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In Vitro Mic Determination of *Haemophilus Influenzae* Strains Using E-Test

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Abstract: *H. influenzae* is a fastidious bacteria, so the selection of a reliable method for in-vitro susceptibility tests is particularly important. The E-test is a practical method for detecting in-vitro antimicrobial susceptibility of antibiotics by MIC values. This study was performed using the E-test for direct patient management and in order to obtain epidemiological data on *H. influenzae*.

The detection of antimicrobial agent resistance among fifty-seven *H. influenzae* isolates from lower respiratory tract infections in childhood was studied by the E-test. The E-test was performed on HTM agar according to the instruction manual

recommendations. The susceptibility ratios and the MIC₉₀ values of the isolates for ampicillin, cefaclor, cefotaxime, chloramphenicol, ciprofloxacin and imipenem were 89 % and 1.5µg/ml, 98.2 % and 6µg/ml, 100 % and 0.047µg/ml, 98.2 % and 1.5µg/ml, 100 % and 0.075µg/ml, and 100% and 1.0µg/ml respectively. All of the antibiotics tested were found to be effective against *H. influenzae*.

Key Words: *Haemophilus influenzae*, microbial drug resistance, E-test, respiratory tract infections

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Introduction

Following reports of β -lactamase-producing *H. influenzae* in 1974, the need for a standardized method for antimicrobial susceptibility testing of *H. influenzae* became apparent (1). The E-test is a new product for quantitative antimicrobial susceptibility testing. It is a practical method like the disk diffusion method (2).

It is well known that antimicrobial susceptibility tests must be performed for direct patient management as well as to obtain epidemiological data especially when detailed results from microbiological tests are not available (3).

The aim of the present study was to obtain data on the susceptibility pattern of *H. influenzae* to some antibiotics by means of the E-test.

Material and Methods

Fifty-seven *Haemophilus influenzae* strains were used in this study. After visual examination of the predominant cell types, acceptable sputum specimens were used as the samples. Some of the strains were isolated twice from

three patients and three times from one patient at different times, but the serotyping and biotyping results were different. All of the strains were isolated in 1996 and *Haemophilus influenzae* ATCC 49247 was used as the control strain.

The strains used in this study were isolated from patients with acute exacerbation of lower respiratory tract infections, and most of them had cystic fibrosis. The ages of the patients ranged from 4 months to 17 years and the median age was 7.3.

The E-tests for *Haemophilus influenzae* were performed on Haemophilus test medium (HTM) agar (Oxoid, UK). For inoculum preparation, overnight cultures were inoculated on chocolate agar and incubated at 35°C in the presence of 5-10% CO₂. The inoculum was prepared by direct suspension of the colonies. A bacterial suspension with a turbidity equivalent to that of the McFarland 0.5 standard was prepared in Mueller-Hinton broth supplemented with 15µg/ml bovine hematin (Sigma, IL), 15µg/ml NAD (Sigma, IL) and yeast extract (Difco, IL). The 150-mm diameter agar plates

were inoculated by confluent swabbing of the surface with the adjusted inoculum suspensions. The E-test strips (AB Biodisk, Sweden) were placed in an equidistant radial fashion on the surface of the plates. The incubation was conducted at 35°C in 5% CO₂ for 16-18 hours. E-test MIC evaluations were interpreted by noting the point of intersection of the growth ellipse margin with the MIC scale on the E-test strip when viewed from the upper agar surface with the plate lids removed. The antibiotics used were ciprofloxacin, ampicillin, chloramphenicol, cefotaxime, cefaclor and imipenem and the breakpoints used for interpretation were 1µg/ml, 1µg/ml, 2µg/ml, 2µg/ml, 8µg/ml, and 4µg/ml respectively. The results were evaluated according to NCCLS and the E-test instruction manual recommendations (4).

Results

The in-vitro MIC results and susceptibility rates of 57 *H. influenzae* strains are shown in Table 1. All of the

strains were susceptible to cefotaxime (MIC90 0.047 µg/ml) and imipenem (MIC90 1.0 µg/ml), and 98.2% of the strains were susceptible to cefaclor (MIC90 6 µg/ml) and chloramphenicol (MIC90 1.5 µg/ml). The susceptibility rate of the strains to ciprofloxacin was 94.7 % (MIC90 0.75 µg/ml), and all of these antibiotics were found to be effective against *H. influenzae*. The lowest susceptibility rate was that of ampicillin, with 89 % (MIC90 1.5 µg/ml), but it was still found to be effective. Data from the literature on the susceptibility patterns of *H. influenzae* strains are reviewed in Table 2.

Table 1. MIC results and susceptibility percentages of *Haemophilus influenzae* strains tested (n=57).

	MIC50	MIC90	Range	Susceptibility (%)
Ciprofloxacin	0.023	0.75	0.008-4	94.7
Ampicillin	0.38	1.5	0.064-6	89
Chloramphenicol	0.50	1.5	0.064-3	98.2
Cefotaxime	0.016	0.047	0.016-2	100
Cefaclor	2	6	0.38-12	98.2
Imipenem	0.50	1.0	0.002-4	100

Table 2. Data of other investigations of susceptibility rate and MIC50/90 values.

Reference list by authors country and test method	Ampicillin		Ciprofloxacin		Chloramphenicol		Cefotaxime		Cefaclor		Imipenem	
	S. (%)	MIC50/90 mg/ml	S. (%)	MIC50/90 mg/ml	S. (%)	MIC50/90 mg/ml	S. (%)	MIC50/90 mg/ml	S. (%)	MIC50/90 mg/ml	S. (%)	MIC50/90 mg/ml
Doern G V, USA (5)		0.25/1/64/256*				0.5/1/0.5/1*						4/16/4/32*
Gür D, Turkey (6) E-test	92		100				100		100			
Şener B, Turkey (7)	85.4				96.4				97.1			
Özalp M, Turkey (8) Disk diffusion	89				96				96			
Aytuğ Ş, Turkey (9)										1/6		
Sidorenc S V, USA (12) E-test	90				93				97			
Dornbush K, Sweden(13) E-test				0.032/0.047				0.125/0.75				
Hasegawa M, Japan (14) E-test		-/1/1/64*										-/2/-/2*
Jones RN, USA (15) Broth-microdilution		0.25/8								4/32		
Moloney AC, Ireland (16) Broth-microdilution	83.2	0.25/64			97.9	0.25/0.25	100	0.01/0.01				
Jones RN, USA (17)										-/8		
Yeo SF, UK (18) Disk diffusion										4/8		
Thege MK, Hungary (19)	88				98.7					94.7		
Hryniewics W, Poland (20)	80									73		

*β-lactamase (-)/(+)

Discussion

Laboratory testing of the in-vitro activity of antimicrobial agents is of use when deciding upon a therapeutic regimen, and can also provide important epidemiological data to assist the selection of empiric therapy. This study revealed the susceptibility pattern of *H. influenzae* for ciprofloxacin, ampicillin, chloramphenicol, cefotaxime, cefaclor and imipenem. All of the antimicrobials tested were found to be effective against *H. influenzae*. Ampicillin exhibited the lowest susceptibility rate (89%). Ampicillin resistance may be mediated by TEM-1 type or ROB-1 type β -lactamase enzyme as well as alterations both in the penicillin-binding proteins and in the cell wall, resulting in reduced permeability of the cell membrane to antimicrobial agents, including β -Lactamase-negative but ampicillin-resistant (BLNAR) strains (5). This relatively low susceptibility rate may be due to the frequent use of ampicillin in Turkey. Ampicillin-resistant strains show diminished susceptibility to some cephalosporins, and cefaclor is one of these. This is because of the β -lactamase enzyme, and the fact that cefaclor is a β -lactam antibiotic. In the present study, the cefaclor susceptibility rate was 98.2%, and there was no correlation between the ampicillin-resistant and cefaclor-resistant strains when a direct comparison of susceptibility to the two antibiotics was carried out. The chloramphenicol susceptibility rate was 98.2%. Thus, both cefaclor and chloramphenicol appear to be suitable for empiric therapy. The ciprofloxacin susceptibility rate was also highly favorable (94.7%). Cefotaxime and imipenem, with a susceptibility rate of 100%, exhibited the highest effectiveness.

We searched the literature for other investigations of *H. influenzae* susceptibility patterns carried out during the years 1990-1998, in the U.S.A., Europe and Japan, as well as Turkey. The data from these investigations are shown in Table 2. Our results are similar to those obtained in these previous studies. However, when the MIC 50/90 values are evaluated, in some studies β -lactamase positive and β -lactamase negative strains were separated and the β -lactamase positive strains produced higher MIC values for ampicillin, cefaclor and imipenem

than in our study (Table 2). In our study, we did not separate the strains into β -lactamase positive or negative, and as for the BLNAR strains, we wanted to obtain a total result. It is, therefore, not surprising that the MIC values in β -lactamase-positive groups were higher than our overall MIC values.

In those Turkish studies which investigated ampicillin, cefaclor and cefotaxime, both the susceptibility patterns and MIC50/90 values were within the range of results obtained in our study (6-9).

The standardization of methods of antimicrobial susceptibility testing for *H. influenzae* has been hampered by the fastidious growth of the bacterium. Thus, it is important to assess the ability of in-vitro systems in order to enable the detection of antimicrobial agent resistance in *H. influenzae* isolates (1). The E-test represents a new and innovative approach in the determination of antimicrobial susceptibility which is potentially applicable to a wide array of drugs and microorganisms (10). The E-test may be well suited to the testing of certain fastidious bacteria like *H. influenzae*. In a study carried out in the U.S.A., 4 in-vitro susceptibility test techniques were assessed (11). These were: Vitek, the E-test, microscan and micromedia. In terms of ampicillin susceptibility, the rate varied from 83 to 100%. The E test result was 100%. For chloramphenicol and cefotaxime all of the tests exhibited a 100% susceptibility rate. For cefaclor, the susceptibility rates varied from 83 to 95%, and the E-test result was 95%. Some of the studies we evaluated used the E-test (3,6,11-14), while the others used agar dilution, disk diffusion and broth microdilution (8,9,11,15-18). The E-test results correlated well with those obtained by the other methods.

In conclusion, the E-test has a potential for use for *H. influenzae* and the tested antimicrobials are effective. All of the agents tested, with the exception of ampicillin, can be used in the empirical treatment of infections suspected to be due to *H. influenzae*. However, in-vitro susceptibility tests are required for the identification of the susceptibility patterns of individual isolate since resistance is still likely.

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