The Trends Of Penicillin Resistant Streptococcus Pneumoniae In A Training Hospital

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Citation

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Abstract

Objective: Seven-year period of penicillin resistant in pneumococcal strains isolated in a training hospital in Turkey is presented.

Methods: Penicillin resistant was investigated in 148 isolates by Mueller Hinton Broth Microdilution test between 1993-1997 (Period I), and by E-test for 101 bacteria between 1997-2000 (period II).

Results: The penicillin resistant was 12% in period I and 31% in period II ($p<0.001$). Intermediate resistant increased from 12% to 27% and highly resistant from 0% to 4%. Especially it is observed that the resistant trend accelerates during 1999-2000 with respect to the preceding years. Like penicillin resistant, a gradual increase in multiresistant was detected.

Conclusion: Our study emphasizes that the burden of antibacterial resistant in pneumococci has reached noteworthy levels.

INTRODUCTION

Although pneumococcal infections have been treated with penicillin G and beta-lactam antibiotics for many years, penicillin resistant pneumococci (PRP) have widened steadily thoroughout the world and reached alarming levels in certain regions of the world, but the prevalence of PRP varies regionally ([1], [2]).

The first datum in Turkey was reported by Erbas et al. in 1990 as 12.3% ([3]). There were some differences between hospitals in the studies performed in subsequent years. Intermediate resistant (IR) was between 12.2-50%, while highly resistant (HR) lingered around 0-17% ([4], [5], [6]). It is deduced that PRP have been encountered more frequently in our country in recent years. However, the studies performed have only reflected short term PRP rates of the hospitals. That is, long term results are not known on a regular basis. In this article, a 7-year follow up results of the GATA Haydarpasa Training Hospital were presented.

MATERIAL AND METHODS

In our study, 249 pneumococcal strains recovered from clinical materials and healthy nasopharyngeal carriers in our hospital were included between June 1993 and June 2000. The healthy carriers and patients which pneumococci were isolated divided into 4 age groups as 0-2, 3-10, 11-60 and elderly beyond 61. Our study was conducted in two steps: 1993-1997 (period I) and 1997-2000 (period II). Penicillin resistant was determined for 148 isolates in period I with Mueller Hinton Broth (MHB) microdilution method and in period II for 101 isolates by using E-test method.

Alpha-haemolytic, catalase-negative, optochin-sensitive, bile-soluble colonies and gram-positive, lancet-like bacteria under microscobic evaluation has been defined as pneumococci. ([7]). Penicillin resistant in pneumococci has been detected with Kirby-Bauer disk diffusion test, minimal inhibitor concentrations (MICs) were determined by the MHB microdilution test, and E-test recommended by the National Committee and Clinical Laboratory Standarts (NCCLS) ([8]). One µg oxacillin disk was used in Kirby-Bauer disk diffusion test. Lysed horse serum was used in MHB for microdilution method.

MUELLER-HINTON BROTH MICRODILUTION TEST

A suspension of organisms was prepared in MHB equivalent to 0.5 McFarland density and diluted 1 in 100 to give 10^6 cfu / ml. Serial dilutions of antimicrobial agent were prepared in broth, then a standardized bacterial suspension was added.

Quantites of antibiotic were serially diluted from 100 µg / ml to 0.4 µg / ml. The following antimicrobial agents were tested in the range of 0.03-32 µg / ml: penicillin G, erythromycin, cefaclor, cefotaxime, chloramphenicol, tetracycline and vancomycin. Well number 12 was free of antibiotic and served as a growth control. Each of the 12 wells were inoculated with a calibrated suspension of the microorganism to be tested and incubated at 35°C for 18 hours. At the end of the incubation period, the wells were visually examined for turbutidy. Cloudiness indicated that bacterial growth had not been inhibited by concentration of antibiotic contained in medium. Quality control stain S.pneumoniae 29546 was included in all runs. Results were intrepreted according to the MIC breakpoints recommended by NCCLS ([8]).

E TEST METHOD

Susceptibility testing was performed by E test (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar with 5% sheep blood and incubation in presence of 5% CO_2. The E test was performed according to maufacturer's instructions. By examining the plate with oblique angle, intersection point of eliptic growth zone with the E test strip was accepted as the MIC value. The values obtained at the middle of two numerics on E test strips were extrapolated to upper gradient ([9]).

DEFINITION OF RESISTANT

Zone diameter of ≥20 mm was considered as sensitive while a diameter of < 20 mm zone was considered as resistant in disk diffusion test ([8]). For MIC values 1999 NCCLS standards were used. Values of ≥2 µg/ml were defined as HR and for those between 0.12-1 µg/ml as IR and for the lower values under 0.06 g/ml the isolate was defined as sensitive ([8]).The values obtained between 0.06-0.12 as 1-2 g/ml were extrapolated to upper values according to E test. Multiresistant was defined as the resistant patterns to at least three different antibiotics ([1])

STATISTICS

Statistical analysis of the results were done by chi-square test and Pearson correlation coefficient analysis and $p<0.05$ was accepted as significant.

RESULTS

In this study, 148 and 101 pneumococcal strains were included in period I and period II respectively. In period I, 49 (33.2%) of them were isolated from the clinical materials and 99 (66.8%) were recovered from nasopharyngeal carriers, while in period II 25 (24.7%) isolates were obtained from clinical materials and 75 (74.0%) strains were recovered from nasopharyngeal carriers . Table-1 shows the distrubution of the pneumococci with respect to ages.

Figure 1

Table 1: Distrubution of the isolates according to ages.

PERIOD	*AGES*			
	0-2	3-10	11-60	>60
Period I	10	27	86	25
Period II	3	36	52	10

Sputum was the most frequently encountered clinical material in both periods. Isolates recovered from otorrhoea in period I and blood culture in the latter came in the second (Table-2). The penicillin resistant was elevated from 12% during 1994-97 to 31% in 1997-2000 ($p<0.001$). In addition to IR elevation from 12 % to 27 % ($p<0.001$), a 4 % HR isolates were found in period II as opposed to 0 % in period I (Table-3) (Fig-1).

Figure 2

Table 2 : Distrubution of the pneumococci with regard to the clinical materials.

	PERIOD I		*PERIOD II*	
MATERIAL	n	%	n	%
Nasopharyngeal carriers	99	67	75	74
Otorrhoea	11	7	-	-
Adenoid vegetation	2	1	-	-
Vaginal culture	-	-	1	1
Blood culture	4	3	6	6
CSF	3	2	5	5
Sputum	29	20	11	11
Pleura	-	-	2	2
Burn wound	-	-	1	1
TOTAL	148	100	101	100

Figure 3

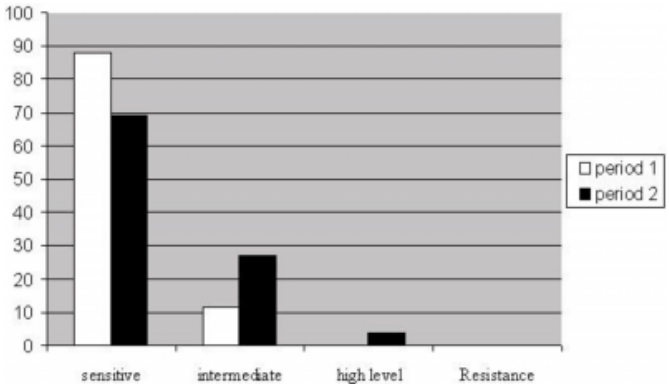
Table 3 : Penicillin susceptibility in the pneumococci

	S		*IR*		*HR*		
PERIOD	n	%	n	%	n	%	
Period I (n=148)	130	88	18	12	0	0	$X^2=15.480$, $p<.001$
Period II (n=101)	70	69	27	27	4	4	

(IR: Intermediate resistant HR:High level resistant)

Figure 4

Figure 1: Penicillin susceptibility in the pneumococci



In period I 12% of the nasopharyngeal isolates were penicillin resistant when compared to 27% ratio seen in the following period (p<0.001). Three HR strains were recovered from nasopharyngeal carriers and one from cerebrospinal fluid of the patient with meningitis. The most prominent increase in resistant between two intervals was seen in blood (0% vs. 33%), in cerebrospinal fluid (0% vs. 20%) and in sputum cultures (3% vs. 45%) (p<0.001) (Table-4).

When both periods were compared, the penicillin resistant was found as elevated in all age groups excepting 0-2 years' interval. The resistant increased from 26 % to 42 % in 3-10 age group, 9 % to 23 % in 11-60 years and 8 % to 30 % in elderly. In comparing both periods, resistant decreased to 0 % from 10 % in 0-2 age group. HR was seen 3 % in 3-10 age group and 6 % in 11-60 age group (Table 5). The MIC values of all HR strains were 2 µg/ml.

Figure 5

Table 4: Penicillin susceptibility of the pneumococci with respect to clinical materials

	SENSITIVE				INTERMEDIATE RESISTANT				HIGH RESISTANCE			
	Period I		Period II		Period I		Period II		Period I		Period II	
SAMPLES	n	%	n	%	n	%	n	%	n	%	n	%
Nasopharynx	87	88	53	71	12	12	19	25	-	-	3	4
Sputum	28	97	6	55	1	3	5	45	-	-	-	-
Blood Culture	4	100	4	67	-	-	2	33	-	-	-	-
Otorrhoea	7	64	-	-	4	36	-	-	-	-	-	-
Cerebrospinal fluid	3	100	4	80	-	-	-	-	-	-	1	20
Adenoid Vegetation	1	50	-	-	1	50	-	-	-	-	-	-
Pleura	-	-	1	50	-	-	1	50	-	-	-	-
Burn Wound	-	-	1	100	-	-	-	-	-	-	-	-
Vaginal Culture	-	-	1	100	-	-	-	-	-	-	-	-
TOTAL	130		70		18	-	27		0		4	

Figure 6

Table 5: Distribution of resistant with regard to age groups

	SENSITIVE				INTERMEDIATE RESISTANT				HIGH RESISTANT			
	Period I		Period II		Period I		Period II		Period I		Period II	
AGE (Years)	n	%	n	%	n	%	n	%	n	%	n	%
0-2	9	90	3	100	1	10	-	-	-	-	-	-
3-10	20	74	21	58	7	26	14	39	-	-	1	3
11-60	78	91	40	77	8	9	9	17	-	-	3	6
>60	23	92	7	70	2	8	3	30	-	-	-	-
TOTAL	130		70		18		27		0		4	

Table-6 shows the predominant antibiotic resistant patterns encountered among the 148 S.pneumoniae isolates in period I, and 101 pneumococci in period II. Six isolates showed resistant to more than one antibiotic and one were multi-resistant in period I, whereas 12 isolates were multiresistant in period II. All isolates with HR to penicillin in period II were multi-resistant strains.

Figure 7

Table 6: Distribution of multiple resistant S.pneumoniae

Resistant pattern	Period I (n=148)	Period II (n=101)
Penicillin+ertythromycin	2	4
Penicillin+chloramphenicol	1	1
Penicillin+tetraciycline	2	2
Penicillin+cefotaxime	-	1
Penicillin+ertythromycin+chloramphenicol	-	1
Penicillin+ertythromycin+tetracycline	1	2
Penicillin+ertythromycin+chloramphenicol+tetracycline	-	1
TOTAL	6	12

DISCUSSION

The control of resistant among the bacteria necessitates the surveillance of the alterations in antibiotic susceptibilities due to time. When this alteration is in favour of elevation, effective struggle with the resistant is a necessity. These surveillance data are also important in recognizing the potential hazards that may cause morbidity, mortality and increased management costs ([10]).

Our study, which was performed throughout 7 years regarding two periods, demonstrated that penicillin resistant in pneumococci has increased. According to CDC data, only one of the isolates in USA was resistant to penicillin between 1978-87, and this trend has reached to 1.3% with an sixty-fold increase till 1991. In the following years, with some regional differences, penicillin resistant increased a rate of 40-50% in USA ([11]). In South Africa, the rates were 4.9%in 1979, 14.4% in 1990 and 40% in 1996 ([12], [13]). Penicillin resistant has also been observed as an increasing patterns in various European countries. In France 1.1% in 1984-86 has reached 12% in 1990 and a 41-55% non-susceptibility was seen around 1997-98 ([14], [15], [16]). In Germany, a slowly increase in resistant was observed with penicillin, where the resistant rate rose from 0% in 1981 to

5.8% in 2000 ([17, 18]). Our study showed the regional details of penicilllin resistant and emphasized that penicillin resistant pneumococci should be considered as an important issue in our country concordant with the other parts of the world.

Although there is a considerable geographical variation in penicillin susceptibility among pneumococci, this gradual increase in pneumococcal penicillin resistant has become a global problem so that it continues to spread clonally through hospitals, cities, countries and continents. In a recent multi-center study, it is reported that 36% of the respiratory isolates showed decreased penicillin susceptibility and 23% was HR to penicillin. The higher resistan- rates were seen in these countries: 80% in South Korea, 65% in Japan, 63% in France, 59% in Hong Kong and 57% in Mexico ([19]).

In various studies carried in Turkey, it was shown that penicillin resistant increased year by year. The resistant rate of 12.3% by Erbas et al in 1990 was reported as 22% in 1992-94 by Sumerkan et al ([2, 5]). The resistant was announced by Kanra as 30% between 1994-1995 and by Gonullu as 41.3% in 1998 ([20, 21]). Another noteworthy point in our study is the escalation of IR from 12% to 27 and HR to 4% from zero. These data indicate an increase in resistant of more than two folds in IR and four folds in HR in our hospital. Since most of the isolates were community acquired, it is reasonable to postulate that neighbouring western parts of our country may experience similar resistant patterns.

Pneumococcal penicillin resistant rises in accordance with the frequency of the infections at different ages. In community, resistant strains seems to be recovered from day nurseries in preschool era, at school ages or in overcrowded living conditions and from patients with underlying diseases ([22]). In our study, resistant was encountered mostly during 3-10 and 11-60 age groups for both periods. Because of the increased possibility of these individuals encountering pathogenic bacteria and sharing the same media that might result in the transfer of resistant strains, the inflation in resistant is an expected result. In this study, it was also shown that the number of carriers of PRP was higher in period II compared with in period I. On the other hand, decreasing resistant in 0-2 age groups between two periods might be related with small sample size in this group.

The MIC value in the pneumococci is a good determinant in the treatment of pneumococcal infections. Its value is a marker of performance of antibiotics in vitro ([7]). The standard parenteral penicillin treatment except cerebrospinal fluid infections has a reasonable theraupatic efficacy for strains with 4 µg/ml or lower MIC values ([23]). As in our study, any pneumococcus with a MIC value exceeding 2 µg/ml was not reported in our country. On the other hand, it seems reasonable to postulate the ongoing increase in resistant will result in the higher MIC values and will continue complicating the problem further in the very near future for our country. For this reason the struggle against resistant must be considered crucial and the surveillance must be performed as it should be.

Strains with reduced susceptibilty to penicillin are usually cross-resistant to other antibiotics ([7]). In this study, it was observed that some penicillin-resistant isolates were also resistant to one or more of tetracycline, erythromycin and chloramphenicol. The majority of the isolates in the present study remained susceptible to chloramphenicol, whereas about 8% were resistant to erythromycin in period II. These resistant rates make erythromycin inappropriate for empirical therapy. Resistant to erythromycin probably results from the frequent use of macrolides for the treatment of upper respiratory tract infections in population in our country. Like penicillin resistant, the gradual increase in erythromycin resistant was found in US, where the macrolide resistant increased from 10.6% in 1995 to 20.4% in 1999 ([24]). In the present study, multi-resistant strains were more in period II than in period I. Our results showed that this gradual increase of multi-resistant S. pneumoniae has become a problem in our country.

All starins were susceptible to vancomycin in both periods, in agreement with recent reviews, so vancomycin may be an important alternative for use in the treatment of infections caused by pneumococci resistant to penicillin and other antimicrobial agents. On the other hand, of considerably greater concern is the possibility that vancomycin resistant genes and the enterococci may be transferred to more virulent organisms such as S.pneumoniae ([25]).

CONCLUSIONS

In conclusion, these results emphasize that antibacterial resistant in pneumococci has been increasing in Turkey as another parts of the world. For this reason, strategies to establish training programs for health care professionals on rational antibiotic use, to perform comprehensive epidemiologic studies and to provide strong health policies will result in decreases in penicillin resistant.

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