

## The Effect of Cholesterol on Growth of Anaerobes with Special Reference to *Clostridium perfringens*

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### Abstract

This is a prospective analytical case control study that was used to investigate the effect of cholesterol on growth of *Clostridium perfringens*. The study was conducted in the department of Microbiology, Tropical Medicine Research Institute in the period between November 2012 and February 2013. Blood agar was spread with various concentrations of cholesterol solution simulating those concentrations found in human sera. Blood agar plates without cholesterol were used as controls. Predetermined serially diluted cultures of the *Clostridium perfringens* at dilution  $10^6$  were used as 0.05ml and spread all over the plates. The cultured plates were then incubated anaerobically at 37° C for 24 hours. Number of colonies in each plate was counted using digital colony counter. The study showed the clear effect of cholesterol on growth of *Clostridium perfringens*. The presence of cholesterol in high concentration contributes to the proliferation of *Clostridium perfringens* ( $P>0.05$ ). These results proved that the presence of high cholesterol levels in serum may contribute to the proliferation of *Clostridium perfringens*.

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## Introduction

The *Clostridia* are large anaerobic, gram-positive, motile rods. Many decompose proteins or form toxins, and some do both. Their natural habitat is the soil or the intestinal tract of animals and humans, where they live as saprophytes (Brooks *et al.*, 2007).

Clinically significant species of *Clostridium* include *C. perfringens*, which causes histotoxic (tissue destructive) infections (myonecrosis) and food poisoning; *C. difficile*, which causes pseudomembranous colitis associated with antibiotic use; *C. tetani*, which causes tetanus and *C. botulinum*, which causes botulism (Harvey *et al.*, 2007).

*C. perfringens* is classified into five types (A-E) depending on the pattern of the toxin ( $\alpha$ ,  $\beta$ ,  $\epsilon$  and  $\iota$ ) it produces: type A produces only the  $\alpha$  toxin; type B produces the  $\alpha$ ,  $\beta$  and  $\epsilon$  toxins; type C produces the  $\alpha$  and  $\beta$  toxins; type D produces the  $\alpha$  and  $\epsilon$  toxins; and type E produces the  $\alpha$  and  $\iota$  toxins (Hagiya *et al.*, 2012)..

Cholesterol is a lipid with a structure quite different from that of phospholipids. It is a steroid, built from four linked hydrocarbon rings. A hydrocarbon tail is linked to the steroid at one end, and a hydroxyl group is attached at the other end. In membranes, the orientation of the molecule is parallel to the fatty acid chains of the phospholipids, and the hydroxyl group interacts with the nearby phospholipid head groups. Cholesterol is absent from prokaryotes but is found to varying degrees in virtually all animal membranes. It constitutes almost 25% of the membrane lipids in certain nerve cells but is essentially absent from some intracellular membranes (Jeremy *et al.*, 2007).

Hassan (1996) demonstrates that sera collected from fattened animals (sheep) supported a better growth of *S. aureus* subspecies *anaerobius* than sera collected from non-fattened animals.

Lamis (2011) reported that cholesterol enhances growth of *S. aureus* and reduce the growth duration.

## Methodology

### Study design

This is a prospective analytical case control study that used to assess *invitro* the effect of cholesterol on growth of *Clostridium perfringens*. The study was carried out at Tropical Medicine Research Institute, Khartoum, Sudan, in the period between November 2012 and February 2013.

### Bacterial strains

Clinical isolate of *C. perfringens* was obtained from the laboratory administration- Khartoum state; it was purified by culture anaerobically, using commercial anaerobic kits on blood agar culture media. Cooked meat media (CMM) was used for preservation of *C. perfringens* at 4°C.

### Confirmatory tests of bacterial strain

*C. perfringens* strain was examined by Gram- stain followed by cultivation on Blood agar, cooked meat media and neomycin blood agar for confirmation and purification.

### Preparation of cholesterol

Cholesterol powder was used. It was obtained from (sdFiNE-CHEM LiMiTED). Certain concentrations of cholesterol solution were selected to simulate those concentration found in total blood cholesterol level in human (i.e normal rang <200 mg/dl, border line 200-240 mg/dl and high >240 mg/dl).

Different concentrations of cholesterol solution were used: 100, 200, 300, 400, and 500 mg/dl. These added to blood agar base powder, then added 100 ml of distilled water (D.W), well mixed and autoclaved at 121°C for 15 min.

### Preparation of ten-fold serial dilution

Tenfold serial dilution were prepared using sterile peptone water; by taking 1ml from cooked meat media contain overnight growth of *C. perfringens*, to select suitable dilution which gives countable colonies.

### **Growth of *Cl. perfringens* on cholesterol containing media**

Cooked meat media were inoculated with an overnight culture of *C. perfringens* grown on blood agar, well mixed and incubated at 37°C overnight.

One milliliter from overnight growth culture was removed and mixed with 9ml of sterile peptone water to prepare a tenfold serial dilution.

0.05ml from predetermined serially diluted culture of *C. Perfringens* at dilution  $10^6$  was removed and spread over each plate by using glass rod. The cultured plates were then incubated anaerobically by using commercial anaerobic kits at 37°C for 24 hours.

### **Bacterial count**

Miles and Misra (1938) method was used for bacterial count; the number of colonies was counted by Digital colony counter.

### **Statistical analysis**

Data was summarized and a descriptive statistical analysis was calculated, using Microsoft Excel (2007), the data was subjected to completely randomized design .Analysis of variance (ANOVA), mean separation and linear regression were conducted to test significant difference of groups with the aid of SPSS version 17.

### **Result**

The result showed that there is a positive correlation between colonies number and different concentrations of cholesterol as shown in **Tables (1)**.

The correlation between the bacterial viable count (CFU /ml) and different levels of cholesterol solution concentrations in Blood agar, when they were compared with control is shown on **Figure (1)**.

### **Effect of cholesterol powder on bacterial growth**

The following results of *Clostridium perfringens* illustrated a significant increase of mean number of colonies when compared with different concentrations of cholesterol ( $P \geq 0.05$ ).

Also there is a significant increase of mean number of colonies with different groups (levels) of cholesterol concentrations (normal, border line and high), **Figure (2)**.

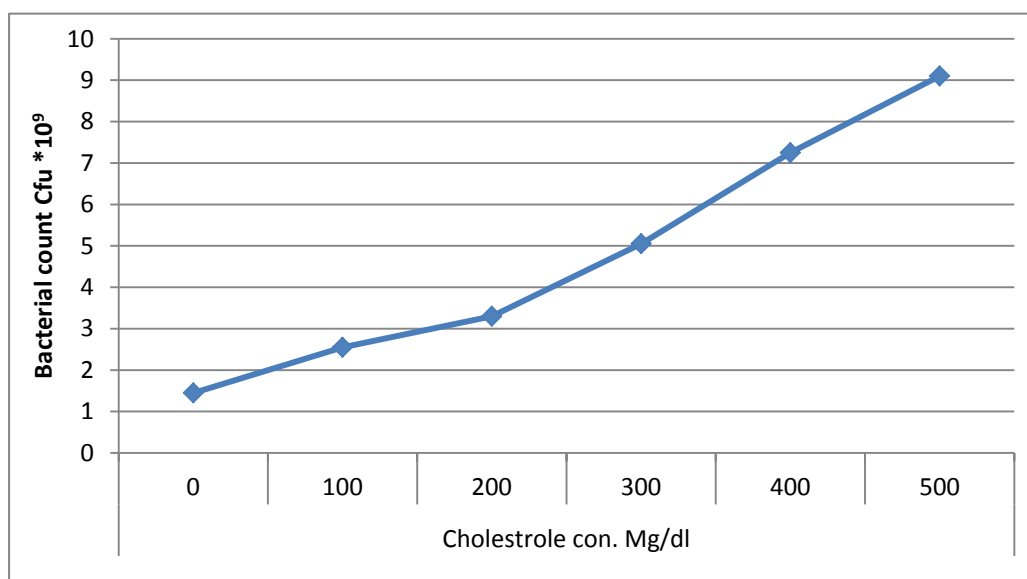
**Table (1): Bacterial count on medium with different concentrations of cholesterol solution.**

Cholesterol solution Concentrations (mg/dl)	Bacterial count CFU/ml
Control 0	$1.45 \times 10^9$
N* 100	$2.55 \times 10^9$
B** 200	$3.3 \times 10^9$
H*** 300	$5.05 \times 10^9$
H*** 400	$7.25 \times 10^9$
H*** 500	$9.3 \times 10^9$

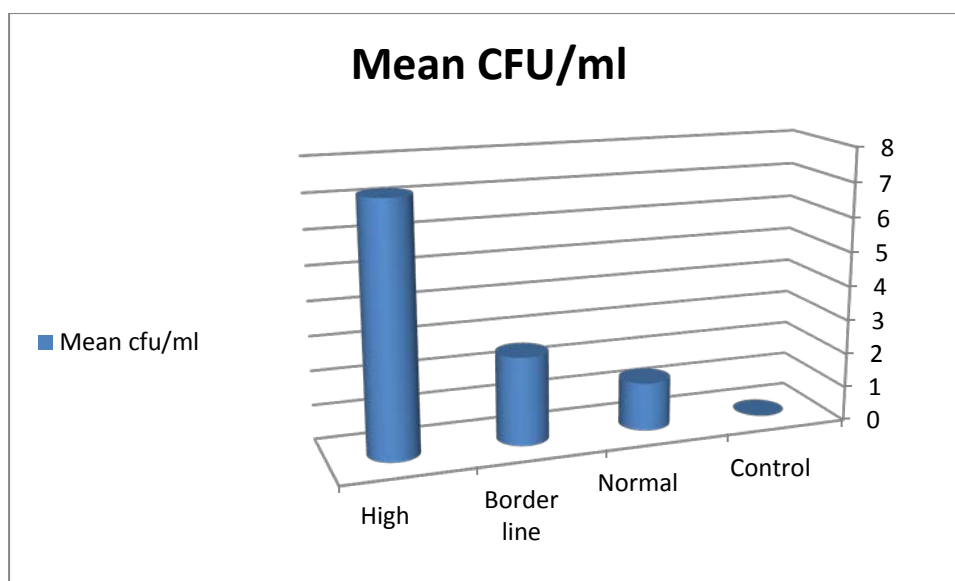
\*N: normal cholesterol solution concentrations.

\*\*B: border line high cholesterol solution concentrations.

\*\*\*H: high cholesterol solution concentrations.



**Figure (1): Correlation between the growth of *C. perfringens* and different levels of cholesterol solution concentrations.**



**Figure (2): Mean of the colony count in control and; normal, border line, and high cholesterol powder concentrations.**

## Discussion

In Sudan, there was no relevant published data on the effect of cholesterol on growth of pathogenic bacteria apart from that published by Hassan (1996), Lamis (2011) and Rihab (2013); accordingly, our results will be compared with studies published by Lamis (2011), Rihab (2013) and researchers outside the Sudan.

Our findings, in general, agrees with Lamis (2011) who demonstrated that the presence of blood cholesterol with high levels in sera of obese and non-obese persons contribute to the proliferation of *Staphylococcus aureus* that causes skin infections and also the role of different concentrations of cholesterol powder in enhancing growth of *Staphylococcus aureus*.

This study agrees with Robert *et al.* (2009) who found that cholesterol gall bladder stones facilitate the presence of bacterial biofilms on gallstones from typhoid carriers in both mice and man.

Our findings also agree with Bukhet (2005) who demonstrated the relationship between abscesses size (cm<sup>2</sup>) and body weight (kg) of fattened and unfattened sheep, where abscess size increased after infection proportionally to the body weight.

Our study also agrees with Hassan (1996) who demonstrated that sera collected from fattened animals (sheep) supported a better growth of *Staphylococcus aureus sub spp anaerobius* than the sera collected from non-fattened animals.

This result also agrees with Rihab (2013) who demonstrated that cholesterol enhances the growth of six isolates of Gram positive and Gram negative bacteria including: *Staphylococcus aureus*, *Bacillus subtilis*, *Corynebacterium ulcerans*, *Escherichia coli*, *Salmonella typhi* and *Kelbsiella pneumoniae sub spp. pneumoniae*.

### Conclusion

The results proved that the presence of cholesterol solution in different concentrations in blood agar may contribute to the proliferation of *Clostridium perfringens*. It also showed a significant increase in means of colony count due to impact of cholesterol solution on growth of the isolates of *Clostridium perfringens*.

### Recommendations

From results of this study it is recommend the followings:

- 1- Further researches are needed to cover the role of cholesterol on others Microorganisms including viruses, fungi, parasites and other various bacteria not studied.
- 2- Prospect research is needed to cover the impact of cholesterol on growth of *Clostridium perfringens* invivo.
- 3- Future research is required to explain how cholesterol enhances the growth of *Cl. perfringens*.
- 4- More studies are needed to cover the relationship between skin infection and soft tissue infections and fattening. With respect to blood cholesterol levels.

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