# Relation Between The Presence Of Clostridial Spores And Soil Constituents With Special Reference To Antibiogram

N Maitra, N Nag, R Ghosh

**Citation**

N Maitra, N Nag, R Ghosh. *Relation Between The Presence Of Clostridial Spores And Soil Constituents With Special Reference To Antibiogram*. The Internet Journal of Microbiology. 2007 Volume 5 Number 1.

**Abstract**

The Clostridial group of organisms are ubiquitous in nature and distributed in soil, water, milk, dust, faeces, fish meal, meat and meat products etc. The present study was carried out taking 120 soil samples from eight different districts of the state of West Bengal India to find out the presence of Clostridial spores and its relation to soil constituents. Comparatively high population of Clostridial spore found in the district where soil texture were silty loam in nature and high significant ($P<0.01$) positive correlation between Clostridial spore count and Exchangeable Calcium, Magnesium, Iron content of the soil were observed where as non significant correlation between Clostridial spore and Organic carbon, exchangeable potassium and Sodium content were found. Antibiotic sensitivity test revealed that Clostridial group of organisms are resistant to Gentamycin, Kanamycin, Neomycin, Streptomycin, Tetracycline, Amikacin Cotrimoxazole and sensitive to Amixycillin, Ampicillin, Bacitracin, Chloramphenicol, Metronidazole, Erythromycin and penicillin-G.

## INTRODUCTION

The Clostridial group of organisms are ubiquitous in nature and distributed in soil, water, milk, dust, faeces, fish meal, meat and meat products etc. The main habitat of anaerobic Clostridial organisms are undoubtedly soil (Wilson et al., 1983-84). Das (1977) and Wilkins et al., 1988 isolated the Clostridium tetani from pasture and cultivated soil where spores are capable of persisting for many years (Blood et al., 1979). The viability of the spores may be dependent on some factors of the soil. Weller (1941) suggested that soil texture may determine the spore population of Clostridium tetani and found that heavy clay soil are richer in spore population than sandy soil. It has been observed that heat resistance and dormancy of bacterial spores are dependent upon Calcium (Ca ++) and depicolonic acid content where a better yield of spores is seen in magnesium sulphate peptone medium of Duncan and Strong, 1968.

There is hardly any referred publication in India that whether the viability of incidence of Clostridial spores are influence by different physio-chemical status of the soil. So the present study was undertaken for the physio-chemical analysis of the soil samples, population of Clostridial spores in soil samples and to find out the relation of Clostridial spores with the soil Ingredients with special reference to its Antibiogram.

## MATERIALS AND METHODS

One hundred and twenty samples were collected from different villages of eight districts of West Bengal, India viz. Nadia, North-24 Parganas, South 24 Parganas, Howrah, Hooghly, Burdwan, Murshidabad and Malda. Each soil sample amounting 500 gms were collected from the local meat shop and were brought to Veterinary Microbiology Department, West Bengal University of Animal & Fishery Sciences, Kolkata, India. Each soil sample was divided into two parts, one part containing 100 gms sample was kept in sterile polythene container for microbiological works and 400 gms in other container for chemical analysis. The pH of the soil samples were measured by Glass electrode (Jackson, 1967), Organic carbon was determined for calculation of organic matter by Walkley and Blacks method (Jackson, 1967). Exchangeable calcium and magnesium of the soil samples were determined by leaching the soil with neutral normal ammonium acetate solution. Then the calcium and magnesium were estimated by Complexometric titration with sodium salt of EDTA using Erichrome Black T and Calcon indicators (Black, 1965). Exchangeable potassium and sodium were determined by leaching the soil with neutral normal ammonium acetate solution and estimation were made by the help of Systronix type 121 Flame photometer as described by Jackson, 1967. The extraction of Iron from soil samples was conducted following the

procedure developed by Lindsay and Norvell (1979). For conducting microbiological study, the media like Nutrient Broth, Nutrient Agar, Blood Agar, Neomysin Blood Agar, Cooked Meat Broth (CMB) were used and to provide anaerobic condition during incubation of tested culture Alkaline Pyrogallol anaerobic jar was used. Number of colonies was counted by Electronic Colony meter and colonies were counted from 2-3 plates and mean were taken. Then the number of Clostridial spores per gm of soil sample was calculated by multiplying the colony count (Average number per plate) with the dilution figure ($1:10^4$) as each spore was presented by each colony. For Antibiogram, the antibiotic discs were placed on the Blood Agar media containing culture of the organism with the help of a sterile forceps. The plates were placed inside the Anaerobic Pyrogallol jar which was incubated at 37 C for 24 hours and the zone of inhibition was measured by taking the diameter of zone of inhibition around every antibiotic discs with the help of a scale.

## RESULTS AND DISCUSSIONS

### Figure 1

Table 1: Showing the Mean and Standard Error of Clostridial spores present in soil samples of different districts of West Bengal, India

| Districts | Mean ± Standard Error |
|---|---|
| Murshidabad | $83.866^{c} \pm 4.224$ |
| Nadia | $69.333^{b} \pm 3.594$ |
| Howrah | $85.933^{c} \pm 3.587$ |
| Malda | $74.133^{b} \pm 4.429$ |
| Burdwan | $65.533^{ab} \pm 3.802$ |
| North-24 Parganas | $82.200^{c} \pm 3.745$ |
| South-24 Parganas | $72.733^{b} \pm 3.324$ |
| Hooghly | $60.600^{a} \pm 1.989$ |

According to critical difference test, mean spore count of the district Murshidabad was significantly differed from Nadia, Malda, Burdwan, South-24 Parganas and Hooghly but did not differed significantly from Howrah and North- 24 Parganas. Mean spore count of district Nadia was significantly differed from other district except Malda, Burdwan and South– 24 Parganas. Similarly the mean spore count of Howrah and North- 24 Parganas significantly differed from other districts except Murshidabad. The mean spore count of Malda and South-24 Parganas significantly varied from other districts except Nadia and Burdwan. Lastly the mean spore count of Hooghly district varied significantly from all other district except Burdwan.

In the present study, higher spore population was found among the districts of Howrah, Murshidabad and North- 24 Parganas where the soil texture was silty loam in nature. This higher population of Clostridial spores in the silty soil also agreed with the findings of Weller (1941) and Kazdovina et al. (1976).

### Figure 2

Table 2: Showing the Analysis of Variance of Clostridial spore count

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Sum of Squares | Calculated F |
|---|---|---|---|---|
| Between districts | 7 | 9114.39 | 1302.0557 | 6.6231** |
| Within districts (Error) | 112 | 22018.40 | 196.5928 | |

From Table-2 it was found that the variation of Clostridial spore count between different districts were highly significant (P<0.01).

### Figure 3

Table 3: Showing Correlation and Regression co-efficient of variable Clostridial spore count with soil pH, Organic Carbon, Exchangeable Calcium, Magnesium, Potassium, Sodium and DTPA extractable Iron

| Parameter | Spore Count | |
|---|---|---|
| | Correlation Co-efficient | Regression Co-efficient |
| Soil pH | $0.1875^{*} \pm 0.0904$ | $4.2638^{*} \pm 2.0568$ |
| Organic Carbon | $0.1415^{NS} \pm 0.0911$ | $3.9180^{NS} \pm 2.5217$ |
| Exchangeable Calcium | $0.3777^{**} \pm 0.0852$ | $2.8576^{**} \pm 0.6449$ |
| Exchangeable Magnesium | $0.2344^{**} \pm 0.0895$ | $4.5370^{**} \pm 1.7320$ |
| Exchangeable Potassium | $0.0894^{NS} \pm 0.0917$ | $21.1594^{NS} \pm 21.9641$ |
| Exchangeable Sodium | $0.1130^{NS} \pm 0.0915$ | $8.3654^{NS} \pm 6.7723$ |
| DTPA extractable Iron | $0.2471^{**} \pm 0.0892$ | $0.0780^{**} \pm 0.0281$ |

$^{NS}$ denotes non significant
* denotes P< 0.05; ** denotes P< 0.01

From the Table-3 it is revealed that there was positive and significant (P< 0.05) correlation between Clostridial spore count and soil pH but non significant correlations were found with Organic Carbon, Exchangeable Potassium and Sodium content of the soil samples. At the same time highly significant (P< 0.01) positive correlation between Clostridial spore count and exchangeable Calcium, Magnesium and Iron content was also observed. Similarly the regression coefficient of Clostridial spore count on soil pH was found significant (P< 0.05) where as high significance (P< 0.01) was observed in exchangeable Calcium, Magnesium and Iron content of the soil samples. These findings mostly correlate with the findings of Tavares (1974), Gould and

Drink (1974) and Das (1977).

**Figure 4**

Table 4: Showing the antibiotic sensitivity test against different anti-microbial agents

| Sl. No. | Anti-microbial agent | Zone of inhibition (mm) | Interpretation |
|---|---|---|---|
| 1. | Gentamycin | - | Resistant |
| 2. | Kanamycin | - | Resistant |
| 3. | Neomycin | - | Resistant |
| 4. | Streptomycin | - | Resistant |
| 5. | Tetracycline | - | Resistant |
| 6. | Amikacin | - | Resistant |
| 7. | Cotrimoxazole | - | Resistant |
| 8. | Cloxacillin | 13 | Intermediate Sensitive |
| 9. | Amoxycillin | 20 | Sensitive |
| 10. | Ampicillin | 29 | Sensitive |
| 11. | Bacitracin | 16 | Sensitive |
| 12. | Chloramphenicol | 19 | Sensitive |
| 13. | Metronidazole | 20 | Sensitive |
| 14. | Erythromycin | 19 | Sensitive |
| 15. | Penicillin- G | 24 | Sensitive |

From the above table it is revealed that the isolates were found resistant to seven anti-microbial agents viz. Gentamycin, Kanamycin, Neomycin, Streptomycin, Tetracycline, Amikacin, and Cotrimoxazole where as Intermediate sensitive to Cloxacillin with inhibition zone of 13 mm. Isolates were sensitive to rest seven anti-microbial agents with inhibition zone ranging from 16 - 29 mm.

Clostridial organisms in general were reported to be resistant to Amikacin, Gentamicin, Kanamycin, Neomycin and Streptomycin (Rood et al., 1985; Gabay et al., 1981) which corroborates with the findings of the present study. Ampicillin and Amoxycillin were very effective against the organisms where as Cloxacillin was intermediately sensitive which corroborates with the published reports of Verma (1988).

## ACKNOWLEDGEMENT

Authors are thankful to all the teaching and non-teaching staffs of the department of Veterinary Microbiology, West Bengal University of Animal & Fishery Sciences, Kolkata, India for their kind cooperation during the research work.

## References

r-0. Black C A 1965 Methods of soil analysis; Part- II, Americal Soc. Agraon. Inc. Publisher, Madison, Wisconsin, U.S.A.: 771-1572.
r-1. Blood D C, Gay C C and Radostits O M 1979 Veterinary Medicine; 4 th Ed., The English Language Book Society.
r-2. Das S 1977 Incidence of Clostridium tetani in the excreta of domesticated animals and man its geographical distribution in West Bengal with particular reference to chemical composition of soil sample; Ph.D. thesis (Vet. Sc. Microbiology), Calcutta University.
r-3. Gabay E L, Rolfe R D and Finegold S M 1981 Susceptibility of Clostridium septicum to 23 anti-microbial agents; Antimicrob. Agents chemother. 20(6): 852-853.
r-4. Gould G W and Drink G J 1974 Mechanisms of spore heat resistance; Adv. Microbiol. Physiol. 11: 137-142.
r-5. Jackson M L 1967 Soil chemical analysis; Prentice Hall of India Pvt. Ltd., New Delhi.
r-6. Kazdobina I S, Matveev K I and Bultova T I 1976 Clostridium botulinum in the soil of the coast of the Aral Sea; Hyg. Sanit. 2: 18-20.
r-7. Lindsay W L and Norvell W A 1969 Development of DTPA micronutrient soil test; Agron. Abst. P: 84.
r-8. Rood J I, Buddle J R, Wales A J and Sidhu R 1985 The occurrence of antibiotic resistance in Clostridium perfringens from Pigs; Aust. Vet. J. 62(8): 276-279.
r-9. Tavares W 1974 Soil contamination by Clostridium tetani in Nio-de-Janeria, Brazil and contribution to the knowledge natural distribution of the tetani; Health Lab. Sciences. 6(2): 72-76.
r-10. Verma N D 1988 Antibiotic susceptibility of Clostridium perfringens Type A isolated from false black-leg of cattle; Ind. Vet. J. 65: 263-264.
r-11. Weller E 1941 Regional distribution of Clostridium tetani infection; J. Ber. Vet. Med. 71: 217-218.
r-12. Wilkins C A, Richter M B, Hobbs W B, Whitcomb M, Bergh N and Carstens J 1988 Occurrence of Clostridial tetani in soil and Horse; South African Med. J. 73(12): 718-720.
r-13. Wilson G, Miles A and Parker M T 1984 Principles of bacteriology, virology and immunity; 7 th Ed., Edward Arnold, London.

## Author Information

**N.J. Maitra, M. V. Sc. (Veterinary Microbiology)**
Ramkrishna Ashram Krishi Vigyan Kendra

**N.C. Nag, Ph. D. (Veterinary Microbiology)**
West Bengal University of Animal & Fishery Sciences

**R.K. Ghosh, M. V. Sc. (Vety. & A. H. Extension Education)**
Ramkrishna Ashram Krishi Vigyan Kendra