 strains (strains No. 4 & 14) showed intermediate sensitivity.

* Conversely, nearly all strains which produce beta-lactamase (11 strains) were susceptible to amoxicillin-clavulanate, erythromycin, cefotaxime, cephradine and TMP-SMX. Out of these 11 beta-lactamase-positive strains:

- Only one strain (strain No. 10) showed intermediate sensitivity to amoxicillin - clavulanate, while the others were susceptible.

- Only one strain (strain No. 7) showed intermediate sensitivity to erythromycin, while the others were susceptible.

- Only one strain (strain No. 13) showed intermediate sensitivity to cefotaxime, while the others were susceptible.

- Only 2 strains (strains No. 1 & 13) showed intermediate sensitivity to cephradine, while the others were susceptible.

- Only 3 strains (strains No. 7, 13 & 17) showed intermediate sensitivity to TMP-SMX, while the others were susceptible.

Table 4.13:

Results of beta-lactamase testing of the 18 Branhamella catarrhalis strains isolated from 300 children with AOM in the study.

No. of Branhamella catarrhalis strains	Beta-lactamase	
	+ve	-ve
1	+ve	
2	+ve	
3		-ve
4	+ve	
5		-ve
6		-ve
7	+ve	
8	+ve	
9		-ve
10	+ve	
11	+ve	
12		-ve
13	+ve	
14	+ve	
15	+ve	
16		-ve
17	+ve	
18		-ve
Total	18	11	7
%	100%	61.1%	38.9%

N.B.:
Beta-lactamase was detected by the nitrocefin test using beta-lactamase BR66 identification sticks (Oxoid).

Table 4.14:

Results of the in vitro antibiotic susceptibility testing (Disc diffusion method) of 18 Branhamella catarrhalis strains isolated from 300 children with acute otitis media in the study.

Antimicrobial agents	Isolated Strains			Total
	R*	I*	S*	
Penicillin G	8	4	5	18
Ampicillin	5	4	9	18
Amoxicillin	9	2	7	18
Amoxicillin-clavulanate	-	1	17	18
Erythromycin	-	1	17	18
TMP-SMX	-	3	15	18
Cephradine	-	2	16	18
Cefotaxime	-	1	17	18

* R = Resistant.

* I = Intermediate.

* S = Susceptible.

* TMP-SMX = Trimethoprim-Sulphamethoxazol.

Table 4.15:

Relation between beta-lactamase production by the isolated strains of Branhamella catarrhalis and the sensitivity to the antimicrobial agents included in the study.

No. of Branhamella strains	Beta lactamase		Antimicrobial agents																						
			Penicillin G			Ampicillin			Amoxicillin			Amoxicillin-clavulanate			Erythro-mycin			TMP-SMX*			Cephradine			Cefotaxime		
	+ve	-ve	R*	I*	S*	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
1	+ve		R			R			R					S			S			S		I				S
2	+ve		R					S	R					S			S			S			S			S
3		-ve			S			S			S			S			S			S			S			S
4	+ve			I			I			I				S			S			S			S			S
5		-ve			S			S			S			S			S			S			S			S
6		-ve		I				S			S			S			S			S			S			S
7	+ve		R			R			R					S		I			I				S			S
8	+ve			I			I		R					S			S			S			S			S
9		-ve			S			S			S			S			S			S			S			S
10	+ve		R			R			R				I				S			S			S			S
11	+ve		R				I		R					S			S			S			S			S
12		-ve			S			S			S			S			S			S			S			S
13	+ve		R			R			R					S			S		I			I			I	
14	+ve			I			I			I				S			S			S			S			S
15	+ve		R					S	R					S			S			S			S			S
16		-ve			S			S			S			S			S			S			S			S
17	+ve		R			R			R					S			S		I				S			S
18		-ve			S			S			S			S			S			S			S			S
TOTAL 18	11	7	8	4	6	5	4	9	9	2	7	-	1	17	-	1	17	-	3	15	-	2	16	-	1	17

* TMP-SMX : Trimethoprim - Sulphamethoxazol.
* R : Resistant. * I : Intermediate. * S : Susceptible.

* **TABLE 4.16 and TABLE 4.17** show the MICs and MBCs, respectively, for the 7 beta-lactamase-negative isolates of B. catarrhalis after 24 hours incubation, while **TABLE 4.18** lists the values of MICs and MBCs of each antimicrobial agent for each strain of such 7 isolates after 24 hours incubation. The geometric mean MICs and MBCs are also shown.

  ** A narrow range of MICs ($\leq$ 8-fold difference) was observed with all antimicrobial agents tested. Based on MIC susceptibility correlates derived from achievable serum concentrations, all the 7 beta-lactamase-negative strains of B. catarrhalis were susceptible to each of the antimicrobial agent tested.

  ** The MBCs of the strains were generally equal to or two-fold higher than the MICs.

Table 4.16:

Minimal inhibitory concentrations(MICs) for the 7 beta-lactamase -ve isolates of Branhamella catarrhalis after 24 hours incubation.

Antimicrobial agents	No. of isolates inhibited at concentration (mg/L) of antibiotic												Geometric mean MIC
	25	12.5	6.25	3.13	1.56	0.78	0.39	0.2	0.1	0.05	0.02	0.01	
Penicillin G					1		5	1					0.54
Ampicillin									1		6		0.04
Amoxicillin									2	5			0.06
Amoxicillin-clavulanate										2	5		0.03
Erythromycin						3	4						0.56
TMP-SMX				3	4								2.2
Cephradine				1	2	4							1.3
Cefotaxime						2			5				0.3

* TMP-SMX : Trimethoprim-Salphamethoxazol.

Table 4.17:

Minimal bacteriocidal concentrations (MBCs) for the 7 beta-lactamase -ve isolates of Branhamella catarrhalis after 24 hours incubation.

Antimicrobial agents	No. of isolates killed at concentration (mg/L) of antibiotic												Geometric mean MBC
	25	12.5	6.25	3.13	1.56	0.78	0.39	0.2	0.1	0.05	0.02	0.01	
Penicillin G					1	4	2						0.8
Ampicillin									3	4			0.07
Amoxicillin								3	4				0.14
Amoxicillin-clavulanate								1	3	3			0.09
Erythromycin				3	4								2.2
TMP-SMX			2	3	2								3.5
Cephradine				2	5								2
Cefotaxime						2		2	3				0.32

* TMP-SMX : Trimethoprim-Sulphamethoxazol.

Table 4.18:

Minimal inhibitory concentrations and minimal bacteriocidal concentrations of antimicrobial agents for the 7 beta-lactamase -ve isolates of Branhamella catarrhalis after 24 hours.

No.	Beta lactamase -ve Branhamella catarrhalis strains	Antimicrobial agents.															
		Penicillin G		Ampicillin		Amoxicillin		Amoxicillin-clavulanate		Erythromycin		TMP-SMX		Cephradine		Cefotaxime	
		MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)
1	Strain No.3	0.39	0.78	0.02	0.05	0.10	0.20	0.02	0.05	0.39	1.56	3.13	3.13	0.78	1.56	0.10	0.10
2	Strain No.5	0.39	0.78	0.02	0.05	0.05	0.10	0.05	0.10	0.78	3.13	3.13	6.25	0.78	1.56	0.10	0.10
3	Strain No.6	1.56	1.56	0.10	0.10	0.05	0.10	0.02	0.05	0.78	3.13	1.56	3.13	3.13	3.13	0.10	0.20
4	Strain No.9	0.20	0.39	0.02	0.05	0.05	0.20	0.02	0.10	0.39	1.56	1.56	1.56	1.56	1.56	0.78	0.78
5	Strain No.12	0.39	0.78	0.02	0.10	0.10	0.10	0.05	0.20	0.39	1.56	3.13	6.25	0.78	1.56	0.10	0.20
6	Strain No.16	0.39	0.39	0.02	0.05	0.05	0.20	0.05	0.10	0.39	1.56	1.56	3.13	1.56	3.13	0.78	0.78
7	Strain No.18	0.39	0.78	0.02	0.10	0.05	0.10	0.05	0.05	0.78	3.13	1.56	1.56	0.78	1.56	0.10	0.10
Geometric mean MIC & MBC		0.54	0.80	0.04	0.07	0.06	0.14	0.03	0.09	0.56	2.20	2.20	3.50	1.30	2.00	0.30	0.32

* TMP-SMX : Trimethoprim-Sulphamethoxazol.
* MIC : Minimal inhibitory concentration.
* MBC : Minimal bacteriocidal concentration.

* **TABLE 4.19 and TABLE 4.20** show the MICs and MBCs, respectively, for the 7 beta-lactamase negative isolates of B. catarrhalis after 24 and 48 hours incubation, while **TABLE 4.21** lists the values of MICs and MBCs of each antimicrobial agent for each strain of such 7 isolates after 24 and 48 hours incubation.

** There was an increase of one dilution in the MIC as well as the MBC of each antimicrobial agent for 2 strains only, while 5 strains had the same values unchanged.

Table 4.19:

Minimal inhibitory concentrations (MICs) for the 7 beta-lactamase -ve isolates of Branhamella catarrhalis after 24 and 48 hours incubation.

Antimicrobial agents	Incubation time	No. of isolates inhibited at concentration (mg/L) of antibiotic												Geometric mean MIC
		25	12.5	6.25	3.13	1.56	0.78	0.39	0.2	0.1	0.05	0.02	0.01	
Penicillin G	24 H					1		5	1					0.54
	48 H					1	1	5						0.6
Ampicillin	24 H									1		6		0.04
	48 H									1	2	4		0.04
Amoxicillin	24 H									2	5			0.06
	48 H									4	3			0.08
Amoxicillin-clavulanate	24 H										2	5		0.03
	48 H									1	4	2		0.05
Erythromycin	24 H						3	4						0.56
	48 H					2	1	4						0.78
TMP-SMX	24 H				3	4								2.2
	48 H				5	2								2.7
Cephradine	24 H				1	2	4							1.3
	48 H				1	4	2							1.6
Cefotaxime	24 H						2			5				0.3
	48 H						2		2	3				0.32

* TMP-SMX : Trimethoprim-Sulphamethoxazol.

Table 4.20:

Minimal bacteriocidal concentrations (MBCs) for the 7 beta-lactamase -ve isolates of Branhamella catarrhalis after 24 and 48 hours incubation.

Antimicrobial agent	Incubation time	No. of isolates killed at concentration (mg/L) of antibiotic												Geometric mean MBC
		25	12.5	6.25	3.13	1.56	0.78	0.39	0.2	0.1	0.05	0.02	0.01	
Penicillin G	24 H					1	4	2						0.8
	48 H					1	6							0.9
Ampicillin	24 H									3	4			0.07
	48 H									4	3			0.08
Amoxicillin	24 H								3	4				0.14
	48 H							1	3	3				0.18
Amoxicillin-clavulanate	24 H								1	3	3			0.09
	48 H								2	3	2			0.1
Erythromycin	24 H				3	4								2.2
	48 H			2	1	4								3
TMP-SMX	24 H			2	3	2								3.5
	48 H			2	5									4
Cephradine	24 H				2	5								2
	48 H				4	3								2.4
Cefotaxime	24 H						2		2	3				0.32
	48 H						2	1	2	2				0.37

* TMP-SMX : Trimethoprim-Sulphamethoxazol.

Table 4.21 :

Minimal inhibitory concentrations and minimal bacteriocidal concentrations of antimicrobial agents for the 7 beta - lactamase - ve isolates of Branhamella catarrhalis after 24 and 48 hours incubation.

Beta - lactamase - ve Branhamella catarrhalis Strains			Strain No. 3	Strain No. 5	Strain No. 6	Strain No. 9	Strain No. 12	Strain No. 16	Strain No. 18	Geometric mean MIC&MBC
Penicillin G	MIC (mg/L)	24H	0.39	0.39	1.56	(0.2)	0.39	(0.39)	0.39	0.54
		48H	0.39	0.39	1.56	(0.39)	0.39	(0.78)	0.39	0.6
	MBC (mg/L)	24H	0.78	0.78	1.56	(0.39)	0.78	(0.39)	0.78	0.8
		48H	0.78	0.78	1.56	(0.78)	0.78	(0.78)	0.78	0.9
Ampicillin	MIC (mg/L)	24H	(0.02)	0.02	0.1	0.02	(0.02)	0.02	0.02	0.04
		48H	(0.05)	0.02	0.1	0.02	(0.05)	0.02	0.02	0.04
	MBC (mg/L)	24H	(0.05)	0.05	0.1	0.05	0.1	0.05	0.1	0.07
		48H	(0.01)	0.05	0.1	0.05	0.1	0.05	0.1	0.08
Amoxicillin	MIC (mg/L)	24H	0.1	(0.05)	0.05	0.05	0.1	(0.05)	0.05	0.06
		48H	0.1	(0.1)	0.05	0.05	0.1	(0.1)	0.05	0.08
	MBC (mg/L)	24H	0.2	(0.1)	0.1	0.2	0.1	(0.2)	0.1	0.14
		48H	0.2	(0.2)	0.1	0.2	0.1	(0.39)	0.1	0.18
Amoxicillin-clavulanate	MIC (mg/L)	24H	0.02	0.05	0.02	(0.02)	0.05	0.05	(0.05)	0.03
		48H	0.02	0.05	0.02	(0.05)	0.05	0.05	(0.1)	0.05
	MBC (mg/L)	24H	0.05	0.1	0.05	(0.1)	0.2	0.1	(0.05)	0.09
		48H	0.05	0.1	0.05	(0.2)	0.2	0.1	(0.1)	0.1
Erythromycin	MIC (mg/L)	24H	0.39	0.78	(0.78)	0.39	0.39	0.39	(0.78)	0.56
		48H	0.39	0.78	(1.56)	0.39	0.39	0.39	(1.56)	0.78
	MBC (mg/L)	24H	1.56	3.13	(3.13)	1.56	1.56	1.56	(3.13)	2.2
		48H	1.56	3.13	(6.25)	1.56	1.56	1.56	(6.25)	3
TMP - SMX	MIC (mg/L)	24H	3.13	3.13	1.56	(1.56)	3.13	1.56	(1.56)	2.2
		48H	3.13	3.13	1.56	(3.13)	3.13	1.56	(3.13)	2.7
	MBC (mg/L)	24H	3.13	6.25	3.13	(1.56)	6.25	3.13	(1.56)	3.5
		48H	3.13	6.25	3.13	(3.13)	6.25	3.13	(3.13)	4
Cephradine	MIC (mg/L)	24H	(0.78)	0.78	3.13	1.56	(0.78)	1.56	0.78	1.3
		48H	(1.56)	0.78	3.13	1.56	(1.56)	1.56	0.78	1.6
	MBC (mg/L)	24H	(1.56)	1.56	3.13	1.56	(1.56)	3.13	1.56	2
		48H	(3.13)	1.56	3.13	1.56	(3.13)	3.13	1.56	2.4
Cefotaxime	MIC (mg/L)	24H	0.1	(0.1)	(0.1)	0.78	0.1	0.78	0.1	0.3
		48H	0.1	(0.2)	(0.2)	0.78	0.1	0.78	0.1	0.32
	MBC (mg/L)	24H	0.1	(0.1)	(0.2)	0.78	0.2	0.78	0.1	0.32
		48H	0.1	(0.2)	(0.39)	0.78	0.2	0.78	0.1	0.37

N.B Values in parentheses are those for strains which show a change in the 24 & 48 hours MIC & MBC

* MIC = Minimal inhibitory concentration .
* MBC = Minimal bacteriocidal concentration .
* TMP - SMX = Trimethoprim - Sulphamethoxazol .

* **TABLE 4.22 and TABLE 4.23** show the MICs and MBCs, respectively, for the 11 beta-lactamase - positive isolates of B. catarrhalis after 24 hours incubation, while **TABLE 4.24** lists the values of MICs and MBCs of each antimicrobial agent for each strain of such 11 isolates after 24 hours incubation. The geometric mean MICs and MBCs are also shown.

** A reasonably narrow range of MICs (≤ 8-fold difference) was observed with amoxicillin-clavulanate, erythromycin, cefotaxime, cephradine and TMP-SMX. A wider variation was observed with penicillin G (32-fold difference), ampicillin (62-fold difference) and amoxicillin (32-fold difference).

** Based on MIC susceptibility correlates derived from achievable serum concentration, nearly all the 11 beta-lactamase positive strains of B. catarrhalis were resistent to penicillin G, ampicillin and amoxicillin. The only exceptions were: one strain (strain No. 14) which was susceptible to penicillin G (MIC: 1.56 mg/L) and 2 strains (strains No. 2 & 15) which were susceptible to ampicillin (MIC : 0.2 mg/L). On the other hand, all the 11

beta-lactamase-positive strains were susceptible to amoxicillin - clavulanate, erythromycin, cefotaxime, cephradine and TMP-SMX.

** The MBCs of the strains were generally equal to, two-fold or sometimes four-fold higher than the MICs.

Table 4.22:

Minimal inhibitory concentration (MICs) for the 11 beta-lactamase +ve isolates of Branhamella catarrhalis after 24 hours incubation.

Antimicrobial agents	No. of isolates inhibited at concentration (mg/L) of antibiotic												Geometric mean MIC
	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.2	0.1	0.05	
Penicillin G		1	1	2	4	2	1						12
Ampicillin				2	3	4				2			5
Amoxicillin		1	1	2	5	1	1						12.4
Amoxicillin-clavulanate								2	1	8			0.32
Erythromycin								7	4				0.64
TMP-SMX					1	2	5	2					2
Cephradine					2		4	5					2
Cefotaxime						1		5	5				0.8

* TMP-SMX : Trimethoprim-Sulphamethoxazol.

Table 4.23:
Minimal bacteriocidal concentrations (MBCs) for the 11 beta-lactamase +ve isolates of Branhamella catarrhalis after 24 hours incubation.

Antimicrobial agents	No. of isolates killed at concentration (mg/L) of antibiotic												Goemetric mean MBC
	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.2	0.1	0.05	
Penicillin G		2	3	3	2	1							20.7
Ampicillin			2	3	3	1			2				10
Amoxicillin		1	3	5	2								18
Amoxicillin-clavulanate							2	3	4	2			0.68
Erythromycin					5	3	3						4
TMP-SMX					2	4	5						3
Cephradine				1	1	3	5	1					3.3
Cefotaxime						2	7	2					1.7

* TMP-SMX : Trimethoprim-Sulphamethoxazol.

Table 4.24:

Minimal inhibitory concentrations and minimal bacteriocidal concentrations of antimicrobial agent for 11 beta-lactamase +ve isolates of Branhamella catarrhalis after 24 hours.

No.	Beta lactamase +ve Branhamella catarrhalis strains	Antimicrobial agents															
		Penicillin G		Ampicillin		Amoxicillin		Amoxicillin clavulanate		Erythromycin		TMP-SMX		Cephradine		Cefotaxime	
		MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)
1	Strain No.1	25	25	12.5	12.5	25	25	0.20	0.39	0.39	1.56	1.56	1.56	6.25	6.25	0.78	1.56
2	Strain No.2	6.25	12.5	0.20	0.39	12.5	25	0.20	0.20	0.78	6.25	1.56	3.13	0.78	1.56	0.78	0.78
3	Strain No.4	3.13	6.25	3.13	6.25	1.56	6.25	0.39	1.56	0.78	6.25	0.78	1.56	0.78	1.56	0.39	1.56
4	Strain No.7	12.5	50	6.25	25	12.5	12.5	0.20	0.39	0.39	1.56	6.25	6.25	1.56	3.13	0.78	1.56
5	Strain No.8	3.13	6.25	3.13	6.25	6.25	12.5	0.78	1.56	0.78	3.13	1.56	3.13	1.56	1.56	0.39	1.56
6	Strain No.10	50	50	12.5	25	50	50	0.78	0.78	0.78	6.25	1.56	1.56	0.78	1.56	0.78	3.13
7	Strain No.11	6.25	12.5	3.13	3.13	6.25	12.5	0.20	0.39	0.78	6.25	0.78	1.56	1.56	3.13	0.39	0.78
8	Strain No.13	12.5	25	6.25	12.5	6.25	12.5	0.20	0.20	0.39	3.13	3.13	6.25	6.25	12.5	3.13	3.13
9	Strain No.14	1.56	3.13	3.13	6.25	3.13	6.25	0.20	0.78	0.78	3.13	1.56	3.13	0.78	0.78	0.39	1.56
10	Strain No.15	6.25	25	0.20	0.39	6.25	12.5	0.20	0.39	0.78	6.25	0.78	1.56	0.78	3.13	0.78	1.56
11	Strain No.17	6.25	12.5	6.25	12.5	6.25	25	0.20	0.78	0.39	1.56	3.13	3.13	1.56	1.56	0.39	1.56
Geometric mean MIC & MBC		12	20.7	5	10	12.4	18	0.32	0.68	0.64	4	2	3	2	3.30	0.80	1.70

* TMP-SMX : Trimethoprim-Sulphamethoxazol.

* MIC : Minimal inhibitory concentration.

* MBC : Minimal bacteriocidal concentration.

* **TABLE 4.25 and TABLE 4.26** show the MICs and MBCs, respectively, for the 11 beta-lactamase positive isolates of B. catarrhalis after 24 and 48 hours incubation, while **TABLE 4.27** lists the values of MICs and MBCs of each antimicrobial agent for each strain of such 11 isolates after 24 and 48 hours incubation.

    ** There was an increase of one dilution either in the MIC only or in the MIC as well as the MBC of each antimicrobial agent for 4 strains only, while 7 strains had the same values unchanged.

* **TABLE 4.28** shows a comparison of geometric mean MICs of various antimicrobial agents for beta-lactamase negative and positive isolates of B. catarrhalis after 24 and 48 hours incubation.

* **TABLE 4.29** shows a comparison of geometric mean MBCs of various antimicrobial agents for beta-lactamase negative and positive isolates of B. catarrhalis after 24 and 48 hours incubation.

Table 4.25:
Minimal inhibitory concentrations (MICs) for the 11 beta-lactamase +ve isolates of Branhamella catarrhalis after 24 and 48 hours incubation.

Antimicrobial agents	Incubation time	No. of isolates inhibited at concentration (mg/L) of antibiotic											Geometric mean MIC
		100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.2	0.1	0.05	
Penicillin G	24 H		1	1	2	4	2	1						12
	48 H		1	1	4	4		1						14
Ampicillin	24 H				2	3	4				2			5
	48 H			2		5	2				2			8
Amoxicillin	24 H		1	1	2	5	1	1						12.4
	48 H		1	2	2	5	1							14.5
Amoxicillin-clavulanate	24 H								2	1	8			0.32
	48 H								3	3	5			0.4
Erythromycin	24 H								7	4				0.64
	48 H							4	3	4				0.92
TMP-SMX	24 H					1	2	5	3					2
	48 H					1	3	7						2.4
Cephradine	24 H					2		4	5					2
	48 H					2	1	7	1					2.5
Cefotaxime	24 H						1		5	5				0.8
	48 H						1	3	3	4				1.1

* TMP-SMX : Trimethoprim-Sulphamethoxazol.

Table 4.26:

Minimal bacteriocidal concentrations (MBCs) for the 11 beta-lactamase +ve isolates of Branhamella catarrhalis after 24 and 48 hours incubation.

Antimicrobial agents	Incubation time	No. of isolates killed at concentration (mg/L) of antibiotic											Geometric mean MBC
		100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.2	0.1	0.05	
Penicillin G	24 H		2	3	3	2	1							20.7
	48 H		2	4	4		1							23
Ampicillin	24 H			2	3	3	1			2				10
	48 H			3	3	3				2				12
Amoxicillin	24 H		1	3	6	1								18.8
	48 H		1	4	5	1								20
Amoxicillin-clavulanate	24 H							2	3	4	2			0.68
	48 H							2	5	2	2			0.75
Erythromycin	24 H					5	3	3						4
	48 H					7	1	3						4.7
TMP-SMX	24 H					2	4	5						3
	48 H					2	8	1						3.6
Cephradine	24 H				1	1	3	5	1					3.3
	48 H				1	1	5	4						3.7
Cefotaxime	24 H						2	7	2					1.7
	48 H						3	7	1					1.9

* TMP-SMX : Trimethoprim-Sulphamethoxazol.

Table 4.27 :

Minimal inhibitory concentrations and minimal bacteriocidal concentrations of antimicrobial agents for the 11 beta - lactamase + ve isolates of Branhamella catarrhalis after 24 and 48 hours incubation.

Beta - lactamase + ve Branhamella catarrhalis Strains			Strain No. 1	Strain No. 2	Strain No. 4	Strain No. 7	Strain No. 8	Strain No. 10	Strain No. 11	Strain No. 13	Strain No. 14	Strain No. 15	Strain No. 17	Geometric mean MIC&MBC
Penicillin G	MIC (mg/L)	24H	25	6.25	(3.13)	12.5	(3.13)	50	(6.25)	12.5	1.56	(6.25)	6.25	12
		48H	25	6.25	(6.25)	12.5	(6.25)	50	(12.5)	12.5	1.56	(12.5)	6.25	14
	MBC (mg/L)	24H	25	12.5	(6.25)	50	(6.25)	50	(12.5)	25	3.13	25	12.5	20.7
		48H	25	12.5	(12.5)	50	(12.5)	50	(25)	25	3.13	25	12.5	23
Ampicillin	MIC (mg/L)	24H	(12.5)	0.2	3.13	6.25	(3.13)	(12.5)	(3.13)	6.25	3.13	0.2	6.25	5
		48H	(25)	0.2	3.13	6.25	(6.25)	(25)	(6.25)	6.25	3.13	0.2	6.25	8
	MBC (mg/L)	24H	(12.5)	0.39	6.25	25	(6.25)	25	(3.13)	12.5	6.25	0.39	12.5	10
		48H	(25)	0.39	6.25	25	(12.5)	25	(6.25)	12.5	6.25	0.39	12.5	12
Amoxicillin	MIC (mg/L)	24H	25	(12.5)	(1.56)	12.5	6.25	50	(6.25)	6.25	(3.13)	6.25	6.25	12.4
		48H	25	(25)	(3.13)	12.5	6.25	50	(12.5)	6.25	(6.25)	6.25	6.25	14.5
	MBC (mg/L)	24H	25	25	6.25	12.5	12.5	50	(12.5)	12.5	(6.25)	12.5	25	18
		48H	25	25	6.25	12.5	12.5	50	(25)	12.5	(12.5)	12.5	25	20
Amoxicillin-clavulanate	MIC (mg/L)	24H	(0.2)	0.2	(0.39)	(0.2)	0.78	0.78	0.2	0.2	0.2	0.2	(0.2)	0.32
		48H	(0.39)	0.2	(0.78)	(0.39)	0.78	0.78	0.2	0.2	0.2	0.2	(0.39)	0.4
	MBC (mg/L)	24H	(0.39)	0.2	1.56	(0.39)	1.56	0.78	0.39	0.2	0.78	0.39	0.78	0.68
		48H	(0.78)	0.2	1.56	(0.78)	1.56	0.78	0.39	0.2	0.78	0.39	0.78	0.75
Erythromycin	MIC (mg/L)	24H	0.39	0.78	(0.78)	0.39	(0.78)	0.78	0.78	0.39	(0.78)	(0.78	0.39	0.64
		48H	0.39	0.78	(1.56)	0.39	(1.56)	0.78	0.78	0.39	(1.56)	(1.56)	0.39	0.92
	MBC (mg/L)	24H	1.56	6.25	6.25	1.56	(3.13)	6.25	6.25	3.13	(3.13)	6.25	1.56	4
		48H	1.56	6.25	6.25	1.56	(6.25)	6.25	6.25	3.13	(6.25)	6.25	1.56	4.7
TMP - SMX	MIC (mg/L)	24H	(1.56)	1.56	(0.78)	6.25	1.56	1.56	(0.78)	3.13	1.56	(0.78)	3.13	2
		48H	(3.13)	1.56	(1.56)	6.25	1.56	1.56	(1.56)	3.13	1.56	(1.56)	3.13	2.4
	MBC (mg/L)	24H	(1.56)	3.13	(1.56)	6.25	3.13	1.56	(1.56)	6.25	3.13	(1.56)	3.13	3
		48H	(3.13)	3.13	(3.13)	6.25	3.13	1.56	(3.13)	6.25	3.13	(3.13)	3.13	3.6
Cephradine	MIC (mg/L)	24H	6.25	(0.78)	0.78	1.56	(1.56)	0.78	1.56	6.25	(0.78)	(0.78)	1.56	2
		48H	6.25	(1.56)	1.56	1.56	(3.13)	0.78	1.56	6.25	(1.56)	(1.56)	1.56	2.5
	MBC (mg/L)	24H	6.25	(1.56)	0.78	3.13	(1.56)	1.56	3.13	12.5	(0.78)	3.13	1.56	3.3
		48H	6.25	(3.13)	1.56	3.13	(3.13)	1.56	3.13	12.5	(1.56)	3.13	1.56	3.7
Cefotaxime	MIC (mg/L)	24H	0.78	(0.78)	0.39	(0.78)	0.39	(0.78)	0.39	3.13	0.39	0.78	(0.39)	0.8
		48H	0.78	(1.56)	0.39	(1.56)	0.39	(1.56)	0.39	3.13	0.39	0.78	(0.78)	1.1
	MBC (mg/L)	24H	1.56	(0.78)	1.56	(1.56)	1.56	3.13	0.78	3.13	1.56	1.56	1.56	1.7
		48H	1.56	(1.56)	1.56	(3.13)	1.56	3.13	0.78	3.13	1.56	1.56	1.56	1.9

N.B Values in parentheses are those for strains which show a change in the 24 & 48 hours MIC & MBC

* MIC = Minimal inhibitory concentration .
* MBC = Minimal bacteriocidal concentration .
* TMP - SMX = Trimethoprim - Sulphamethoxazol .

Table 4.28:

Comparison of geometric mean minimal inhibitory concentrations (MICs) of various antimicrobial agents for beta-lactamase -ve and +ve isolates of Branhamella catarrhalis after 24 and 48 hours incubation.

Antimicrobial agents	Beta-lactamase negative strains (No. = 7)		Beta-lactamase positive strains (No = 11)	
	Geometric mean MIC (mg/L)		Geometric mean MIC (mg/L)	
	24 H	48 H	24 H	48 H
Penicillin G	0.54	0.60	12	14
Ampicillin	0.04	0.04	5	8
Amoxicillin	0.06	0.08	12.4	14.5
Amoxicillin-clavulanate	0.03	0.05	0.32	0.40
Erythromycin	0.56	0.78	0.64	0.92
TMP-SMX	2.20	2.70	2	2.40
Cephradine	1.30	1.60	2	2.50
Cefotaxime	0.30	0.32	0.80	1.10

* TMP-SMX : Trimethoprim-Sulphamethoxazol.

Table 4.29:

Comparison of geometric mean minimal bacteriocidal concentrations (MBCs) of various antimicrobial agents for beta-lactamase -ve and +ve isolates of Branhamella catarrhalis after 24 and 48 hours incubation.

Antimicrobial agents	Beta-lactamase negative strains (No. = 7)		Beta-lactamase positive strains (No. = 11)	
	Geometric mean MBC (mg/L)		Geometric mean MBC (mg/L)	
	24 H	48 H	24 H	48 H
Penicillin G	0.8	0.9	20.7	23
Ampicillin	0.07	0.08	10	12
Amoxicillin	0.14	0.18	18	20
Amoxicillin-clavulanate	0.09	0.1	0.68	0.75
Erythromycin	2.2	3	4	4.7
TMP-SMX	3.5	4	3	3.6
Cephradine	2	2.4	3.3	3.7
Cefotaxime	0.32	0.37	1.7	1.9

* TMP-SMX : Trimethoprim-sulphamethoxazol.

D I S C U S S I O N

For many years, the aerobic Gram-negative diplococcus "Branhamella catarrhalis" was described as a species within the genus Neisseria, and was known as Neisseria catarrhalis.

Catlin & Cunningham (1961 & 1964), and **Bovre (1967)** found that there is genetic incompatibility between Neisseria catarrhalis and other members of the genus Neisseria, through their studies on DNA base composition of the Gram-negative cocci, by DNA hybridization.

Lewis et al. (1968) found that all strains of Neisseria catarrhalis yielded large amount of decanoic acid, a compound found in no other species of Neisseria.

In 1970, the organism named Neisseria catarrhalis was transferred to a new separate genus "Branhamella" named in honor of a distinguished American microbiologist: Sarah Branham **(Catlin, 1970).**

Other genetic, biochemical and serologic properties also support separate classification of B. catarrhalis **(Russel & McDonald, 1976; Johnson et al., 1976; Fox & McClain, 1974 & 1975; Holten & Jyssum, 1974; Russel et al., 1978 and Eliasson, 1980).**