STAPHYLOCOCCAL RESISTANCE IN TURKEY TO SEVEN COMMONLY USED ANTIBIOTICS

In Vitro Studies on Cultures from 300 Children *

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Introduction

The increasing resistance of pathogenic staphylococci to antibiotics presents a serious problem to physicians throughout the world. It is well known that penicillin, which was exceedingly effective in the treatment of staphylococcal infections not more than twelve years ago, is now effective against only a small percentage of pathogenic staphylococci. Unfortunately the development of resistant strains has occurred with all of the more recently introduced antibiotics, and this

TABLE 1. PERCENTAGE OF ANTIBIOTIC RESISTANT STRAINS OF STAPHYLOCOCCI FOUND BY VARIOUS INVESTIGATORS

ANTIBIOTIC	AUTHOR	% OF STRAINS RESISTANT
Penicillin	Andreoni [7]	73 %
	Kempe [15]	90 %
Streptomycin	Josephson [6]	42 %
	Kempe [15]	80 %
Oxytetracycline	Lutz [2]	59 %
	Kirby [5]	61 %
Chloramphenicol	Koch [3]	30 %
	Kempe [15]	50 %
Chlortetracycline	Lutz [2]	59 %
	Kirby [5]	61 %
Erythromycin	Juel [4]	24 %
	Kempe [15]	69 %

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phenomenon will undoubtedly operate with others yet to be discovered.

In recent years many reports have been published demonstrating an increase in the number of strains resistant to antibiotics commonly in use.[1-11] Some of the highest figures reported on the percentage of antibiotic resistant strains of staphylococci are shown in table 1.

In Turkey the problem of staphylococcal antibiotic resistance has become evident both from clinical experience and from small scale *in vitro* testing. So far as we have been able to determine, however, no large scale studies have been made nor have resistance rates been established in Turkey. The study to be reported here was undertaken in order to obtain data for the treatment of staphylococcal infections and to provide information for comparative studies of the incidence of antibiotic resistant staphylococci.

Cultures from 300 cases of staphylococcal infection in the Hacettepe Children's Hospital between October 25, 1958 and August 15, 1959 were tested for antibiotic sensitivity and resistance. Seven commonly used antibiotics were tested in three dilutions each.

Materials and Methods

Strains: The staphylococci tested were obtained from outpatients between 6 days and 12 years of age. Of the 300 samples, 89 were isolated from patients with purulent otitis media, 69 from throat and nose, 2 from blood cultures, 123 from skin lesions, 3 from urine and 14 from different kinds of exudates. In order to avoid duplication of samples only one culture from each patient was used. Staphylococci which coagulated human plasma, demonstrated hemolysis and were capable of fermenting mannitol were considered pathogenic regardless of the type of pigment they formed. About 95 % grew out as *Staphylococcus aureus*.

Strains to be tested were taken from single colonies on the inital plate and cultured in nutrient broth for 18 to 24 hours at 37 C.

Media: Nutrient agar prepared with Bacto-beef extract was poured into Petri dishes to a depth of 7 mm. Plates, with the lids removed, were kept in the incubator in an inverted position for a reasonable period of time to remove excess moisture prior to use.

Antibiotics Tested: The seven antibiotics and their dilutions are shown in the following table : *

Antibiotic	Dilutions used		
	1 (low)	2 (medium)	3 (high)
1. Penicillin (crystalline G, Sodium, Farmitalia)	2 I. U./ml	5 I. U./ml	10 I. U./ml
2. Streptomycin (dihydrosulfate, Farmitalia)	2 mcg/ml	10 mcg/ml	100 mcg/ml
3. Oxytetracycline HCI (Terramycin, Pfizer)	5 mcg/ml	10 mcg/ml	30 mcg/ml
4. Chloramphenicol (Chloromycetin, Parke-Davis)	5 mcg/ml	10 mcg/ml	30 mcg/ml
5. Chlortetracycline HCI (Aureomycin, Lederle)	5 mcg/ml	10 mcg/ml	30 mcg/ml
6. Tetracycline HCI (Ambramycin, Lepetit)	5 mcg/ml	10 mcg/ml	30 mcg/ml
7. Erythromycin (Erythrocin, Abbott)	2 mcg/ml	5 mcg/ml	15 mcg/ml

* The dilutions used in our tests were identical to those of Difco antibiotic discs and the Difco method of interpretation was followed. [12]

Stock solutions were prepared in buffers with the proper pH necessary for each antibiotic (pH 7 for penicillin, 8 for streptomycin and erythromycin, 4.5 for oxytetracycline, chloramphenicol, tetracycline and chlortetracyline). These solutions were kept at -20 C for a period of not more than two months. Test solutions were prepared with sterile saline once a month and kept in dropper bottles at the ordinary refrigerator temperature.

Technic: The agar diffusion method was used in testing the sensitivity of the various strains. Two nutrient-agar plates were prepared for each strain: using a sterile swab, the plates were inoculated by smearing the broth culture over the surface of the agar. Twenty-one holes were then made on the two plates with a 7mm corkborer creating cylindrical cavities penetrating the depth of the agar, and one drop from each antibiotic test solution was placed in each hole. Preliminary experimentation had shown the optimum pattern of the holes necessary

to provide enough space for each inhibition zone, as illustrated in figure 1. The plates were incubated at 37 C. for 18 to 24 hours and then read.

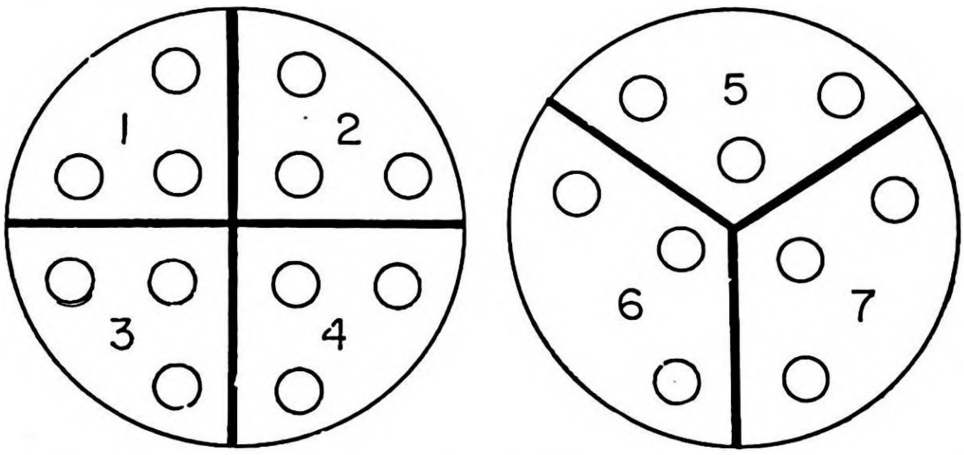

Fig. 1. — The order of holes for three dilutions each of seven antibiotics: two Petri dishes used for each strain.

Recording and Interpretation: A zone of any size around a hole was accepted as a sign of an inhibitory effect of the antibiotic. Since the width of the inhibition zone is influenced by many factors such as the quantity of agar used in the medium, depth, pH value, type of medium, diameter of holes, size of drops, incubation time and temperature, in our opinion it is reasonable to consider any size zone of inhibition as proof of effectiveness of the antibiotic in question. Therefore we interpreted the results as follows:

Antibiotic dilution:

1 (low)	3 (medium)	3 high)	*Sensitivity of strain*
-	-	-	Resistant
-	-	+	Slightly sensitive
-	+	+	Sensitive
+	+	+	Very sensitive

(-): no zone
(+): zone

The number of strains of staphylococci resistant and sensitive to each antibiotic and the relative percentages are shown in table II and in figure 2.

It will be noted from table 2 and figure 2 that none of the strains tested was resistant to erythromycin. The number of strains sensitive to the last four antibiotics in table 2 (and the first four in figure 2) was very high compared to the number of strains sensitive to penicillin, streptomycin and chloramphenicol. Moreover, most of the

strains sensitive to four highly effective antibiotics were "very sensitive" (See sixth column in table 2 and white areas in figure 2).

TABLE 2. RESULTS OF ANTIBIOTIC SENSITIVITY TESTS OF 300 STRAINS OF PATHOGENIC STAPHYLOCOCCI

ANTIBIOTIC	Resistant strains		SENSITIVE STRAINS				
	Number	%	Slightly sensitive	Sensitive	Very Sensitive	Total Number	%
Penicillin	199	**66.3**	40	21	40	101	**33.6**
Chloramphenicol	117	**39**	141	36	6	183	**61**
Streptomycin	82	**27.3**	53	129	36	218	**72.6**
Oxytetracycline	36	**12**	11	19	234	264	**88**
Tetracycline	34	**11.3**	5	13	248	266	**89.6**
Chlortetracycline	18	**6**	10	193	259	282	**94**
Erythromycin	0	**0**	3	11	286	300	**100**

The most effective antibiotic among the seven tested was erythromycin, followed by chlortetracycline, tetracycline, oxytetracycline, streptomycin, chloramphenicol and finally by penicillin. The data obtained in our tests correspond to those reported elsewhere in the world, except that the newest antibiotics, which are not as widley used in Turkey as are penicillin and streptomycin, are more effective against a higher proportion of the strains of pathogenic staphylococci than in other countries where these new agents are in greater use. For example, some of the recent figures reported elsewhere on resistance rates of staphylococci to erythromycin, chlortetracycline and oxytetracycline are from 5 to 69 times higher than the results in this study. [5,15]

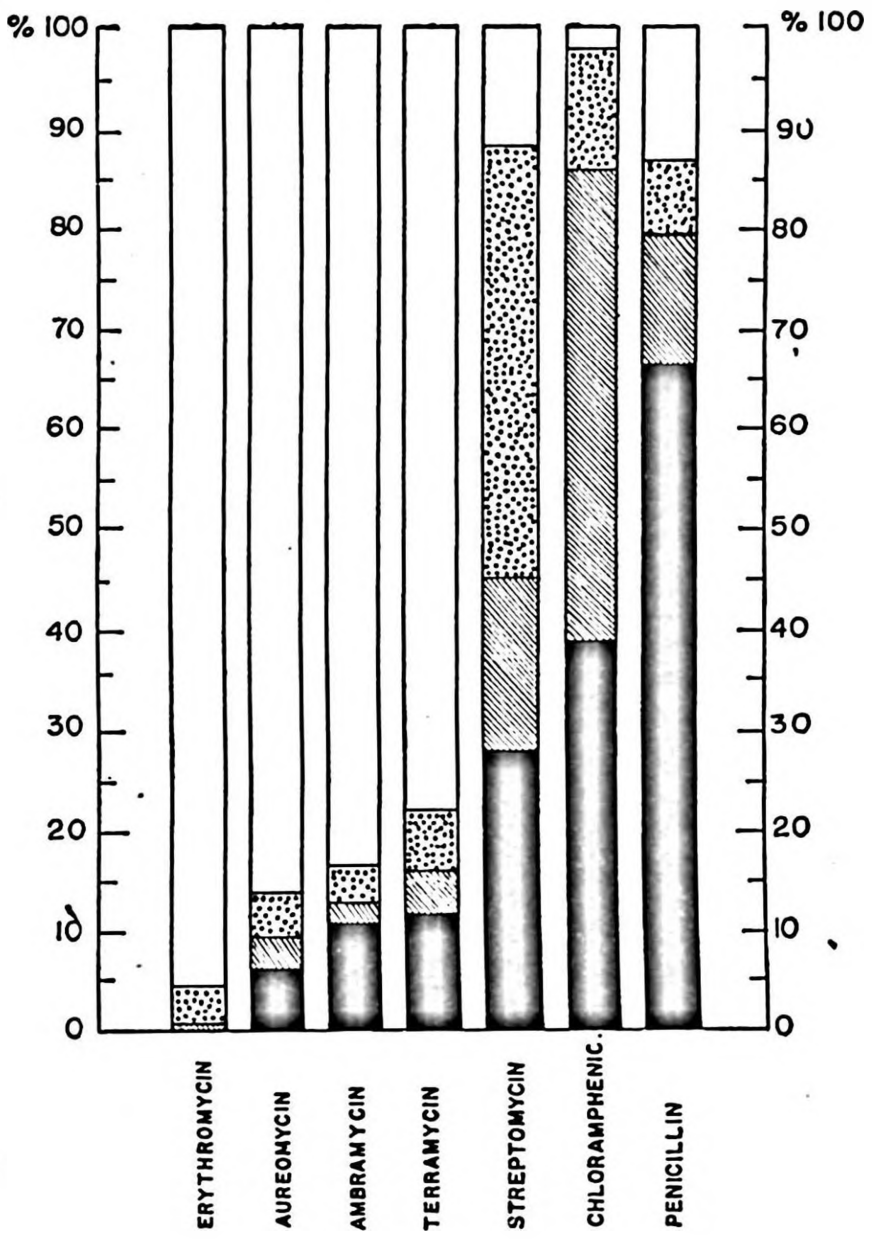

Fig. 2. — Degree of Antibiotic Resistance or Sensitivity of 300 Strains of Staphylococci :

Black areas : resistant
Striped areas : slightly sensitive
Dotted areas : sensitive
White areas : very sensitive

Since some strains may be naturally resistant to an antibiotic, as shown by Chabbert et al.,[13] it is a general rule that the increase in the number of strains resistant to an antibiotic is related to the amount of antibiotic used and especially to the misuse of the drug. Rountree's observation on the pathogenic staphylococci isolated from New Guinea tribes is a good example. He could not find any strain resistant to any antibiotic among 23 strains of pathogenic staphylococci from these people living almost isolated from the civilized world. In

Turkey penicillin is the antibiotic most generally used and erythromycin, chlortetracycline and oxytetracycline are less frequently employed. In countries where high resistance rates to the newer antibiotics have been reported, these agents have been used much more extensively. Postulating that a relationship exists between antibiotic resistance and the frequency of drug administration, we attempted to correlate the differences in resistance to various antibiotics with the quantities of these antibiotics being used in Turkey. Detailed information on the administration of antibiotics was not available, but we were able to obtain information concerning the quantities of each antibiotic used in the six largest hospitals in Ankara during 1958. These quantities are illustrated graphically in figure 3, and the percentage of staphylococcal strains resistant to each antibiotic is shown superimposed. There does, indeed, seem to be a correlation and we believe that this picture reflects the existing situation very well.

The close correlation between the frequency of administration of an individual antibiotic and the resistance rate is evident. However, there are two major exceptions. In the case of streptomycin and chlortetracycline it appears that the number of resistant strains is not related to the amounts used. The following considerations might be of some value in explaining these discrepancies: There is no doubt that most of the streptomycin was used in Ankara in the treatment of tuberculosis and thus might not have come in contact with pathogenic staphylococci, and this in turn would explain the relatively low number of resistant strains. As for chlortetracycline, the amount shown in this figure surely could not be taken as a real yearly average. While chlortetracycline was not widely available in Turkey during 1958, other structurally similar tetracyclines were fairly readily available and it would seem possible that the relatively high rate of resistant strains could be explained on a basis of "cross-resistance".

Future studies will help to determine the reliability of this relationship in our country. In the light of our present knowledge it is not unrealistic to presume that in the near future, with th increasing use of newer antibiotics in Turkey, the number of resistant strains and the degree of antibiotic resistance will incr ase.

Having obtained the basic data reported above, we then sought to learn whether the number of resistant strains could be correlated with other factors, including the source of the strain within the individual (pus, urine, blood, etc.), the age and sex of the children from

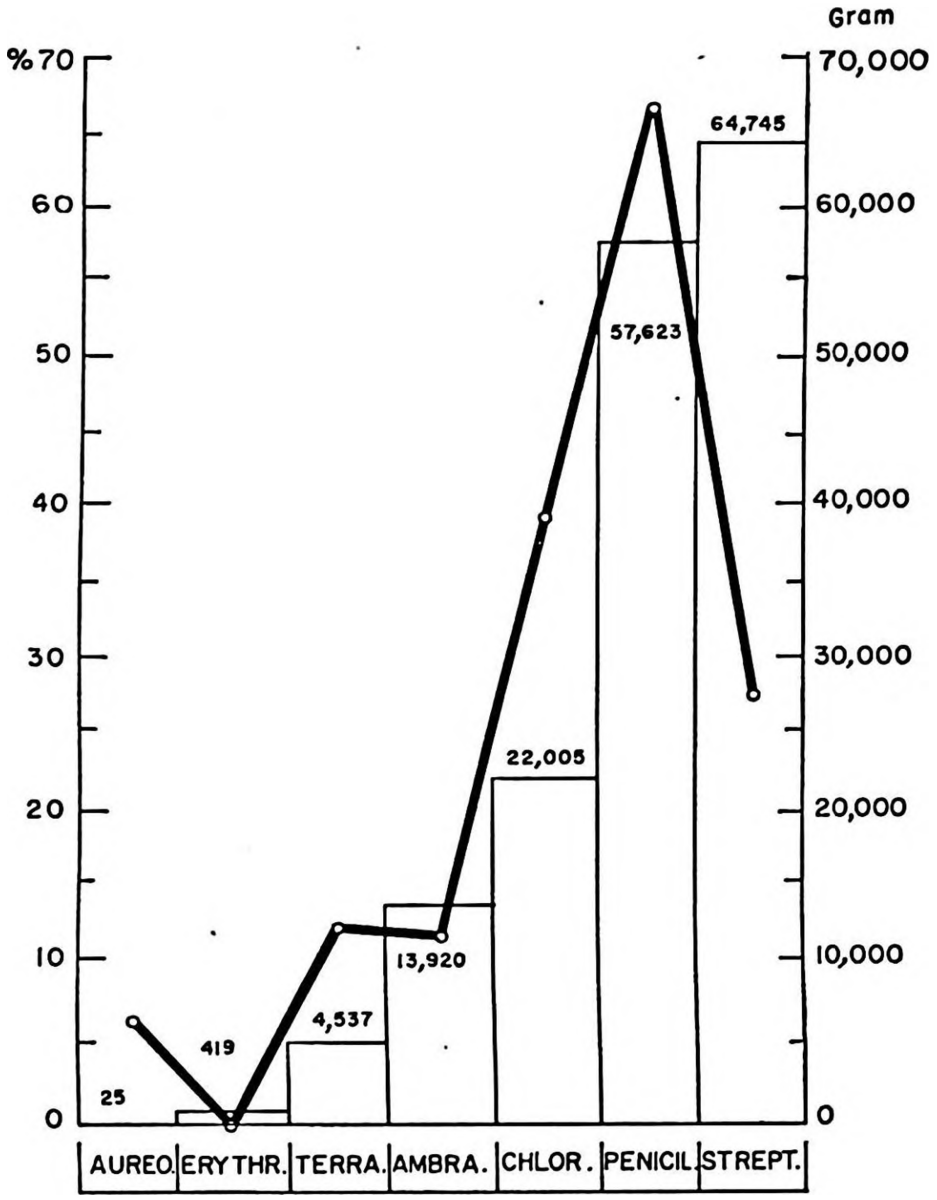

Figure 3. — Correlation between the total quantities of antibiotics used in six hospitals in Ankara in 1958 and antibiotic resistance rates of 300 strains of staphylococci.
Grams of individual antibiotics are shown in bars and the graph line indicates percentage of resistant strains to each antibiotic.

whom the strains were isolated, and whether the child came from the city or from villages where exposure to antibiotics in the past might have been less extensive. Results were as follows:

(a) No significant difference was apparent in the antibiotic resistance rates of the strains from different age groups or from different sexes.

(b) This was also true for city and village populations. Although the rate of resistant strains to penicillin was 64.4 % for patients from the city and 55.5% for patients from the villages, the difference is not important for two reasons: first, the difference

is not statistically significant, and second, in a city like Ankara it is not possible to draw a distinction between some parts of the city and the surrounding villages which are in close contact with the city. Theoretically one should be able to distinguish between the two groups and might reasonably expect differences, but at the moment we do not believe the figures are representative of actually distinct environments.

(c) It was interesting to observe some antibiotic resistance differences for staphylococci isolated from various sources. When the strains were classified according to their origin, namely, from nose and throat lesions, skin lesions, etc., no significant difference was apparent. However, between strains isolated from nose and throat lesions and those from cases of purulent otitis media a marked difference was found as shown in table 3.

Although at first glance there appear to be significant differences in the rates for streptomycin, chloramphenicol, tetracycline and chlortetracycline, they are not in fact statistically significant. In fact, the chi square test indicates that differences for penicillin and oxytetracycline only are significant. From this we could conclude that the strains isolated from otitis media cases are less likely to be resistant to these antibiotics than those isolated from throat and nose lesions.

This phenomenon provokes questions for which we could not find satisfactory answers. Are there measurable differences in strains from infection of the ear and from the nose and throat in regard to their biochemical behavior, phage types, antigenic structure or in some other respects? Could we suppose that there is some tropism, some affinity of strains for certain organs or tissues which are, somehow, correlated with their resistance pattern? We hope that future surveys may help to answer these questions.

Summary

1. Results of antibiotic resistance tests of 300 strains of pathogenic staphylococci isolated from children at the Hacettepe Children's Hospital in Ankara, Turkey, were as follows: 66.3 % resistant to penicillin; 39 % to chloramphenicol; 27 % to streptomycin; 12% to oxytetracycline; 11.3% to tetracycline; 6% to chlortetracycline, and 0 % to erythromycin.

2. A correlation exists between the frequency of drug administration for the treatment of staphylococcal infections and the development of staphylococcal resistant strains.

TABLE 3. ANTIBIOTIC RESISTANCE RATES OF STAPHYLOCOCCI ISOLATED FROM NOSE AND THROAT LESIONS AND FROM CASES OF PURULENT OTITIS MEDIA

Origins of the strains	Total Number of Samples	Penicillin		Streptomycin		Oxytetracycline		Chloramphenicol		Tetracycline		Chlortetracycline	
		Resistant	%	Resistant	%	Resistant	%	Resistant	%	Resistant	%	Resistant	%
Throat-nose	69	54	78.2	20	28.9	13	18.8	30	43.4	11	15.9	6	8.6
Otitis media	89	45	50.5	23	15.8	7	7.8	33	37	7	7.8	4	4.4
CHI SQUARE VALUES		12.6		0.2		4.21		0.69		2.53		1.18	
P.		<.001		.50-.70		.05-.02		.50-.30		.20-.10		.30-.20	

3. Among the strains studied a statistically significant difference was found in resistance to penicillin and oxytetracycline as between strains isolated from infections of the middle ear and of the nose or throat, resistant strains being isolated more frequently from the latter sites.

Acknowledgement

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