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SPORE BEARING PATHOGENIC MICROBES ON ANCIENT ARTIFACTS OF CHANDRAKETUGARH

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ABSTRACT
Artifacts and ecofacts of Chandraketugarh were studied simultaneously for isolation of spore bearing pathogenic microbes. It was found that *Bacillus anthracis* were found in plenty numbers in animal bones, while *Clostridium tetani* were present in terracotta materials. These bacteria were not isolated from surrounding ecofacts.. Thus personal protection should be taken by all workers at these sites particularly against these diseases.

KEYWORDS: Chandraketugarh, terracotta, ancient bones, tetanus, anthrax.

INTRODUCTION

It is extremely difficult to prove the infections which were prevalent in ancient times. We may assume some infections from different writers of the ancient time, but we could not prove it by modern laboratory techniques. Important problems are that the genomes of microorganisms are not stable, external contamination from excavation sites is very common, and the competitive survival of pathogenic microbes among overwhelming nonpathogenic microbes in the environment is a remote possibility. However, in this gloomy scenario there is a ray of hope of finding atleast some pathogens. If we target the spore bearing pathogenic bacteria belonging to the genera *Bacillus* and *Clostridium*, then probably we may isolate them easily this is because bacterial spores may Survive for more than several thousand years in dormant state without any active biological activities. Thus if we can prove that bacterial pathogenic spores are present in a significantly more numbers over the excavated artifacts then probably this may indicate the prevalence of those diseases at that ancient time although it would be restricted for only few pathogens. Thus in this study we targeted two important spore bearing pathogens - *Bacillus anthracis* and *Clostridium tetani*, to see whether they were significantly present in more numbers on the excavated artifacts in comparison to surrounding materials both during excavation and the preservation time.

MATERIALS AND METHODS

Among the large settlements which occurred in the early historic period of Bengal, Chandraketugarh (22°41'N, 88°42'E) in the coastal region of southern West Bengal is important. It is situated about 37 km north-east of the city of Kolkata in North 24 Parganas District in the alluvial delta of lower Bengal, besides the dying stream of river Vidyadhari, which was once thrived as a large and important branch of the river Bhagirathi (Haque, 1996; Chakraborty, 2002; Sengupta, Roy Chowdhury and Chakraborty, 2007). It was an ancient port city (De and De, 2004). Thus in this study we selected Chandraketugarh- an ancient settlement in which seven cultural periods were extended – Pre-Maurya, Maurya, Sunga, Kushana, Gupta, Post-Gupta, Pala-Chandra-Sena (Haque,1996) extending from *c.* 600 BCE to *c.* 1250 CE. The site is enriched with terracotta artifacts. Besides terracotta materials there are bone artifacts, pottery, beads, bangles, coins, seals, metal artifacts etc. How and when this civilization was destroyed is very difficult to say but according to the local people this port city was destroyed by a devastating flood or a tsunami like condition as this was very close to Sundarbans – the world's largest mangrove forest.

The studied materials

We studied artifacts like terracotta materials, wheels, other artifacts, bones as preserved materials from the excavated site with permission taken from the Principal, Chandraketugarh Sahidullah Smriti Mahavidyalay, in charge of the Chandraketugarh museum, P.O. Debalaya (Berachampa), Dist.-North 24 Parganas, West Bengal.

Swab collections

Sterile cotton swabs were collected from the artifacts and bones and were kept in sterile containers with proper labeling. Control swabs were also taken from all adjoining materials for comparison.

Culture

Collected swabs were inoculated on Mueller Hinton medium, which is a very good bacterial growth medium with plenty nutrients, incubations were also done in anaerobic condition for isolation of *C. tetani* following all standard procedures.

Identification of isolates

All organisms were identified after their growth by standard procedures like Gram's stain, motility test, biochemical tests, and special tests as usual..

RESULTS

Nonpathogenic *Bacillus* species and coagulase negative staphylococcus which is also non pathogenic were widespread both in control and test cultures (Fig 1) sometimes producing confluent colonies. However, in this study we could isolate *C. tetani* from swabs taken from terracotta materials (Fig 1) and *Bacillus anthracis* was isolated from swabs taken from the bones (Fig 5). These two pathogenic spore bearing microorganisms were not isolated from any control and other experimental samples. One spiral elongated bacteria (Fig. 1) was also isolated from one terracotta figure of elephant which was later found to be a variety of *Bacillua* spp.

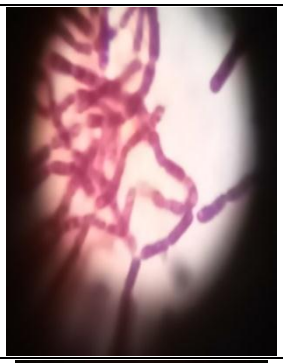
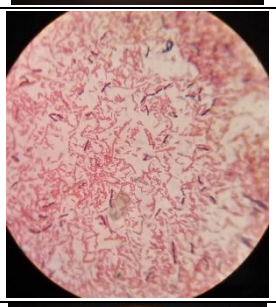
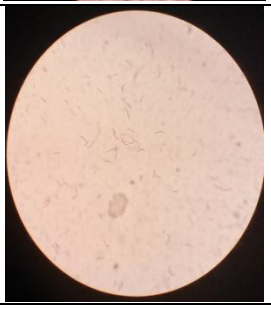
Sample name	picture	plate	staining	Result
Elephant toycartt				Bacillus confluent
Bones of different animals				*Bacillus anthracis*
Designed stand				*Clostridium tetani*
Terracotta figure of elephant				Spiral bacteria

Figure1: Artifacts, bones, cultures and isolates of Chandraketugarh.

DISCUSSION

There is no study so far regarding demonstration of *C. tetani* and *B. anthracis* from ancient artifacts in India. This study indicates probably these two diseases were prevalent at that time. Terracotta art was very famous in the early history of Bengal. Brick temples decorated with terracotta panels was very common at that time. *Clostridium tetani* spores were found abundant in the terracotta materials particularly in the designed stands with terracotta probably originating from Gupta Period (*c.* 300 CE – 750 CE). Tetanus has been known from antiquity (Sow et al, 19901). The disease is caused by a gram positive anaerobic spore bearing bacilli known as *Clostridium tetani*. This bacterium naturally remains in the soil and stools of the animals (Wilkins et al, 1988). The spores of *C. tetani* are abundant in the environment around the habitation of animals (Edlich et al, 2003) and in areas where farm animals are grazed. In this way the disease was common in ancient time. Brahmi and Kharoshti inscriptions are found on the pots, seals and plaques in Chandraketugarh which may indicate that there were trade connections with northwest region of India (Mukherjee, 1996; Sarma, 1991; Chakravarti, 2002) probably in relation to horse trade (Tripati, 2017) and tetanus spore contamination through horses is well known. Asʹvattha (*Ficus religiosa*) which means horse stand is similar to *Ficus bengalenesis* which were commonly used as horse shades and was associated with flourishing market places around the area, tetanus spores were easily spread in the general population at that time in those localities.

Anthrax was common in ancient times. The ‘plague’ of Athens (c. 430-427 BCE), epizootic affecting horse, cattle, sheep and other animals in Rome as described by Virgil (c. 70-19 BCE) are examples of anthrax infection in ancient times (McSherry and Kilpatrick, 1992; The University of Chicago Press, 1956). Anthrax is a zoonotic disease characterised by black eschars, oedema, sudden death syndrome, and haemorrhaging from the orifices mainly affecting livestock with episodic spill over to humans and carnivores (Turnbull 2008). After death the animal carcass becomes a potential source of infection (Dragon et al. 2005).The causative agent is a gram-positive, encapsulated, square ended ‘box-shaped’ appearance, aerobic and endospore-forming bacterium *B. anthracis*. Except demonstration of spores from bones during archaeological excavations at a site in KNP by De Vos (1990), there is no important study regarding this. It was found that anthrax spores can survive easily for a long period in alkaline soils rich in calcium and organic matter, and in this condition it can survive for centuries (De Vos 1998; Wilson & Russell 1964). As according to some local persons the ancient settlement was destroyed due to a devastating flood or tsunami probably the clumps of spores are deposited and concentrated when water evaporates, as the spores are highly resistant to UV radiation as described earlier by some scientists (Hugh-Jones & Blackburn 2009; Hugh-Jones & De Vos 2002; Vilas-Boas et al. 2007). It is likely that anthrax spores may be present at sites where animal related materials are present.

As these two important pathogens are present in bones and artifacts, at all stages of excavations all persons should take appropriate steps to prevent infection from these pathogens as well as to prevent contamination. If one wants to study the DNA, then they should follow the standard guidelines for this (Brown and Brown 1992, Brown 1998, Brown 2000). DNA may survive in animal or human teeth and bones. Personal protective equipment (PPE) like washable safety footwear, disposable over-suits, gloves, hard hats, a face mask system with air filtration to PP3 standard are some important materials which may be used if possible. Waste disposal from the site should also be done taking all precautions as it is well known that bacterial, viral and fungal pathogens may be present in the excavated materials.

CONCLUSION

This study revealed presence of significantly high numbers of tetanus and anthrax spores on ancient artifacts and bones which may indicate prevalence of those diseases in that ancient times. Thus the persons handling all these materials should take all personal protective measures during handling of all those materials.

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