Reciprocal Sensitivities of *Staphylococcus aureus* to Streptomycin, Streptothricin, and Penicillin

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Some strains of *Staphylococcus aureus* are inhibited or destroyed by each of the antibiotic agents—penicillin, streptomycin, and streptothricin. It is well known that susceptible strains of *Staph. aureus* may develop fastness to penicillin under both *in vitro* and *in vivo* conditions. The studies reported below were aimed at determining reciprocal sensitivities of strains of *Staph. aureus* to each of the other two antibiotics, after developing *in vitro* resistance of a susceptible strain to streptomycin, streptothricin, and penicillin separately.

EXPERIMENTAL METHOD

Staph. aureus (F.D.A. 209) and *Escherichia coli* (Waksman's assay strain) were used in this work.

The penicillin used was a commercial sodium salt. The streptomycin and streptothricin were crude extracts from *Streptomyces griseus* and *Streptomyces lavendulae* cultures, respectively, which contained 900 and 1,100 units/ml. Various dilutions of each antibiotic were prepared. One ml. of each dilution was then added to 9 ml. of nutrient agar (N-Z Case nutrient agar[1] for penicillin), and the media were poured into Petri dishes. The agar plates were streaked with a 24-hour tryptose-phosphate broth culture of *Staph. aureus* and then incubated for 24 hours at 37° C. Colonies from plates containing the highest concentration of antibiotic agent permitting growth were selected, and transfers made to tryptose-phosphate broth. After incubation, the broth culture was streaked on nutrient agar containing a higher concentration of antibiotic than previously. Transfers of colonies after incubation were again made to tryptose-phosphate broth. This procedure was repeated many times with each antibiotic agent until highly resistant strains were obtained. The parent *Staph. aureus* was transferred daily in tryptose-phosphate broth and served as a control.

Each resistant strain and the parent culture were then tested for susceptibility to each antibiotic. *E. coli* was also streaked on the plates containing streptomycin and streptothricin to determine the unitage of the preparations. The procedure followed was the agar streak method commonly used for assaying these antibiotics.

[1] Methods used by F.D.A. for assay of penicillin, revised January 1945.

RESULTS

Strains of *Staph. aureus* obtained by the procedure outlined above were highly resistant to penicillin, streptothricin, and streptomycin. The development of maximum resistance was accomplished after 12 transfers for streptomycin, 25 transfers for streptothricin, and 32 transfers for penicillin.

The principal purpose of the investigation was to determine whether the development of resistance to one of the antibiotic agents would result in resistance to one or both of the others. An inspection of Table 1 reveals that the strain which was resistant to streptothricin was also more resistant to streptomycin than was the parent culture; however, the reverse of this was not true. With the exception noted, the development of resistance to one antibiotic agent did not result in increased resistance to either of the others.

TABLE 1

RESISTANCE OF STREPTOMYCIN-FAST, STREPTOTHRICIN-FAST, AND PENICILLIN-FAST STRAINS OF *Staph. aureus* TO:

Streptomycin

Strain	Dilution, 1: 20	40	80	100	200	300	400	500	600	700	800	900	1,000
Staph. aureus													
Sm R	3	3	3	3	3	3	3	3	3	3	3	3	3
St R	0	0	0	0	0	1	2	3	3	3	3	3	3
Pen R	0	0	0	0	0	0	0	0	0	0	0	1–	1
Parent	0	0	0	0	0	0	0	0	0	0	0	1–	1
E. coli	0	0	0	0	0	0	0	0	0	0	0	1–	1

Streptothricin

Strain.	Dilution, 1: 20	50	100	200	300	400	500	600	700	800	900	1,000	1,100	1,200
Staph. aureus														
St R	1	2	3	3	3	3	3	3	3	3	3	3	3	3
Sm R ...	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pen R ..	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Parent ..	0	0	0	0	0	0	0	0	0	0	0	0	0	1
E. coli	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Penicillin

Strain	Dilution, 1: 5	10	20	40	50	80	100	150	200	300	400	500	600
Staph. aureus													
Pen R ..	1	1	2	2	3	3	3	3	3	3	3	3	3
Sm R ...	0	0	0	0	0	0	0	0	1–	1	1	1	2
St R	0	0	0	0	0	0	0	0	0	0	1–	1	2
Parent .	0	0	0	0	0	0	0	0	0	1–	1	1	2

Sm R = streptomycin resistant; St R = streptothricin resistant; Pen R = penicillin resistant.
Growth: 3 = good; 2 = fair; 1 = slight; 1– = a few scattered colonies.

In vivo confirmation of the findings reported here would be highly significant to the clinician in the treatment of infectious diseases where penicillin fastness is encountered.

In Vitro Action of Monopyridine Iodine (I) *p*-Nitrobenzoate Against Ringworm Fungi

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In a previous report (*2*) it was demonstrated that compounds of positive univalent iodine, stabilized by coordination with pyridine, were toxic to some common pathogenic bacteria and to the saprophytic fungus, *Aspergillus niger*. The present communication deals with the action of various concentrations of the dry powder of monopyridine iodine (I) *p*-nitrobenzoate in sterile talc against the ringworm fungi, *Trichophyton gypseum* and *Microsporum audouini*. The evidence presented shows that low concentrations of the drug not only are effective against the above-mentioned fungi but also are extremely active toward the dried spores of these fungi.

EXPERIMENTS WITH *T. gypseum*

Strain #9533, obtained from the American Type Culture Collection, was used in this portion of the work.

The fungicidal activity of the *p*-nitrobenzoate against the dried spores was determined in the following manner: A saline suspension containing 24,000,000 conidia/ml. was prepared as described in a proposed A.P.H.A. method for testing fungicides against *Trichophyton* (*1*). Sterile pieces (5 mm.^{2}) of Whatman filter paper No. 40 were soaked in this suspension and dried at 37.5° C. for two to six days. Duplicate dried impregnated papers were immersed in various concentrations (3, 5, 10, 15, 20, 25, and 50 per cent) of the dry powder in sterile talc for intervals of 1, 5, and 10 minutes, after which they were washed twice in Sabouraud's liquid medium and deposited on Sabouraud's agar slants. Dry and washed controls were set up in duplicate. The slants were examined daily for two weeks. With two exceptions (10 per cent-5 minutes and 25 per cent-1 minute), no growth resulted from those papers which had been immersed in *p*-nitrobenzoate in concentrations of 10 per cent or greater. All controls grew out rapidly.

To test for fungistatic and fungicidal activity using the agar cup-plate method, the following experiments were performed: Sterile 9-cc. Petri dishes, in the center of which were sublimation rings, were filled with Sabouraud's agar. After the agar had hardened, the sublimation rings were removed and *T. gypseum* was streaked over the entire surface with a dry, sterile cotton swab. Then the cup was filled with the appropriate concentration of the drug in sterile talc. These plates were incubated at room temperature for approximately 10 days, and the zones of inhibition measured. The results are shown in Table 1. A portion of agar from the clear zone was transferred to a Sabouraud's glucose agar slant. The absence of growth on the slant after an incubation period of two weeks indicated that the compound is fungicidal toward *T. gypseum*.

TABLE 1

FUNGISTATIC ACTION ON *T. gypseum* BY MONOPYRIDINE IODINE (I) *p*-NITROBENZOATE

Per cent drug	3	5	10	15
Zone of inhibition* (in mm.)	12	16	19	23

* The averages of triplicate plates.

EXPERIMENTS WITH *M. audouini*

A saline suspension of conidia of a freshly isolated strain of *M. audouini* from a case of ringworm of the scalp was diluted to 18 per cent light transmittance as measured against a saline standard on a Lumetron photoelectric colorimeter, Model 400-G.[1] Sterile filter papers (5 mm.^{2}) were soaked in the suspension and dried for only 24 hours at 37.5° C., since the spores of *M. audouini* proved to be extremely sensitive to drying as compared to those of *T. gypseum*. Duplicate test papers were immersed in various concentrations (3, 5, 10, and 15 per cent) of the dry drug in sterile talc for intervals of 1, 5, and 10 minutes and treated as described previously for *T. gypseum*. At the time the slants were discarded after one month of observation, growth had occurred only in the controls and the papers treated with 3 per cent of the compound.

The agar cup-plate technique described for testing the fungistatic activity of monopyridine iodine (I) *p*-nitrobenzoate against *T. gypseum* proved less successful with *M. audouini*, since regular zones of inhibition could not be obtained because of the lack of uniform growth. However, the areas of inhibition in the case of the latter were greater than those obtained with the former for corresponding concentrations of compound.

DISCUSSION

Monopyridine iodine (I) *p*-nitrobenzoate, I (C_5H_5N),

[1] It proved impossible to make a count of the conidia in a haemocytometer.