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Susceptibility of Clinical Isolates of Staphylococcus Aureus Strains to 32 Antibiotics

by

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Previous studies in this department on the activity of various antibiotics against clinical isolates showed that Staphylococcus aureus strains were the most frequent microorganisms encountered in clinical specimens (Tan et al., 1968; Tanzil et al., 1971; Tanzil and Warsa, 1971, 1972).

In the present paper the purpose is to determine the activity of 32 antibiotics by using the single disk method against strains of Staphylococcus aureus isolated consecutively from clinical sources, and to establish differences in antibiotic resistance in relation to the origin of the biological material.

Material and methods

The material subjected to bacteriological investigation was derived from a variety of clinical specimens sent to the department between May 1970 and December 1971.

The presence of Staphylococcus aureus was confirmed by colonial appearance on blood-agar, haemolysis, presence of golden pigment, Gram reaction and microscopic morphology.

All isolated Staphylococcus aureus strains were tested for antibiotic-resistance by the single disk method. The tests were performed on pure cultures isolated from single colonies. From an overnight broth culture inoculation was made on Oxoid Diagnostic Sensitivity Test Agar in 10 cm petri dishes in a streak at a right angle against the edge of a sensitivity disk. On each petri dish, 4 different disks were placed at a suitable distance from each other, and from each disk 8 streaks of culture inoculation were made (Eriksen and Eriksen, 1966). Following overnight incubation of the plates at 37° C, the presence or absence of a zone of inhibition of growth from

Received 13th Sept. 1972

the edge of the disk, was used to determine susceptibility. For example, a definite zone of inhibition indicated that the organism was sensitive, whereas absence of zone indicated resistance.

Disks containing the following amounts of antibiotics were used throughout this study:

Albamycin-T (sodium novobiocin 15 mcg and tetracycline hydrochloride 5mcg), the Upjohn Company, Kalamozoo, Michigan, U.S.A.

Ampicillin 25 mcg, Oxoid Limited, London SE1, England. Ampiclox (ampicillin 25 mcg and cloxacillin 5 mcg) Mast Laboratories Ltd., Liverpool, England.

Bactrim [trimethoprim sulphamethoxazole (1:5) 25 mcg], Oxoid Limited, London SE1, England.

Baycillin (propicillin 3 Units), Bayer, Leverkusen, Germany.

Binotal (ampicillin 75 mcg), Bayer, Leverkusen, Germany.

Cephaloridin 15 mcg, Oxoid Limited, London SE1, England.

Chloromycetin (chloramphenicol 25 mcg), Oxoid Limited, London SE1, England.

Erythrocin (erythromycin 15 mcg), Abbott Laboratories, North Chicago, U.S.A.

Fultrexin (hydroxymethylnitrofurantoin 100 mcg, thiamphenicol 50 mcg and sulfaisoxazole 400 mcg), Zambon, Milano-Bresso, Italy.

Furadantin (nitrofurantoin 200 mcg), Oxoid Limited, London SE1, England.

Gabbromycin 60 mcg, Farmitalia, Mast Laboratories Ltd., Liverpool, England.

Gentamycin 10 mcg, Difco Laboratories for Schering Corporation, U.S.A.

Kanamycin 10 mcg, Difco Laboratories, U.S.A.

Kelfizina (2-sulfa-3-methoxy-pyrazine), Farmitalia, S.A. Farmaceutici Italia Milano, Italy.

Ledermycin (dimethylchlortetracycline 5 mcg), BBL Cockeysville, Maryland, U.S.A.

Lincocin (lincomycin hydrochloride 10 mcg), The Upjohn Company, Kalamazoo, Michigan, U.S.A.

Micoflavin (tetracycline 30 mcg and chloramphenicol 30 mcg), Zambon, Milano-Bresso, Italy.

Orbenin (cloxacillin 5 mcg), Beecham Research Laboratories, Mast Laboratories Ltd., Liverpool, England.

Penbritin (ampicillin 25 mcg), Beecham Research Laboratories, Mast Laboratories Ltd., Liverpool, England.

Penicillin-G 1.5 Units, Oxoid Limited, London SE1, England.

Pristinamycin (pyostacine 15 mcg), Institut Pasteur, Paris, France.

Pyopen (carbenicillin 100 mcg), Beecham Research Laboratories,

TABLE 1 : *The frequency of occurrence of staphylococcus aureus in bacteriologically positive sample cultures.*

Sources of material	Number of bacteriologically positive sample cultures	Samples containing Staphylococcus aureus	
		Number	Percentage
Urine	3737	1715	45.9
Blood	233	171	73.4
Pus	452	301	66.6
Cerebrospinal fluid	58	10	17.3
Pleural fluid	161	65	40.4
Sputum	1431	578	40.4
Nose	165	82	49.7
Throat	1752	824	47.1
Mouth	43	13	30.2
Eye	54	29	53.7
Ear	280	92	32.9
Skin	29	18	62.1
Urogenital tract	306	183	59.8
Faeces	61	2	3.3
Bone	3	2	66.7
T o t a l	8765	4085	46.6

TABLE 2 : *Number and percentage of strains of staphylococcus aureus from different sources resistant to various antibiotics.*

Antibiotic	Number of strains tested	Resistant strains	
		Number	%
Sulfadiazine	4085	3755	91.9
Kelfizina	1872	1421	75.9
Bactrim	4019	1676	41.7
Furadantin	3894	1391	35.7
Urfadyn	4085	1266	31.0
Penicillin	4085	2872	70.3
Baycillin	4085	2806	68.7
Pyopen	3517	1184	33.7
Stapenor	671	2280	55.8
SQ 11302	4085	389	58.0
Orbenin	4085	2468	60.4
Penbritin	4085	2287	56.0
Binotal	3280	2237	68.5
Ampiclox	3415	1186	34.7
Chephaloridin	1482	559	37.7
Chloromycetin	3887	2117	51.8
Urfamycin	4085	2961	72.5
Fultrexin	3130	2461	63.3
Micoflavin	1576	2050	50.2
Tetracyclin	3886	3354	82.1
Ledermycin	2961	2286	73.0
Albamycin-T	3866	1548	37.9
Erythrocin	2984	623	39.5
Rovamycin	4085	1365	35.1
Lincomycin	4085	1532	51.7
Streptomycin	4085	2658	68.8
Kanamycin	4085	1060	35.9
Soframycin	4085	851	20.8
Gabbromycin	4085	1120	27.4
Gentamycin	3934	1231	31.3
Pristinamycin	2200	1022	46.5
Rifampin	1701	712	41.8

TABLE 3 : *Antibiotic-resistance of staphylococcus aureus strains isolated from urine specimens.*

Antibiotic	Number of strains tested	Resistant strains	
		Number	%
Sulfadiazine	1715	1637	95.5
Kelfizina	814	658	80.8
Bactrim	1715	726	42.3
Furadantin	1715	668	39.0
Urfadyn	1715	618	36.0
Penicillin	1715	1285	74.9
Baycillin	1715	1254	73.1
Pyopen	1454	474	32.6
Stapenor	1715	957	55.8
SQ 11302	270	190	70.4
Orbenin	1715	1070	62.4
Penbritin	1715	1033	60.2
Binotal	1325	933	70.4
Ampiclox	1450	525	36.2
Chephaloridin	578	264	45.7
Chloromycetin	1715	911	53.1
Urfamycin	1715	1357	79.1
Fultrexin	1654	1133	68.5
Micoflavin	1715	993	58.0
Tetracyclin	1715	1487	86.7
Ledermycin	1340	1004	74.9
Albamycin-T	1715	630	36.7
Erythrocin	632	302	47.8
Rovamycin	1650	620	37.6
Lincomycin	1200	685	57.1
Streptomycin	1637	1186	72.5
Kanamycin	1249	463	37.1
Soframycin	1715	353	20.6
Gabbromycin	1715	474	27.6
Gentamycin	1665	513	30.8
Pristinamycin	815	360	44.2
Rifampin	715	280	39.2

TABLE 4 : *Antibiotic-resistance of staphylococcus aureus strains isolated from blood specimens.*

Antibiotic	Number of strains tested	Resistant strains	
		Number	%
Sulfadiazine	171	161	94.2
Kelfizina	73	56	76.7
Bactrim	167	72	43.1
Furadantin	171	62	36.3
Urfadyn	171	47	27.5
Penicillin	171	129	75.4
Baycillin	171	110	64.3
Pyopen	126	48	38.1
Stapenor	171	86	50.3
SQ 11302	25	10	40.0
Orbenin	171	85	49.7
Penbritin	171	95	55.6
Binotal	155	95	61.3
Ampiclox	123	51	41.5
Chephaloridin	86	27	31.4
Chloromycetin	171	95	55.6
Urfamycin	171	114	66.7
Fultrexin	150	95	63.3
Micoflavin	171	83	48.6
Tetracyclin	171	136	79.5
Ledermycin	129	102	79.1
Albamycin-T	171	64	37.4
Erythrocin	88	32	36.4
Rovamycin	150	50	33.3
Lincomycin	130	57	43.8
Streptomycin	169	109	64.5
Kanamycin	121	33	27.3
Soframycin	171	21	12.3
Gabbromycin	171	46	26.9
Gentamycin	149	31	20.8
Pristinamycin	118	78	66.1
Rifampin	88	25	37.9

TABLE 5 : *Antibiotic-resistance of staphylococcus aureus strains isolated from pus specimens.*

Antibiotic	Number of strains tested	Resistant strains	
		Number	%
Sulfadiazine	301	286	95.0
Kelfizina	137	112	81.8
Bactrim	284	118	41.5
Furadantin	285	108	37.9
Urfadyn	301	80	26.6
Penicillin	301	265	88.0
Baycillin	301	220	73.1
Pyopen	267	100	37.4
Stapenor	301	175	58.1
SQ 11302	35	25	71.4
Orbenin	301	167	55.5
Penbritin	301	201	66.7
Binotal	237	178	75.1
Ampiclox	260	98	37.7
Chephaloridin	105	43	41.0
Chloromycetin	301	200	66.4
Urfamycin	301	232	77.1
Fultrexin	273	202	74.0
Micoflavin	301	166	55.2
Tetracyclin	301	236	78.4
Ledermycin	235	167	66.4
Albamycin-T	301	143	47.5
Erythrocin	109	44	40.4
Rovamycin	276	101	36.6
Lincomycin	217	119	54.8
Streptomycin	285	215	75.4
Kanamycin	215	87	40.5
Soframycin	301	51	16.9
Gabbromycin	301	87	28.9
Gentamycin	289	95	32.9
Pristinamycin	226	125	55.3
Rifampin	145	56	38.6

TABLE 6 : *Antibiotic-resistance of staphylococcus aureus strains isolated from sputum specimens.*

Antibiotic	Number of strains tested	Resistant strains	
		Number	%
Sulfadiazine	578	515	89.1
Kelfizina	298	205	68.8
Bactrim	576	245	42.5
Furadantin	569	185	32.5
Urfadyn	578	124	21.5
Penicillin	578	388	67.1
Baycillin	578	359	62.1
Pyopen	468	185	39.5
Stapenor	578	338	58.5
SQ 11302	88	33	37.5
Orbenin	578	327	56.6
Penbritin	578	270	46.7
Binotal	480	308	64.2
Ampiclox	470	143	30.4
Chephaloridin	233	65	28.0
Cloromycetin	578	275	47.6
Urfamycin	578	361	62.5
Fultrexin	569	185	32.5
Micoflavin	578	288	49.8
Tetracyclin	578	428	74.0
Ledermycin	437	301	68.8
Albamycin-T	578	219	37.9
Erythrocin	241	65	27.0
Rovamycin	539	149	27.6
Lincomycin	386	196	50.8
Streptomycin	549	359	65.4
Kanamycin	388	131	33.7
Soframycin	578	106	18.3
Gabbromycin	578	131	22.7
Gentamycin	548	150	27.4
Pristinamycin	303	127	41.9
Rifampin	268	108	40.3

TABLE 7 : *Antibiotic-resistance of staphylococcus aureus strains isolated from ear specimens.*

Antibiotic	Number of strains tested	Resistant strains	
		Number	%
Sulfadiazine	92	89	96.7
Kelfizina	42	36	85.7
Bactrim	91	43	47.3
Furadantin	81	36	44.4
Urfadyn	92	40	43.5
Penicillin	92	64	69.6
Baycillin	92	65	70.6
Pyopen	84	29	34.5
Stapenor	92	54	58.7
SQ 11302	15	10	66.7
Orbenin	92	60	65.0
Penbritin	92	55	59.8
Binotal	71	50	70.4
Ampiclox	78	34	43.6
Chephaloridin	33	13	39.4
Chloromycetin	92	49	53.3
Urfamycin	92	78	77.8
Fultrexin	81	63	84.8
Micoflavin	92	52	56.5
Tetracyclin	92	74	80.4
Ledermycin	67	54	80.6
Albamycin-T	92	36	39.1
Erythrocin	33	16	48.5
Rovamycin	87	31	35.6
Lincomycin	75	24	32.0
Streptomycin	86	63	73.2
Kanamycin	70	23	32.9
Soframycin	92	24	26.1
Gabbromycin	92	38	41.3
Gentamycin	90	34	37.8
Pristinamycin	51	27	52.9
Rifampin	50	23	46.0

TABLE 8 : *Antibiotic-resistance of staphylococcus aureus strains isolated from nose specimens.*

Antibiotic	Number of strains tested	Resistant strains	
		Number	%
Sulfadiazine	82	78	95.1
Kelfizina	33	23	69.7
Bactrim	69	20	29.0
Furadantin	76	21	27.6
Urfadyn	82	20	24.4
Penicillin	82	48	58.5
Baycillin	82	53	64.6
Pyopen	76	43	56.6
Stapenor	82	30	36.6
SQ 11302	17	7	41.2
Orbenin	82	41	50.0
Penbritin	82	51	62.2
Binotal	67	52	77.6
Ampiclox	69	17	24.6
Chephaloridin	22	10	45.5
Chloromycetin	82	42	51.2
Urfamycin	82	64	78.0
Fultrexin	79	56	70.9
Micoflavin	82	44	53.7
Tetracyclin	82	69	84.1
Ledermycin	66	50	75.7
Albamycin-T	82	32	39.0
Erythrocin	24	11	45.8
Rovamycin	82	27	32.9
Lincomycin	62	22	35.5
Streptomycin	77	52	67.5
Kanamycin	61	28	45.9
Soframycin	82	22	26.8
Gabbromycin	82	16	19.5
Gentamycin	81	25	30.9
Pristinamycin	52	17	32.1
Rifampin	36	15	41.7

TABLE 9 : *Antibiotic-resistance of staphylococcus aureus strains isolated from throat specimens.*

Antibiotic	Number of strains tested	Resistant strains	
		Number	%
Sulfadiazine	824	701	85.1
Kelfizina	330	225	68.2
Bactrim	804	309	38.4
Furadantin	761	221	29.0
Urfadyn	729	216	26.2
Penicillin	824	488	59.2
Baycillin	824	528	64.1
Pyopen	729	824	31.3
Stapenor	824	475	57.7
SQ 11302	164	77	47.0
Orbenin	824	533	64.7
Penbritin	824	461	48.7
Binotal	673	430	63.9
Ampiclox	636	220	34.6
Chephaloridin	326	98	30.6
Chloromycetin	824	376	45.6
Urfamycin	824	504	61.2
Fultrexin	796	427	53.6
Micoflavin	824	347	42.1
Tetracyclin	824	650	78.9
Ledermycin	624	431	69.1
Albamycin-T	824	285	34.6
Erythrocin	348	107	30.8
Rovamycin	700	161	23.0
Lincomycin	633	324	51.2
Streptomycin	759	451	59.4
Kanamycin	614	204	33.2
Soframycin	824	177	21.5
Gabbromycin	824	217	26.3
Gentamycin	799	270	33.7
Pristinamycin	454	213	46.9
Rifampin	302	147	48.7

TABLE 10 : *Antibiotic-resistance of staphylococcus aureus strains isolated from urogenital tract specimens.*

Antibiotic	Number of strains tested	Resistant strains	
		Number	%
Sulfadiazine	183	165	90.2
Kelfizina	83	63	75.9
Bactrim	102	69	37.9
Furadantin	133	51	38.3
Urfadyn	183	64	35.0
Penicillin	183	120	65.6
Baycillin	183	126	68.9
Pyopen	143	29	16.8
Stapenor	183	85	46.4
SQ 11302	25	16	64.0
Orbenin	183	105	57.4
Penbritin	183	99	54.1
Binotal	156	108	69.2
Ampiclox	167	50	29.9
Chephaloridin	44	19	43.2
Chloromycetin	183	84	46.0
Urfamycin	183	143	78.1
Fultrexin	179	102	57.0
Micoflavin	183	77	42.1
Tetracyclin	183	158	86.4
Ledermycin	130	100	76.9
Albamycin - T	183	69	37.7
Erythrocin	57	31	54.4
Rovamycin	178	67	37.6
Lincomycin	139	53	38.1
Streptomycin	177	120	67.8
Kanamycin	124	43	34.7
Soframycin	183	46	25.1
Gabbromycin	183	61	33.3
Gentamycin	179	67	37.4
Pristinamycin	99	37	37.3
Rifampin	61	33	54.1

Mast Laboratories Ltd., Liverpool, England.

Rovamycine (spiramycine), Institut Pasteur, Paris, France.

Soframycin (framycetin sulphate 100 mcg), Roussel Laboratories, Mast Laboratories Ltd., Liverpool, England.

SQ 11302 (epicillin 10 mcg), BBL, Cockeysville, Maryland, U.S.A.

Stapenor (oxacillin 10 mcg), Bayer, Leverkusen, Germany.

Streptomycin 5 mcg, Difco Laboratories, U.S.A.

Sulfadiazine 300 mcg, Oxoid Limited, London SE1, England.

Tetracycline 25 mcg, Oxoid Limited, London SE1, England.

Urfadyn (hydroxymethylnitrofurantoin 100 mcg), Zambon, Milano-Bresso, Italy.

Urfamycin (thiamphenicol 50 mcg), Zambon, Milano-Bresso, Italy.

Results

During May 1970 to December 1971, pathological materials were sent to our laboratory for bacteriological diagnosis. Of the 8765 positive culture samples, 4085 (46.6%) were found to contain Staphylococcus aureus. In table 1 are shown the specimens classified according to origin of the material from which Staphylococcus aureus strains were isolated. It can be seen that the proportion of positive samples of 15 different sources of material that contained Staphylococcus aureus strains varied from about 3.3% for faeces and 73.4% for blood specimens. It should be noted that our laboratory has a separate section for the isolation of enterobacteria from faecal material of which only a small number was included in this study.

The number of all Staphylococcus aureus strains tested for resistance to each of the 32 antibiotics or chemotherapeutics mentioned is indicated in tabel 2. The highest resistance was exhibited to the sulfa drugs: sulfadiazine (91.9%) and kelfizina (75.9%). The resistance to bactrim was 41.7% and to the nitrofuranes: furadantin and urfadyn was 35.7% and 31.0%, respectively. The highest resistance to the penicillins was found for penicillin-G (70.3), baycillin (68.7%) and binotal (68.5%), the lowest for pyopen (33.7%) and ampiclox (34.7%); and for the others: stapenor, penbritin SQ 11302 and orbenin the resistance was respectively 55.8%, 56.0%, 58.0% and 60.4%. For cephaloridin the proportion of resistant strains was 37.7%. Resistance to chloramphenicol and derivatives was rather high: to micoflavin it was 50.2%, to chloromycetin 51.8%, to fultrexin 63.3% and to urfamycin 72.5%. Very high resistance was observed for tetracycline (82.1%), ledermycin (73.0%) and streptomycin (68.8%).

A relatively low resistance of the Staphylococcus aureus strains to the macrolide antibiotics: rovamycin

(35.1%), albamycin-T (37.9%) and erythrocin (39.5%), was found. The same was also exhibited to kanamycin (35.9%), gentamycin (31.3%) and gabbromycin (27.4%). To pristinamycin and rifampin the percentage was 46.5% and 41.8%, respectively. The lowest resistance to the isolated strains in this study was observed for soframycin (20.8%).

Differences in antibiotic-resistance of the Staphylococcus aureus strains were established in relation to the source from which the material was obtained. For more detailed analysis of the resistance patterns of strains, they were divided according to the material from which they were isolated, as exhibited in tables 3-10.

Of all the staphylococci isolated from each source, the highest percentages were sulfadiazine-resistant. The lowest percentage of resistance was noted for soframycin-resistant strains isolated from all sources, except nose specimens. From this material the strains were most sensitive to gabbromycin.

Discussion

The rather high proportion of positive culture samples that contained Staphylococcus aureus (46.6%) in comparison with other studies in 1966 by Mustakallio (10-35%), might be due to the omission of the coagulase test in this study. Although the Subcommittee on Taxonomy of Staphylococci and Micrococci (1965) recommends the coagulase production for the identification of pathogenic strains, the test was not performed to save labour, cost and time. In addition, some coagulase negative strains were found to be pathogenic (Cruickshank, 1960; Blair et al., 1970).

As there are many factors which may influence the results of the sensitivity testing, it would not be correct to regard the figures obtained from this study as being comparable with the results of other surveys. The data acquired here are useful solely for comparison of the relative in vitro activities of the 32 antibacterial agents against the Staphylococcus aureus strains tested in this particular study.

The overall high resistance to the drugs was attributed to the use of a proportionately large inoculum. By this method the resistant strains were better recognized.

The true significance of the variations in resistance to the antibacterial agents is difficult to assess, just as is the variation of the percentage rate for the various groups of pathological specimens. But a direct relationship between the incidence of resistant strains and specific antibiotic usage was noted. High resistance rate of the Staphylococci was observed to drugs which have been widely used since a long time in Indonesia, such as the sulfadrugs, penicillin, streptomycin and tetracyclin. On the other hand,

against the relatively new drugs (soframycin, gabbromycin, rovamycin and rifamycin) the tested strains showed to be more sensitive.

Cross-resistance between an antibacterial agent and its related compound, was observed in this study.

Summary and conclusions

The variations in the frequency of occurrence and sensitivity to 32 antibacterial agents of 4085 Staphylococcus aureus strains in samples found to contain bacteria in bacteriological examinations are discussed.

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