

## NASOPHARYNGEAL CARRIAGE RATE AND ANTIMICROBIAL RESISTANCE OF HAEMOPHILUS INFLUENZAE IN ISTANBUL - TURKIYE

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### SUMMARY

We prospectively studied 462 individuals of different age groups attending to outpatient clinics in İstanbul to assess the nasopharyngeal colonization rate and the antibiotic resistance patterns of the isolated haemophilus strains. Prevalence of Haemophilus influenzae in patients under 5 years of age and in patients over 5 years of age was 76.9% (253/329) and 58.6%(78/133), respectively. The antimicrobial susceptibility to chloramphenicol, ampicillin, cefuroxime and ceftriaxone was determined by microbroth dilution tests. Of 106 H. influenzae isolates, 24.5% were resistant to ampicillin, 8.5% to both ampicillin and cephalosporins, and 4.7% to all four antibiotics. Twenty point eight percent of the strains produced beta-lactamase. These results emphasize the emerging resistance to ampicillin among H. influenzae strains in Türkiye and urge to discuss efficacy of the first line agents in H. infections.

**Key Words:** Haemophilus, Ampicillin, Cefuroxime, Ceftriaxone, Chloramphenicol.

### INTRODUCTION

H. influenzae, a constituent of the normal microbial flora of the upper respiratory tract, is responsible for numerous invasive and noninvasive infections such as meningitis, epiglottitis, cellulitis and upper respiratory tract infections. According to some surveys, up to 80 percent of persons are carriers of H. influenzae. When there is a breakdown of the mechanisms that normally protect the respiratory tract, asymptomatic nasopharyngeal colonization with H. influenzae may develop into symptomatic disease.

Since 1974, there have been several reports about the increasing resistance of H. influenzae to antibiotics such as ampicillin and amoxicillin (1). Therefore investigation of the antibiotic resistance patterns of H. influenzae isolates, obtained from nasopharyngeal specimens and development of rational strategies for therapy of infections with H. influenzae appear essential especially in developing

countries. The purpose of the present study was to assess the prevalence of nasopharyngeal carriage among Turkish children and to determine the efficacy of first-line agents used in the treatment of infections with H. influenzae, such as ampicillin. There is a lack of such data in Türkiye.

### MATERIALS AND METHODS

Study subjects from different age groups were chosen at random when attending outpatient clinics in three community hospitals located in different districts of İstanbul, Türkiye. Also, children aged under five years from day care centers and primary schools were included.

Nasopharyngeal specimens were obtained by means of thin, cotton tipped swabs. The swabs were immediately placed in tubes containing 1ml sterile tryptic soy broth. Within 1 hour's time, nasopharyngeal specimens were plated onto media selective for isolating Haemophilus strains, including chocolate agar with 7% heated horse blood in Columbia agar supplemented with bacitracin (300 mg/L) and IsoVitalex (1%). Cultures were incubated overnight at 37°C in 5% CO<sub>2</sub>.

Isolates were subjected to antibiotic susceptibility tests either promptly after isolation or after storage at -20°C in 10% glycerol. Microbroth dilution tests were used according to standard methods (2-5). Briefly, isolates grown on enriched chocolate agar, were suspended in sterile distilled water (pH=7.3) and adjusted to a turbidity equivalent to a 0.5 McFarland standard. To obtain a final inoculum density of approximately  $5 \times 10^5$  colony forming units/ml in each well, this suspension was diluted 1.200 in a phosphate buffered sterile distilled water (pH 7.3, 0.1M), and 50µl was added to 50µl of test antibiotic diluted in Haemophilus Test Medium (HTM). Final inocula were checked by subculturing 10µl of inoculated broth from control wells of each bacteria. Microdilution plates were incubated at 35°C for 20-24 hr. H. influenzae strain ATCC 9327 and a H. influenzae strain known to be resistant to ampicillin (a beta-lactamase producer) were used as a control

strain in each assay. Growth in each well of microdilution plates was detected by visual examination. Resistance to each antibiotic was defined according to the following MIC values: ampicillin 4µg/ml, cefuroxime 16µg/ml, ceftriaxone 2µg/ml, chloramphenicol 8µg/ml. Production of beta-lactamase was detected by a chromogenic cephalosporin substrate nitrocephine (DIFCO) in liquid phase (6).

## RESULTS

A total of 331 strains of *H. influenzae* and 301 strains of *H. parainfluenzae* were isolated from 462 children specimens in the young age group (children younger than 5 years of age), 253 (76.9%) and 198 (60.2%) yielded growth of *H. influenzae* and *H. parainfluenzae*, respectively. In the second age group (133 children older than 5 years of age), 78 strains of

*H. influenzae* (58.6%), and 103 strains of *H. parainfluenzae* (77.4%) were isolated (Table I). There were no statistically significant differences of carriage between the different city districts (not shown).

The antibiotic susceptibility patterns of 106 isolates of *H. influenzae*, and 32 isolates of *H. parainfluenzae* were examined (Table II). Twenty six (24.5%) of *H. influenzae* isolates were resistant to ampicillin, and 22 of them were beta-lactamase producers. Nine of the 26 ampicillin-resistant isolates were also resistant to cefuroxime and ceftriaxone. Five of these 9 isolates were also resistant to chloramphenicol. Beta-lactamase production and resistance to ampicillin were detected in five of the 32 *H. parainfluenzae* isolates. Resistance to cefuroxime, ceftriaxone or chloramphenicol in *H. parainfluenzae* strains was not observed.

**Table I-** Nasopharyngeal Colonization Rate of *H. influenzae* and *H. parainfluenzae* Among Different Age Groups in Istanbul

	H. influenzae	H. parainfluenzae	None
≤ 5 years old	253/329 (77%)	198/329 (60%)	14/329 (4%)
> 5 years old	78/133 (59%)	103/133 (77%)	3/133 (2%)
Total	331/462 (72%)	301/462 (65%)	17/462 (4%)

**Table II-** Antibiotic Resistance of *Haemophilus* spp.

% (Resistant/Total)						
	Nit (+)	Amp	Cef	Cro	C	Sus
H. inf.	21% (22/106)	25% (26/106)	8% (9/106)	8% (9/106)	5% (5/106)	75% (80/106)
H. para.	16% (5/32)	16% (5/32)	0% (0/32)	0% (0/32)	0% (0/32)	84% (27/32)

Nit (+): Nitrocefin positive, Amp: ampicillin, Cef: Cefuroxime, Cro: Ceftriaxone, C: Chloramphenicol, Sus: Susceptible to all antibiotics.

## DISCUSSION

Three mechanisms of ampicilline (Amp) resistance have been described in *H. influenzae*. Plasmid mediated beta-lactamase production is the most common one (6). In more than 90% of the isolates. Amp. resistance is caused by the production of a broad spectrum TEM type beta-lactamase (TEM.1). *H. influenzae* may also produce another plasmid-mediated beta-lactamase with the same substrate profile but with different physicochemical properties than TEM.1, called the ROB type beta-lactamase (ROB.1) (7). Chromosomally mediated resistance, an alteration in penicillin-binding proteins 3A and 3B, has also been described in a small percentage of strains (8, 9). *H. influenzae* isolates resistant to cephalosporins or chloramphenicol have also been reported (10,11). Resistance to cephalosporins is developed by alteration in the penicillin binding proteins as well. Chloramphenicol resistance occurs in most cases via the production of chloramphenicol acetyl transferase (12). In a collaborative study undertaken in nine European countries in 1986, the overall rate of Amp. resistance was 10%, however there were variations between countries the highest rates being recorded in Spain (30.6%) and in Belgium (26.7%) the lowest in Germany (1.6%) (13). In 1990, another multicentre collaborative study was performed in 11 developing countries by BOSTID and this study revealed that 6% of the isolates were resistant to ampicillin (14). In these studies, the *Haemophilus* were obtained from patients with acute *Haemophilus* infections. In our study, however, the strains were obtained from patients admitted to outpatient clinics for other reasons, and from healthy children. When designing this prospective study, our first aim was to find out the nasopharyngeal colonization rate of *H. influenzae* since these strains become the cause of disease when there is a breakdown of the host's protective mechanisms.

It is inconvenient in Türkiye to isolate agents of noninvasive infections in routine practice. Moreover, reliable studies have not been undertaken yet, and data on antibiotic resistance patterns of infecting organisms causing noninvasive infections are lacking. Consequently, antibiotic regimens are usually applied empirically. In our study, the ultimate goal was to obtain data on emerging resistance to first line agents used in *H* infections. The results of this study reveal a high frequency of ampicillin resistant *Haemophilus* strains, and an infrequent, but alarming, presence of multi-resistant strains. These data should be taken into consideration when instituting ampicillin or amoxicillin for *H.* infections in Turkish children.

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