

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

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Citation

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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 Antibioqram.

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

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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 Antibioqram, 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 ^a ± 4.224
Nadia	69.333 ^b ± 3.594
Howrah	85.933 ^a ± 3.587
Malda	74.133 ^b ± 4.429
Burdwan	65.533 ^b ± 3.802
North-24 Parganas	82.200 ^a ± 3.745
South-24 Parganas	72.733 ^b ± 3.324
Hooghly	60.600 ^a ± 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* ± 0.0904	4.2638* ± 2.0568
Organic Carbon	0.1415 ^{NS} ± 0.0911	3.9180 ^{NS} ± 2.5217
Exchangeable Calcium	0.3777** ± 0.0852	2.8576** ± 0.6449
Exchangeable Magnesium	0.2344** ± 0.0895	4.5370** ± 1.7320
Exchangeable Potassium	0.0894 ^{NS} ± 0.0917	21.1594 ^{NS} ± 21.9641
Exchangeable Sodium	0.1130 ^{NS} ± 0.0915	8.3654 ^{NS} ± 6.7723
DTPA extractable Iron	0.2471** ± 0.0892	0.0780** ± 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).

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