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Effect of Nitrogen Dye Laser on Viability, Biochemical Characteristics and Virulence of *Escherichia coli*

S Ananthan* P Saravanan and SV Alavandi

Department of Microbiology, Dr.A.L.M.Post Graduate Institute of Basic Medical Sciences,University of Madras, Taramani Campus, Chennai - 600 113, **India.**

The nitrogen dye laser showed a ten fold bactericidal effect on the *Escherichia coli* isolates obtained from cases of diarrhoea in children. Fermentative metabolism of glucose and dulcitol, production of indole and amino acid decarboxylation activity of the *E.coli* isolates were altered upon exposure to this laser. Exposure of bacteria to the nitrogen dye laser induced no changes in their antibiogram. Out of the 20 *E.coli* isolates, 14 were enterotoxigenic, 4 of which lost their toxigenicity upon exposure to the laser.

Key words: Lasers, *Escherichia coli*, enterotoxin, antibiogram

Lasers are now being increasingly applied for diagnostic and therapeutic purposes and also as surgical tools in medicine (1,2). Application of lasers in microbiology to explore their bactericidal properties have been reported by several workers. Bactericidal properties of helium-neon lasers have been reported on organisms like *Streptococcus mutans, S.sobrinus*, *Lactobacillus casei* and *Actinomyces viscosus* causing dental caries (3). Similarly, ruby lasers have been found to be bactericidal to protozoa (4), neodymium-YAG laser to *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* (5).

The present study was undertaken to explore the role of lasers in intestinal infections by examining the effect of nitrogen dye lasers on the viability, biochemical characteristics, antibiotic sensitivity and virulence properties of *Escherichia coli* isolates obtained from cases of diarrhoea in children.

Materials and methods

Bacterial isolates: Stool samples from cases of diarrhoea in children admitted to the Institute of Child Health and Hospital for Children, Chennai, were routinely cultured for enteropathogens. Bacterial isolates were identified biochemically (6) and 20 *E.coli* isolates obtained during this study were selected for the present study. Antibiotic sensitivity pattern of these isolates was determined by disc diffusion method (7). Bacteria were serotyped by slide agglutination method using polyvalent antisera.

Enterotoxin assay: The bacterial isolates were tested for enterotoxigenicity according to methods of Dean *et al.* (8) with some modifications. The isolates were grown in 10 ml of casamino acids yeast extract broth at 37° C in 25 ml conical flasks and incubated on a shaker for 24 h. The cell free extract of each isolate was prepared by centrifugation of broth at 10,000 rpm at 4° C for 30 min. The supernatant was stored at -20° C till assay. Enterotoxin assay was performed using 2 to 3 d old infant mice (9). Two infant mice were used for each bacterial isolate. 0.1 ml of culture supernatant was injected into the stomach through the body wall using 30 gauge hypodermic needle. After 4 h, mice were sacrificed with chloroform and the abdomen was opened for examination of fluid accumulation in the gut. The small intestines were removed and weighed. A ratio of 0.09 between gut weight and body weight was taken as a positive result in addition to visual observations on fluid accumulation in the gut.

Effect of lasers on viability: The experiment was carried out on one of the enterotoxigenic isolates of *E.coli* serotype 0 8 by exposing the bacteria to the nitrogen dye laser. The bacterium was grown in nutrient broth for 24 hours at 37° C. A loopful of culture was added to 5 ml sterile normal saline and further diluted to 1:10 and 1:50 for laser treatment. Viable counts of bacteria in these suspensions was determined by dilution plate method. The bacterial suspensions (1 ml each) was placed in sterile vials for exposure to laser. Coumarine 7 (0.001%) prepared freshly in methanol was added to each cell suspension prior to irradiation to give a final concentration of 0.25 g ml^{-1}. The cell suspensions were irradiated with nitrogen dye laser (20 mW) at a wave length of 505 nm. Total viable count of the bacteria after exposure to the nitrogen dye laser was determined by serial dilution plate method.

Effect of lasers on biochemical characteristics, antibiogram and enterotoxigenicity: Overnight grown cultures of bacteria in nutrient broth (1 ml each), were exposed to nitrogen dye laser as described above. After exposure to the laser, the bacterial suspensions were

*Corresponding author

plated on to Mac Conkey's agar and the pure cultures were again subjected to biochemical, antibiotic sensitivity and enterotoxigenicity testing as described earlier.

Results and Discussions

Out of 20 isolates of *E. coli* tested, 14 were found to be enterotoxigenic by the infant mouse assay (Table 1). Among these two isolates of serotype O 8 were found to be highly enterotoxigenic considering their cytotonic response in the infant mouse assay. The serotype O 78 also showed significant secretary response. Earlier investigations have demonstrated that these serotypes are toxigenic (10).

Table 1 Enteropathogenicity of *E.coli* isolates using infant mouse assay prior to and after exposure to nitrogen dye laser for 70 sec (20 mW at 505 nm).

Isolate No.	Sero type	Before exposure,		After exposure,	
		gut: body wt Ratio	Inference*	gut: body wt ratio	Inference*
1	0 26	0.082	-	-	-
2	0 86	0.073	-	-	-
3	0 55	0.090	+	0.082	-
4	0 8	0.111	+	0.102	+
5	0 25	0.093	+	0.086	-
6	0 78	0.112	+	0.098	+
7	0 119	0.084	-	0.082	-
8	0 111	0.087	-	0.086	-
9	0 143	0.094	+	0.090	+
10	0 124	0.082	-	0.082	-
11	0 8	0.098	+	0.096	+
12	0 114	0.090	+	0.088	-
13	0 119	0.096	+	0.092	+
14	0 124	0.072	-	0.080	-
15	0 55	0.096	+	0.094	+
16	0 148	0.097	+	0.096	+
17	0 119	0.092	+	0.088	-
18	0 15	0.092	+	0.092	+
19	0 6	0.094	+	0.088	-
20	0 111	0.091	+	0.088	-

*Isolates showing gut:body wt ratio 0f >0.090 are enterotoxigenic.

Among the 14 enterotoxigenic *E. coli* isolates, tested for enterotoxigenicity after exposure to nitrogen-dye laser for 70 sec, 8 isolates were still able to induce diarrheagenic response in the infant mouse assay.

The *E.coli* serotype O 8, when irradiated with nitrogen dye laser after sensitization with coumarine 7 dye, caused significant energy dose related decrease in their viability (Fig.1). The first suspension of bacteria upon exposure to laser for 70 sec caused nearly a ten fold decrease in the bacterial counts. Similarly, the higher dilution of bacterial suspensions upon exposure to the nitrogen dye laser caused nearly 7 times and 4 times decrease in the colony forming units (cfu) of bacteria.

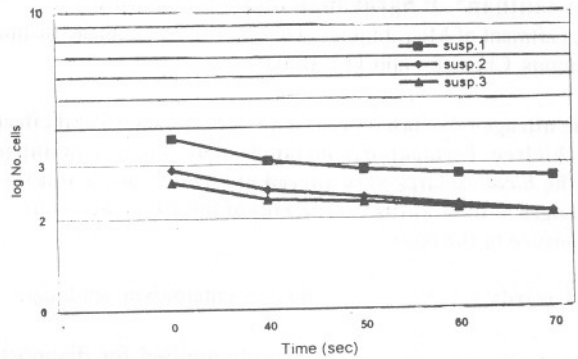

Figure 1. Survival of enterotoxigenic *E,coli* serotype 0 8 exposed to nitrogen dye laser up to 70 sec.

Some of the biochemical characteristics of the *E. coli* isolates upon exposure to nitrogen dye laser are depicted in table 2. Fermentative metabolism of carbohydrates such as glucose and dulcitol was hampered upon exposure of the bacteria to the nitrogen dye laser for 70 sec and 40 sec respectively. The amino acid decarboxylase activities of the bacteria was also affected. The bacteria upon exposure to the laser failed to decarboxylate ornithine and at the same time gained arginine dihydrolase activity. The bacterium also lost its ability to produce indole.

Table 2. Some biochemical characteristics of *E.coli* after exposure to nitrogen dye laser.

Biochemical character	Duration of exposure (sec)				
	C	40	50	60	70
Glucose fermentation	+ (20)	+ (20)	+ (18)	+ (15)	- (20)
Dulcitol fermentation	+ (20)	- (20)	- (20)	- (20)	- (20)
Indole production	+ (20)	+ (20)	- (20)	- (20)	- (20)
Ornithine decarboxylase	+ (20)	+ (20)	+ (16)	- (18)	- (20)
Arginine dihydrolase	- (20)	- (20)	- (18)	+ (13)	+ (20)

Figures in parentheses are number of isolates showing the characteristics.

Exposure of bacteria to nitrogen dye laser did not show any impact on their antibiotic sensitivity/resistance patterns (Table 3).

The study has indicated that nitrogen-dye laser has significant killing effect on the bacteria with possible mutagenic effects on the metabolism of carbohydrates such as glucose and dulcitol and decarboxylation of amino acids in the surviving bacteria. Earlier studies have

Table 3. Antibiogram of *E.coli* isolates (n=20).

Antibiotics	Concentration (μ g ml^{-1})	Sensitivity (S)/ Resistance (R)
Ampicillin	10	S (17)
Cephaloridine	30	R (18)
Erythromycin	15	R (13)
Gentamycin	10	S (17)
Kanamycin	30	S (16)
Nalidixic acid	30	S (19)
Streptomycin	10	S (12)
Sulfadiazine	300	S (17)

No change in antibiogram was noticed upon exposure to nitrogen dye (N2/ D 70 sec) laser.

shown that photosensitization of bacteria with dyes such as methylene blue enhanced the bactericidal properties of YAG laser on *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* (5). Similarly, bactericidal effect of He-Ne laser against cariogenic bacteria such as *Streptococcus mutans, S. sobrinus, Lactobacillus casei* and *Actinomyses viscosus*, when toluidine blue-O (TBO) was incorporated in the cell suspensions (3). In a report concurring lethal photosensitization of cariogenic organisms, Venezio *et al.* (9) reported that *S. mutans* could be killed by irradiation with polychromatic light after the organisms had been sensitised with haematoporhyrin derivative. In the present study, bactericidal effect of the nitrogen-dye laser observed, could be attributed to the coumarine-7 sensitization of the bacterial cells and this may have clinical implications for the treatment of *E. coli* infections.

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