بسم الله الرحمن الرحيم

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Microbiological Studies on *Clostridium perfringens* Type (A)

A THESIS

Submitted in partial fulfillment of the requirements of the degree of Master of Science in Microbiology

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صدق الله العظيم

Declaration

I declare that the thesis titled "**Microbiological Studies on *Clostridium perfringens* Type (A)"** is my own work and has not previously been submitted to any other university. The references were being checked whenever possible; show the extent to which I have availed myself of the work of other authors.

Nada Magdy Mohamed Mohamed Ali

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Dedication

*I dedicate my humble efforts to the memory of **My father**, who always believed in my ability to be successful in the academic arena. He was gone but his belief in me made the journery possible.*

*I also dedicate **My mother**, whose affection, love, encouragement and prays of day and night make me able to get such success and honor.*

Abstract

In this study, four hundred (400) isolates were collected from Fever Abbassia hospital in Cairo, Egypt. Clinical samples were collected from swab wounds of limbs, Two hundred twenty-eight (228) identified as gas gangrene, One hundred twenty eight (128) identified as *Clostridium perfringens* type (A) by biochemicals test {Gram stain : gram positive), Catalase test is negative, Motility test (non motile), skim milk test (stormy fermentation), urease test is negative, Nagler reaction is positive and indole test is negative}. Different media such as Thioglycalate broth, Cooked meat medium, Blood agar and Tryptose Sulfite Cycloserine agar (selective media) were applied. These media help to identify and isolate *Clostridium perfringens* type (A). Multiplex PCR was conducted with the primers mixture of alpha, beta, epsilon and iota toxins genes to identify *Clostridium perfringens* type (A), therefore *Clostridium perfringens* type (A) has alpha toxin only . Different factors can effect on growth of *Clostridium perfringens* type (A) like temperature, incubation period and pH. The optimum conditions for growth of *Clostridium perfringens* (Temperature 37°C, pH 7 and incubation period 24 hrs.).

The antimicrobial susceptibility tests were performed for all of the isolates (128) by two methods (disc diffusion method and MIC (E-test) method):

- Disc diffusion method using antibiotics **(Chloramphenicol (30 µg /disc), Cefoxitin (10 µg /disc), Clindamycin (2 µg /disc), Metrondiazole (5 µg /disc), Tetracyclin (30 µg /disc), Rifampin (5 µg /disc), Penicillin (10 µg /disc),**

Piperacillin (30 µg /disc)) on blood agar and incubate anaerobically in anaerobic jar for 24 hrs at 37°C.

- MIC (E-test) **(µg/mL)** method using antibiotics **(Chloramphenicol, Cefoxitin, Clindamycin , Penicillin G , Tetracycline , Piperacillin)** on blood agar and incubate anaerobically in anaerobic jar for 24 hrs at 37°C . The breakpoints for **Rifampin and Metrondiazole** for *Clostridium perfringens* have not to be determined by EUCAST (**European Committee on Antimicrobial Susceptibility Testing)** or CLSI **(Clinical and laboratory standards institute).**

- **Antiobiotic Combination therapy was done between**

- Chloramphenicol & Cefoxitin
- Chloramphenicol & Clindamycin
- Chloramphenicol & Metrondiazole
- Chloramphenicol & Penicillin G
- Chloramphenicol & Rifampin
- Chloramphenicol & Tetracyclin
- Chloramphenicol & Piperacillin
- Cefoxitin & Clindamycin
- Cefoxitin & Metrondiazole
- Cefoxitin & Penicillin G
- Cefoxitin & Rifampin
- Cefoxitin & Tetracyclin
- Cefoxitin & Piperacillin

- Clindamycin & Metrondiazole
- Clindamycin & Rifampin
- Clindamycin & Piperacillin
- Clindamycin & Penicillin G
- Clindamycin & Tetracyclin

-Metrondiazole & Penicillin G
-Metrondiazole & Tetracyclin
- Metrondiazole & Rifampin
- Metrondiazole & Piperacillin

- Penicillin G & Rifampin
- Penicillin G & Tetracyclin
- Penicillin G & Piperacillin
- Rifampin & Tetracyclin
- Rifampin & Piperacillin
- Tetracyciln & Piperacillin

The present results showed that

1-The disc diffusion method and MIC method give the same results which showing that the best antibioics active for *Clostridium perfringens* type (A) are **Chloramphenicol (30 µg /disc) , Cefoxitin (10 µg /disc), Clindamycin (2 µg /disc), Rifampin (5 µg /disc) , Penicillin G (10 µg /disc), Metrondiazole (5 µg /disc) , Tetracycline (30 µg /disc)** in sequences. ***Clostridium perfringens* type (A)** was resist to **Piperacillin (30 µg /disc)**.

2-Antibiotics Combination therapy was :

- Good treatment for *Clostridium perfringens* type (A), in few cases better than using one antibiotic as **(C+FOX, C+DA, C+RA and C+TE)**
- Not good in other cases.

3- In case of gas gangrene , the best treatment is cleaning the wound with early antibiotic therapy as **(C, FOX, DA, RA, PG, MET and TE).**

LIST OF Abbreviations

Abbreviation	Meaning
AIDS	Acquired immune deficiency syndrome
BSAC	British Society for Antimicrobial Chemotherapy
BEC	Binary enterotoxin of *Clostridium perfringens*
C. perfringens	*Clostridium perfringens*
CLSI	Clinical and laboratory standards institute
CPA	*Clostridium perfringens* alpha toxin
CPE	*Clostridium perfringens* Enterotoxin
CPB	*Clostridium perfringens* beta toxin
FOX	Cefoxitin
C	Chloramphenicol
CPB2	*C.perfringens* beta 2 toxin
DA	Clindamycin
ETX	*Clostridium perfringens* epsilon toxin
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HBO therapy	Hypberic oxygen therapy
H_2O_2	Hydrogen peroxide

List of Abbreviations

H_2S	Hydrogen sulfide
ITX	*Clostridium perfringens* iota toxin
MET	Metrondiazole
MIC	Minimum Inhibitory concentration
MIO	Motility test
NaCL	Sodium chloride
NetB	Necrotic enteritis beta like toxin
NCCLS	National Committee for Clinical Laboratory Standards
PCR	Polymerase chain reaction
PG	Penicillin G
pH	Concentration of hydrogen ions in the solution
PRL	Piperacillin
RCA	Reinforced clostridial agar
RCM	Robertsons cooked meat
SGG	Spontaneous gas gamgrene
TE	Tetracycline
TpeL	Toxin perfringens large
TSC Agar	Tryptose Sulfite Cycloserine Agar
PFO	Perfringolysin O

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