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*With 1 table and 6 figures in the text.*

# Effect of Different Concentrations of Sodium Chloride on Growth of *Clostridium perfringens.*

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**ملخص البحث**

أختبرت ثمان معزولات لبكتريا المطثية الحاطمة (*C. perfringens*) وخمس لبكتريا شبيهة المطثية الحاطمة (*C.* *perfringens*-like organisms) لمعرفة مقدرتها علي النمو في منبـــت لحـــم مطبـــوخ (Cooked Meat Medium) يحتوى علي تراكيز مختلفة لكلوريد الصوديوم (صفر% الي 7%0) ، وقد نمت جميع المعزولات عند إحتواء المنبت علي تركيز صفر إلي 3,5 % من كلوريد الصوديوم، بينما اختلفت مقدرتها علي النمو عند 4% الي 6,5% واخفقت جميعها في النمو عند تركيز 7%0 كما حدثت زيادة في الطول ونقص في العرض لخلايا المعزولات عند إستنباتها في منبت لا يحتوى علي كلوريد الصوديوم (صفر%) بينما حدث العكس وبالتدريج من نقص في الطول وزيادة في العرض لخلاياها عند إستنباتها في منابت تحتوى علي 0,5% إلي 6,5% من كلوريد الصوديوم0 الجدير بالذكر أن المعزولات المختبرة تم الحصول عليها من لحوم طازجة وأخرى مصنعة.

## Summary

**Eight *Clostridium perfringens* and five *Clostridium perfringens* –like organisms isolates from fresh and processed meat were subjected to phenotypic analysis on the basis of growth in different concentrations of Sodium Chloride (0.0-7%). All isolates tested grew well in cooked meat medium with 0-3.5% Nacl concentrations, showed variable results in 4-6.5% and failed to grow in 7%.**

**Gram-stained smears of *C. perfringens* and *C. perfringens* - like organisms grown in cooked meat medium containing 0% NaCl revealed an increase in length and a decrease in the bacterial cells width. A decrease in cells length and an increase of their width occurred in media with 0.5% to 6.5% NaCl concentrations.**

## Introduction

*Clostridium perfringens* is probably more wide-spread than any other potentially pathogenic bacterium. It produces different extracelluar toxins and enzymes which have an important role in the production of various disease manifestations in man and animal (Hobbs *et al.*, 1953). Consumption of meats contaminated with *C. perfringens* type A and some strains of type C will cause food poisoning (Jay, 1986). These srains are characterized by production of heat resistant spores that withstand boiling for 1-4 hrs. Toxins which are responsible for food poisoning are produced during sporulation (Layla, 1987).

In the Sudan, researchers have investigated aspects of contamination of fresh beef, mutton (Seriy Eldin and Ibrahim, 1977) and broilers (Abdel

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Salam, 1986) with *C. perfringens*. Fatty acid products (Salma, 1986) and properties of cold-injured cells (Layla, 1987), as well as the effect of freezing (Fatima, 1990) on *C. perfringens* in processed meat were also studied.

The effect of different NaCl concentrations on the growth of *C. perfringens* has been studied by El Sanousi (1975) who reported retardation in growth rate at 3.5% and very poor growth at 4%. Moreover, Layla (1987) reported growth of *C. perfringens* at 4% and 5% NaCl concentrations and complete growth inhibition at 6%.

The present study was undertaken to determine the salt tolerance (Nacl) of some *C. perfringens* and *C. perfringens*-like organisms isolated from fresh and processed meat and to record any morphological changes that may occur during their growth in different concentrations of NaCl.

## Materials and Methods

**Isolates:**

Eight *C. perfringens* (a1, a9, a10, b11, b16, c18, c19 and c20) and five C. *perfringens* – like organisms (a7, b12, b13, b14 and b17) isolates from 20 specimens of fresh and processed meat and a dead bear (Sudan National Zoo), were used. Their primary identification was carried out according to Abdel Salam (1986) and confirmed according to Barrow and Feltham (1993).

**Preparation of salted cooked meat medium:**

Sodium chloride was added to Nutrient Broth during the preparation of Cooked Meat Medium (CMM) in the concentrations (%w/v) 0, 0.5,1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5 and 7% as described by El Sanousi (1975).

**Test procedure:**

Sterile salted CMM was heated for 10 min. and quickly cooled. Using straight wire loop, isolates were each inoculated into the salted media, incubated for 24 hrs at 37°C, and subcultured onto Blood Agar plates that were incubated anaerobically for 24 hrs at 37°C before being examined for bacterial growth.

Gram-stained smears from salted CMM cultures were examined for morphological changes. Calibrated ocular micrometer was used to measure the cell size of *C. perfringens* and *C. perfringens* -like organisms grown in different NaCl concentrations. The calibration factor of the microscope was 1.6 μm.

## Results

All *C. perfringens* and *C. perfringens* –like organisms isolates tested for growth in different concentrations of NaCl (0-7%) at 37°C are shown in Table 1. They grew in 0.0 – 3.5% NaCl. The density of growth decreased gradually with the increase of NaCl concentration.

Eleven isolates (84.6%) grew in 4-5% NaCl, eight (61.5%) in 5.5% three (37.5%) in 6% and only one isolate (7.7%) was able to grow in 6.5%. No growth was detected in 7% NaCl.

**Table 1: Growth of *C. perfringens* and *C. perfringens*-like organisms in different concentrations of sodium chloride (0-7%)**

| NaCl Conc. / Isolate label | 0-3.5% | 4% | 4.5% | 5% | 5.5% | 6% | 6.5% | 7% |
|---|---|---|---|---|---|---|---|---|
| a1 | + | - | - | - | - | - | - | - |
| a7 | + | + | + | + | + | + | - | - |
| a9 | + | + | + | + | + | - | - | - |
| a10 | + | + | + | + | + | - | - | - |
| b11 | + | + | + | + | - | - | - | - |
| b12 | + | + | + | + | + | - | - | - |
| b13 | + | + | + | + | + | + | - | - |
| b14 | + | - | - | - | - | - | - | - |
| b16 | + | + | + | + | - | - | - | - |
| b17 | + | + | + | + | + | - | - | - |
| c18 | + | + | + | + | + | + | + | - |
| c19 | + | + | + | + | - | - | - | - |
| c20 | + | + | + | + | + | - | - | - |

+ = Growth - = No growth, a= Isolates obtained from fresh meat, b=Isolates obtained from processed meat, c = Isolates obtained from the necropsied bear.

Gram-stained smears of *C. perfringens* and *C. perfringens* -like organisms were used to study the morphological changes in the bacterial cells that were grown onto Blood Agar and in CMM with 0.0%, 1.5% and 4.5% Nacl and are shown in figs. 1, 2, 3 and 4, respectively.

Growth in 0.0% NaCl revealed an increase in cells length and a decrease in their width (Fig. 2), whereas a gradual decrease in cell length and an increase in its width was observed in CMM containing 0.5-6.5% NaCl. At higher concentrations of NaCl the classic rod shape of cells changed to a coccoid one (Figs 3; 4).

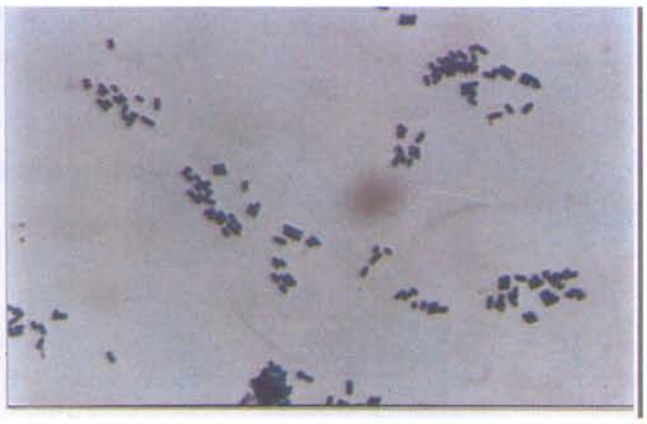

**Fig. 1: *C. perfringens* Gram-stained smear prepared from Blood Agar. X100.**

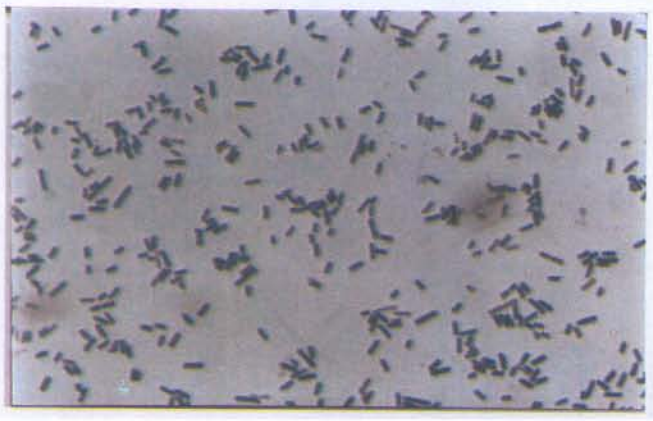

**Fig.2: *C. perfringens* Gram-stained smear prepared from CMM with 0.0% NaC, showing slender cells. X100**

The measurements of the morphological changes observed in the bacterial cells that were grown in CMM with different Nacl concentrations are shown in figs. 5 and 6.

## Discussion

This study revealed that all isolates tested were able to grow in concentrations of NaCl ranging from 0% to 3.5%, but 15.4% (2/13) of the isolates showed poor growth at 3.5% NaCl and complete growth inhibition at 7%. This finding agrees with that of El Sanousi (1975) who has reported the occurrence of severe retardation in growth rate at 3.5% NaCl and very poor growth at 4%. The growth of 84.6% (11/13) of the isolates at NaCl concentration of 4, 4.5 and 5%, is partially in agreement with Layla (1987) who has reported growth of non- injured *C. perfringens* cells at 4% NaCl.

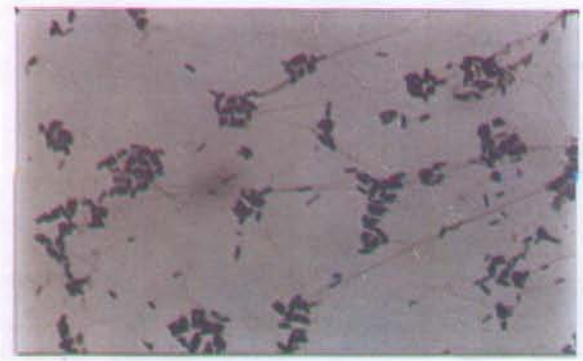

**Fig.3: *C. perfringens* Gram-stained smear prepared from CMM with 1.5% NaCl, showing broad cells. X100**

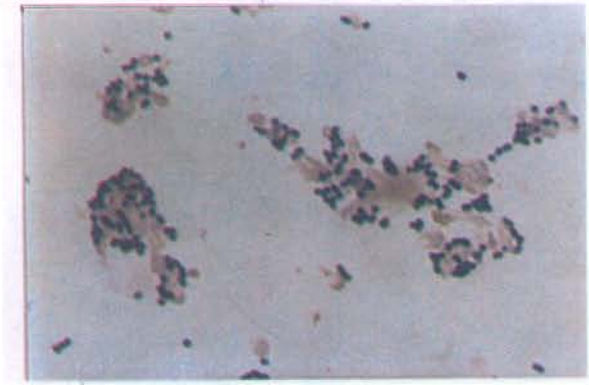

**Fig. 4: *C. perfringens* Gram-stained smear prepared from CMM with 4.5% NaCl, showing coccoid cells. X100.**

Severe retardation in growth rate was detected at high concentrations of NaCl; such finding is consistent with FSC (1999) who found that the use of NaCl in concentrations of 5-8% was inhibitory. Similarly, Layla (1987) has found that 6% NaCl was completely inhibitory to *C. perfringens* cells.

The changes that occurred in the vegetative cells may be attributed to disturbance in the interior osmotic pressure. The presence of vegetative cells in a hypertonic growth media made the water diffuse from cells to the surrounding medium resulting in metabolic disturbances and morphological changes.

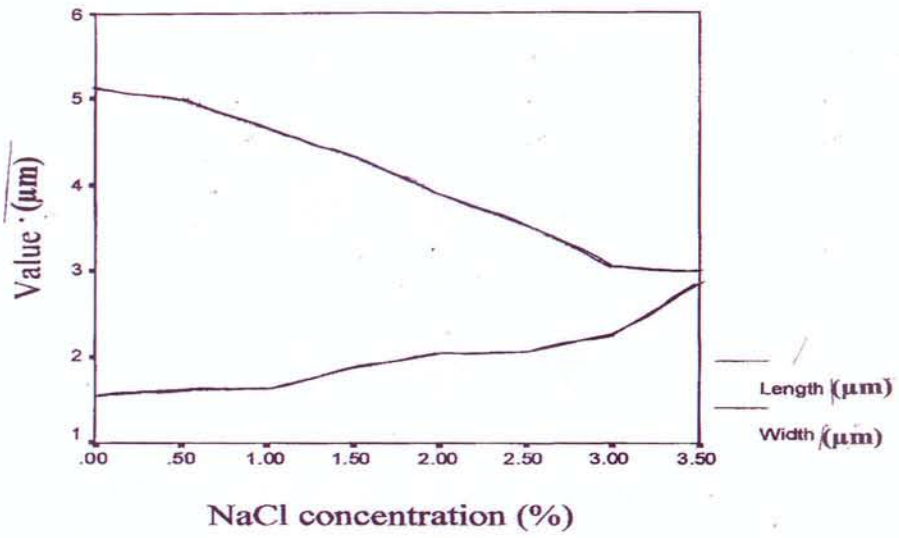


**Fig. 5: Measurements of *C. pefringens* cells (isolate a 1) in different NaCl concentrations**

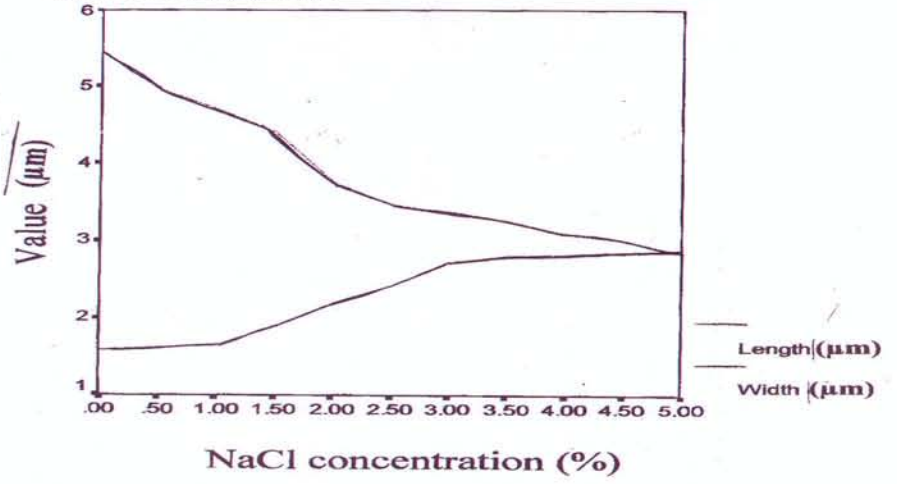


**Fig. 6: Measurements of *C. perfringens*-like organism (isolate a 7) in different NaCl concentrations**

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