Reprinted from the "Proceedings of the Indian Academy of Sciences," Vol. XLVII, 1958

INHIBITION OF BACTERIA FROM MARINE SOURCES BY AUREOMYCIN

By

N. K. Velankar

Reprinted from "The Proceedings of the Indian Academy of Sciences"
Vol. XLVII, No. 2, Sec. B, 1958

INHIBITION OF BACTERIA FROM MARINE SOURCES BY AUREOMYCIN*†

BY N. K. VELANKAR
(Central Marine Fisheries Research Station, Mandapam)

Received August 22, 1957
(Communicated by Dr. S. Jones, F.A.SC.)

INHIBITION of marine bacteria by antibiotics is engaging the attention of researchers in the fields of marine biology and fisheries during recent years. Spencer (1952) used antibiotics in the isolation of bacteria-free cultures of marine phytoplankton organisms. Shewan *et al.* (1954) suggested a method for rapid differentiation of asporogenous rods commonly occurring in the marine environment based on the differences in their sensitivity to certain antibiotics. Oppenheimer (1955) in his studies on the effect of marine bacteria on the development and hatching of pelagic fish eggs, employed various antibiotics including aureomycin, singly and in different combinations, for controlling bacterial growth. Tarr *et al.* (1950) studied the effect of several antibiotics in retarding the spoilage of fish stored in ice and reported aureomycin to be most effective in low concentrations.

The bacteria which cause spoilage in sea fish are largely of marine origin, though other bacteria might also be introduced through handling. Further, certain bacterial genera, *i.e.*, *Achromobacter* and *Pseudomonas* appear to be more significant than the others in fish spoilage (Shewan, 1949; Wood, 1940). In view of the promise held forth by aureomycin as a possible preservative for fish it is of practical importance to know to what extent marine bacteria are affected qualitatively and quantitatively by this antibiotic. In this connection, the inhibiting action of aureomycin in different concentrations on the growth of a number of bacterial species isolated from the marine environment and from sea fish, and on the bacterial population of sea-water, was studied. The results are reported in this paper.

MATERIALS AND METHODS

The bacterial cultures used for these studies were from the stock of well-documented types isolated in this laboratory (Velankar, 1957). A freshly

* Published with the permission of the Chief Research Officer, Central Marine Fisheries Research Station, Mandapam Camp.

† Abstract in *Proc. Ind. Sci. Cong.*, January 1957.

prepared concentrated solution of aureomycin hydrochloride was sterilised by filtration and used for incorporating in agar medium. After melting the agar the aureomycin solution was added aseptically at 42° C. rapidly and slopes prepared. A loopful of growth on agar of the stock culture was taken up in 5 ml. of sterile saline (1% NaCl in distilled water) and a loopful of the suspension was streaked on aureomycin-treated and control agar slopes. In the case of the organisms which required sea-water or 3% NaCl for optimum growth sea-water agar was employed; for the rest of the cultures fresh-water agar was used.

The growth appearing on the inoculated slopes was observed every twelve hours.

Sea-water samples were collected in sterile 1 lb. glass bottles from about 2 miles off the shore in the Palk Bay at Mandapam.

The stability of aureomycin at the level of 2 p.p.m., *i.e.*, the lowest concentration employed in these studies, in sea-water agar at room temperatures was determined empirically as follows: Sea-water agar slopes containing 2 p.p.m. of aureomycin hydrochloride were prepared as described above; on each successive day one of these and a control agar slope (containing no aureomycin) were inoculated with an organism which was known to be very sensitive to 2 p.p.m. of aureomycin. The growth appearing on the control and the aureomycin treated slope was observed after 24 hours. The absence of growth on the test slope and the presence of good growth on the control slope was taken as the indication of the stability of the antibiotic at room temperature in sea-water agar medium.

RESULTS

The results are shown in Tables I to V.

With the exception of two or three all the bacteria were sensitive to aureomycin. Approximately 40% were sensitive to the level of 2 p.p.m., 30% to 5 p.p.m. and 30% to 20 p.p.m. In the case of those sensitive to 2 p.p.m. the lag period (Table I) varied from ½ to 4 days; in those sensitive to 5 p.p.m. from 1 to 3 days and in those sensitive to 20 p.p.m., the period was about 4 days. Bacteria sensitive to 2 p.p.m. included spp. of Gram-negative achromic rods (motile with polar or peritrichous flagellation or non-motile), *Vibrio*, *Flavobacterium*, *Bacterium* and *Bacillus* and an agar digester resembling *Cytophaga*. Yellow, red and violet pigment producing polar flagellated rods and denitrifiers belonging to the *Pseudomonas* were sensitive to higher levels, *i.e.*, 5 and 20 p.p.m. The cocci were more resistant than the Gram-negative rods. In the *Bacillus* the degree of sensitivity varied greatly; the isolate

TABLE I

Duration of lag (in days before growth occurred on agar containing aureomycin in different concentrations)

Sl. No. of culture	Brief description of the organism	Source	Parts per million of aureomycin hydrochloride			
			2	5	20	50
1	Gram-negative, achromic motile rod; polar flagellation	Sea-water & mud	—	—	4	4
2	,,	,,	—	½	4	5
3	,,	Sea-water	—	½	3	×
4	,,	,,	1	2	3	5
5	,,	Marine mud	—	½	1	3
6	,,	Sea-water	—	—	1½	3
7	,,	Spoiled fish	—	½	1	×
8	,,	,,	—	1	1½	3
9	,,	Sea-water	½	3	3	×
10	,, (curved cells also present)	,,	—	1	1½	×
11	,, Denitrifier	,,	—	—	4	×
12	,, Denitrifier	,,	½	½	3	3
13	Gram-negative curved rod (*vibrio*) single polar flagellum	Marine mud	1	1	×	×
14	Gram-negative, non-motile rods in chains	,,	—	1	3	3
15	Gram-negative, non-motile rods; show bipolar staining; slight pinkish pigment	Sea-water	×	×	×	×
16	Gram-negative, non-motile flattened cells (coccoid)	Spoiled fish	1	1	3	4
17	,,	,,	—	—	1	4

TABLE I—*Contd.*

Sl. No. of culture	Brief description of the organism	Source	Parts per million of aureomycin hydrochloride			
			2	5	20	50
18	Gram-negative motile rods with peritrichus flagellation	Marine mud	½	½	3	×
19	,, (Produces gas from lactose)	Spoiled fish	—	—	—	—
20	Small Gram-negative rods non-motile prage pigment	Sea-water	—	—	×	×
21	Gram-negative, non-motile rods, canary, yellow pigment	,,	1	1	×	×
22	Gram-negative-non motile rods; red pigment	Sea-water	4	×	×	×
23	,, Yellow pigment	Fish slime	—	—	×	×
24	Gram-negative-motile rods; polar flagellation; orange yellow pigment and a dark diffusible pigment	Sea-water	—	—	3½	4
25	Gram-negative non-motile long (1·5 μ × 6–12 μ) rods; digest agar; flesh coloured pigment	,,	3	3	3	×
26	Gram-negative motile rods, single polar flagellum	,,	½	1	×	×
27	Gram-negative motile rods; polar flagellation; light yellow pigment	,,	—	½	3	3
28	Gram-negative motile rods; polar flagellation; bright pink (magenta) pigment	,,	—	—	—	1
29	Gram-positive cocci in clusters; rose pigment	,,	—	½	1	2

TABLE I—*Contd.*

Sl. No. of culture	Brief description of the organism	Source	Parts per million of aureomycin hydrochloride			
			2	5	20	50
30	Gram-positive cocci; golden yellow pigment	Fish muscle	—	—	1	1½
31	Gram-positive cocci; lemon yellow pigment	,,	—	—	1	1½
32	Gram-positive cocci (in clusters); no pigment	Shark muscle	1	1	3	4
33	Gram-positive cubical packets; sulphur yellow pigment (*Sorcinæ* sp.)	Sea-water	—	1	2	4
34	Gram-positive cocci; apricot pigment	Spoiled fish	1	1	2	4
35	Gram-positive cocci; shining orange pigment	,,	—	—	—	—
36	Gram-positive cocci; orange pigment	,,	—	—	—	—
37	Gram-positive rods with central spores	Sea-water and mud	1	1	3	×
38	,,	Marine mud	½	½	2	×
39	,,	,,	6	×	×	×
40	Gram-positive rods with central spores; produce yellow pigment	Marine mud	½	2	7	×
41	Gram-positive, large (more than 1·2 μ dia.) with spores	Spoiled fish	—	—	1	2
42	Gram-positive rods with spores; produce red pigment	,,	—	—	1	2
43	Gram-positive rods with spores; medium size (*B. subtilis* sp.)	,,	½	1	1	2

TABLE I—*Contd.*

Sl .No. of culture	Brief description of the organism	Source	Parts per million of aureomycin hydrochloride			
			2	5	20	50
44	Gram-positive rods with central spores	Fish muscle (at 0° C.)	×	×	×	×
45	*Nocardia* sp.; produces dark diffusible pigment	Sea-water	—	½	1	2
46	*Nocardia* sp. produces yellow pigment	,,	—	—	—	1
47	Black pigment producing yeast-like cells	,,	—	—	—	—
48	Gram-negative, asporogenous, motile rods with polar flagellation; violet pigment produced	,,	—	—	—	1

Note.—×, No growth was observed up to 8 days incubation. —, No lag was observed.

from fish muscle at 0° C., No. 44, was inhibited completely by 2 p.p.m. while the isolate from fish muscle at room temperature, No. 41, was unaffected by 5 p.p.m. The black pigment producing yeast was insensitive to aureomycin while the *Nocardia* sp. was slightly inhibited. One sp. of Gram-negative rod was insensitive to all the levels of aureomycin (2 to 50 p.p.m.) employed.

Sensitivity to low concentrations of aureomycin is thus common to a variety of morphological and physiological types of bacteria from marine sources. At 5 p.p.m. level significant lag before growth occurred was observed in 75% of the achromic Gram-negative rods, 50% of Gram-negative chromogenic rods, 50% of the cocci and in over 80% of the spore-forming rods. In about 70% of the total number of bacteria examined growth was delayed from ½ to 3 days at room temperature when 5 p.p.m. of aureomycin was used. The lag period may conceivably extend significantly at 0° C. The use of ice containing aureomycin in fairly low concentrations for enhancing the normal preservation period of fish in ice is therefore justified on theoretical considerations.

Plate counts of fish slime on aureomycin-containing agar (Table II) showed a reduction to ⅓rd at 2 p.p.m. and to 1/25th at 5 p.p.m. In the case of the sea-water sample off the pier the count was reduced to 1/10th at 2 p.p.m. to 1/20th at 5 p.p.m. and 1/80th at 20 p.p.m. (Table III). In the case of the two sea-water samples from 2 miles off the shore the count was reduced to ½ and ⅛th at 2 p.p.m. to 1/20th at 5 p.p.m. and to nil at 20 and 50 p.p.m. levels.

TABLE II

Plate count of fish slime on sea-water agar containing different levels of aureomycin

	Control	p.p.m. of aureomycin	
		2	3
Skin scrapings from 1 sq. cm. area of fresh fish (*Chorinemus* sp.) taken up in 100 c.c. of sterile saline; this was further diluted to 100 times and 1 c.c. of the diluted suspension was plated	154	58	6

TABLE III

Plate count of sea-water samples on agar containing different concentrations of aureomycin

Sample No.		Control	p.p.m. of aureomycin			
			2	5	20	50
I Sea-water sample 2 miles off-shore (Palk Bay)	Surface water (1 c.c.)	910	490	62	nil	nil
	Water from near the bottom (1 c.c.)	760	360	22	1 (mold colony)	..
II Do.	Surface water	1000 (approx.)	208	60	nil	nil
	Water from near the bottom	39	nil	nil	8 (molds)	5 (molds)
III Sea-water off the jetty (Gulf of Mannar)	Surface water 1/10 c.c.	850	84	48	10	nil

At 50 p.p.m. level no growth occurred in 40% of Gram-negative achromic rods, 60% of the Gram-negative chromogenic rods and 60% of the *Bacillus*; in all the cocci strains examined growth occurred even at 50 p.p.m. (Table I). Since 50 p.p.m. levels appear to be bactericidal for a considerable proportion of the asporogenous rods and spore-formers, which are the types concerned in causing fish spoilage "initial dips" in solutions of 50 p.p.m. of aureomycin for enhancing the keeping period may prove useful.

The results shown in Tables I, II and III indicate that a concentration of 5 p.p.m. of aureomycin is likely to be much more effective than 2 p.p.m. Though Tarr *et al.* have reported levels of 2 p.p.m. and even lower to be effective in fish preservation, recent work at Torrey Research Station indicates the need for using levels of 5 p.p.m. in preservative ices (Ingram *et al.*, 1956).

Experiments carried out in this laboratory on the storage of fish in ice have also indicated the efficiency of ice containing 5 p.p.m. of aureomycin in enhancing the storage life [Velankar and Kamasastri (in press)].

In sea-water to which aureomycin was added at 5 p.p.m. level the bacterial count after 24 hours was $\frac{1}{4}$ of that of the control (Table IV); 20 p.p.m.

TABLE IV

Effect of addition of aureomycin at levels of 5 and 20 p.p.m. on the multiplication of bacteria in a sample of sea-water

	Plate counts per ml.		
	0 hrs.	6 hrs.	24 hrs.
Control ..	630	2,500	2,600,000
5 p.p.m. ..	610	3,100	730,000
20 p.p.m. ..	650	2,180	635,000

concentration of aureomycin did not reduce the bacterial population to any greater extent than the 5 p.p.m. level. The bacteria present in the aureomycin-treated sea-water samples consisted of very few spp. unlike the bacteria in the untreated sea-water.

TABLE V

Stability of aureomycin in sea-water agar at room temperature at 2 p.p.m. level

Number of days since preparation of slopes	Growth 24 hrs. after inoculating with the test culture	
	Control	Aureomycin
0	+++	−−−
2	+++	−−−
4	+++	−−−
5	+++	−−−
6	+++	−−−
7	+++	−−−
8	+++	−−−
10	+++	++−

SUMMARY

The effect of aureomycin at levels of 2, 5, 20 and 50 parts per million was observed in the case of about 50 bacterial spp. isolated from marine sources.

Seventy per cent. of the bacteria were sensitive to 5 p.p.m. or lesser concentration of the antibiotic. The cocci were more resistant than the Gram-negative rods while sensitivity to aureomycin varied greatly in the *Bacillus*. The lag period before visible growth occurred on agar slopes at room temperature usually varied from ½ to 4 days.

Plate counts of fresh fish slime on aureomycin containing agar showed a reduction to ⅓rd at 2 p.p.m. and to 1/25th at 5 p.p.m. level. In the case of the sea-water the count was reduced to ⅛th at 2 p.p.m. and to 1/20th at 5 p.p.m. levels.

The probable implication of these observations on the use of aureomycin in ices and in "initial dips" for preservation of fish is discussed.

ACKNOWLEDGEMENT

The author wishes to express his grateful thanks to Messrs. Lederle Laboratories Ltd., Bombay, for the gift of pure aureomycin hydrochloride for experimental use.

REFERENCES

1. Ingram, M. Barnes, Ella, M. and Shewan, J. M. — "Problems in the use of antibiotics for preserving meat and fish," *Food Science Abstracts, London*, 1956, **28** (2), 121.

2. Oppenheimer, Carl H. — "Effect of marine bacteria on the development and hatching of pelagic fish eggs, and the control of such bacteria by antibiotics," *Copeia* (*USA*), 1955, No. 1, 43.

3. Shewan, J. M. — "Some bacteriological aspects of handling, processing and distribution of fish," *J. Roy. Sanit. Inst.*, 1949, **69**, 934.

4. ———, Hodgkiss, W. and Liston, J. — "A method for rapid differentiation of certain non-pathogenic asporogenous bacilii," *Nature* (*London*), 1954, **173**, 208.

5. Spencer, C. P. — "On the use of antibiotics for isolating bacteria-free cultures of marine phytoplankton organisms," *Mar. Biol. Assoc., UK*, 1952, **31**, 97.

6. Tarr, H. L. A., Southcott, B. A. and Bisset, H. M. — "Effect of several antibiotics and food preservatives in retarding bacterial spoilage of fish," *Fish. Res. Bd. Canada, Pac. Coast Prog. Rep.*, 1950, No. 83, 35.

7. Velankar, N. K. — "Bacteria isolated from sea-water and marine mud off Mandapam (Gulf of Mannar and Palk Bay)," *Indian Jour. Fish*, 1957, **4** (1), 208.

8. Wood, E. J. Fergusson — "Bacteriology of fish marketing in Eastern Australia," *Coun. Sci. Industr. Res. Austr. Pamphlet*, 1940, No. 100.