REDUCTION OF AMOEBICIDAL ACTIVITY OF METRONIDAZOLE (FLAGYL) BY BACTERIA

FIVE human gut bacteria *Escherichia coli, Streptococcus faecalis, Pseudomonas aerugimosa, Staphylococcus aureus* and *Proteus mirabilis* and a mixture of these bacteria were used to observe if they reduce the amoebicidal activity of flagyl (metronidazole, May and Baker) against axenically grown *Entamoeba histolytica*, strain NIH–200. It was observed that mixed bacteria reduced the amoebicidal property of flagyl markedly. *P. aeruginosa* reduced the activity moderately; whereas the other four bacteria cause less reduction in the amoebicidal activity when compared with control. This finding may have a direct bearing on the failure of flagyl in curing cases of amoebiasis patients as reported by some workers.

Candy and Ariz[1], Griffin[5], Dutt, Singh and Chuttan[4], Pittman and Pittman[7], reported about the failure of metronidazole in curing amoebiasis. Bacterial inactivation of flagyl has been mentioned by McFaddzean[6]. But no detailed experimental observations have been reported so far. The present communication deals with experimental observations on the reduction of amoebicidal activity of flagyl against axenically grown *E. histolytica* by the intestinal bacteria.

Metronidazole (pure powder) was obtained from May and Baker. Each of the bacteria was grown for 72 h in 1% peptone water. Twenty mg of flagyl was then added to 200 ml of bacterized peptone water and incubated at 37° C for various periods (Table 1). After every 24 h, the sterile filtrates were collected with the help of sietz filters from the flagyl added bacterial peptone water. Amoebicidal activity of each filtrate was tested against axenically grown *E. histolytica* following the method of Das[2]. Flagyl in peptone water, without bacteria served as control. Bacterial culture filtrates with flagyl, collected aseptically at every 24 h, were exposed to trophozoites of axenically grown *E. histolytica* in cavity slides with sealed cover slips. Double drug dilution method was followed in preparing drug concentrations. Concentrations of flagyl required to kill *E. histolytica* trophozoites in the case of the drug exposed to bacteria was estimated and compared with bacteria-unexposed flagyl concentration required to kill trophozoites. Thus the reduction in the amoebicidal action of flagyl by different bacteria was determined. Duplicate sets for each drug concentration were taken into consideration for estimating the amoebicidal action.

Results of the experiments are given in Table 1. Metronidazole exposed *P. mirabilis, Staph. aureus. Strep. faecalis* cultures for 1, 2 and 3 days could kill trophozoites of axenic *E. histolytica* at 3·12 μg/ml, whereas exposure upto 4 and days, to these bacteria 6·25 μg/ml metronidazole could only kill the trophozoites. The drug exposed to *E. coli* for 1 to 2

TABLE 1

Reduction in the amoebicidal activity of metronidazole by some intestinal bacteria

Bacteria	Exposure period (day of 'flagyl' to bacteria)	Amoebicidal end point (μg/ml)
Proteus mirabilis	1	3·12
	2	3·12
	3	3·12
	4	6·25
	7	6·25
Staphylococcus aureus	1	3·12
	2	3·12
	3	3·12
	4	6·25
	7	6·25
Streptococcus faecalis	1	3·12
	2	3·12
	3	3·12
	4	6·25
	7	6·25
Escherichia coli	1	3·12
	2	3·12
	3	6·25
	4	6·25
	7	6·25
Pseudomonas aeruginosa	1	3·12
	2	3·12
	3	6·25
	4	6·25
	5	12·50
	6	25·00
	7	25·00
Mixed bacteria	1	3·12
	2	3·12
	3	6·25
	4	50·00
	5	50·00
	6	50·00
	7	50·00
Control	1	1·00
	2	1·00
	3	1·00
	4	1·00
	5	1·00
	6	1·00
	7	1·00

days and 3 to 7 days killed the trophozoites at 3·12 and 6·25 μg/ml respectively. But flagyl when exposed to *P. aeruginosa* for 1 to 2, 3 to 4, 5 and 6 to 7 days, the concentrations of drug required to kill the trophozoites were 3·12, 6·35, 12·5 and 25·0 μg/ml respectively. When the drug was exposed to the mixture of these bacteria, for 1 to 2, 3, 4 to 7 days, 3·12, 6·25 and 50 μg/ml were required to kill the amoebae respectively. In the case of the control, the amoebicidal end point of flagyl was at 1·0 μg/ml and this activity remained constant for 1 to 7 days exposure to peptone water.

From the results, it can be concluded that all the five bacteria, tried to determine the inactivation of amoebicidal activity of metronidazole, were able to reduce more less, the amoebicidal action of the drug against *E. histolytica.*

Individually *P. aeruginosa* has the maximum reduction capacity among the five bacteria tried. The mixed bacterial population could reduce the amoebicidal activity of metronidazloe two times more than that of *P. aeruginosa* alone when the drug was exposed for 4 to 7 days in the bacterial cultures.

Metronidazole (flagyl) of May and Baker is a drug of choice against clinical amoebiasis. It is highly amoebicidal and kills trophozoites of *E. histolytica* at 1·0 μg/ml[3]. Failure of 'flagyl' in curing invasive and chronic amoebiases cases although are not many, still a few reports have appeared in scientific journals, (see introduction). Inactivation of amoebicidal property of flagyl by bacteria may be the reason. No experimental proof has been reported about the bacterial inactivation of metronidazole. The experimental results presented in this communication clearly reveals that bacteria can reduce the amoebicidal property of 'flagyl' although they may not completely inactivate the drug action against *E. histolytica*.

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NOTE ON SECONDARY INFECTION OF DOWNY MILDEW IN PEARL MILLET

DOWNY MILDEW of pearl millet is a serious disease in India. Its severe infection had eliminated the cultivation of even the most widely adapted productive hybrids like H.B.-3 and H.B.-4. Even the most recently released resistant hybrids show susceptibility to a certain extent (7–10%) as reported in the IPMDMN (International Pearl Millet Downy Mildew Nursery Report[1] by ICRISAT.

The disease is presumed to be both soil as well as air borne. It is also believed to be partly carried by seed. The primary infection takes place from the soil by the oospores while the secondary or asexual infection occurs through air by the dispersal of sporangia.

In the present investigation nearly 1,672 lines of pearl millet, comprising, both segregating as well as non-segregating progenies, were evaluated for the intensity of secondary downy mildew infection. The progenies were sown in a sick plot. Susceptible plants were counted at two stages of plant growth : 1. *seedling (from 4–5 leaf stage to tillering)* and 2. Maturity (after heading to grain filling). Infector rows of old H.B.-3 were planted after each 10 lines to provide the spread of infection. First counting of *susceptible plants showing clear symptoms of infection, viz.,* yellowish broad streaks with whitish down from one end to another end of leaves of various length, was made from 4–5 leaf stage to tillering stage, in each row. *Second counting of infected plants* was made after heading to grain filling stage. The data are presented in Table I. The additional number of

TABLE I

Extent of primary and seconday infection of downy mildew (Sclerospora graminicola) *in pearl millet*

Genotypes	No. of lines observed	Number of susceptible lines		
		Primary infection	Secondary infection	Total infected lines
Inbreds	756	44	194	238
Segregating lines	577	36	81	117
Biparental progenies	144	30	27	57
Do.	78	27	37	64
Downy mildew resistant lines	76	19	19	38
White grained lines	41	2	8	10
Total	1,672	158	366	524
Per cent	—	9·4	21·8	31·2