Bacteriological study of Gas gangerena among burns and surgery patients

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Abstract:

The results of this study showed that the percentage of contamination with *clostridium perfringens* bacteria in the burns wards were (32%) out of (50) specimens samples and the highest rate of contamination was in flooring these wards (50%), followed by burns patients inflamed (40%) , either in the surgery wards the ratio of pollution was (24%) and the highest rate of pollution which has been in the family of the patients (40 %), followed by flooring (30 %) and inflamed wounds of patients (30 %) as well. Was isolated (28) the isolation of these bacteria were classified into four groups serology is the group (A) by (39.8%) and B by (21.4%) ,c(21.4) and E (14.2%) , and all isolates were isolated from patients and exaggeration of (7) isolates produced enzyme Lecithinase which means the percentage (100%) , while the (9) isolates of the total (21) isolated from the hospital environment (surgery and burns wards) were produced for this enzyme above a rate of (42.85%) showed the results of toxicity testing in laboratory mice that ratio percentage of the total toxicity shown by the bacterial isolates were (65.14%) , all isolates burns wounds were toxic at a rate (100%) , while the (28) isolation of isolates environment was toxic also increased (39.28%) , that most isolates toxicity was the group followed by the ECB and that there was a broad consensus between the productivity of these isolates poison Alpha (enzyme Lecithinase) with toxicity in the White mouse .

Key words: *Clostridium Perfringens*, Gas gangerena

blood cells , white and prejudice in penetration the cells to another and thus produces a lack of blood supply and weaken the operations of redox in former textile would receive a tumor in it because of the disruption of permeability and smash the cells , which stimulates the effectiveness of enzymes internal proteolytic and thus produces the decomposition of cellular tissue infected[9] due to the importance of these bacteria in terms of health and the lack of action by any study in Diwaniyah province , we decided to do this study , which aims to : -

• Isolation and diagnostic the bacteria *clostridium perfringens* of surgery and burns wards of Diwaniyah Teaching hospital patients whom admitted from these wards .
• Detection of ability this bacteria to produce an enzyme Lecithinase(toxinAlpha) .
• Susceptibility testing toxicity of these isolates using laboratory rats .

Material & methods

1- Collection of Samples:

One hundred swab from surgery and burns wards patients admitted to the those wards were collected on the period from August 2012 to December 2013 , and followed the way [3] in all samples , were taken by cotton Cotton Swabs of ,these swabs contain Physiological (Normal Salin) , who added her after it was opened in sterile conditions in the laboratory of the hospital and rounded in place burn or wound , either infections that contain pus (pus) has withdrawn part of the pus by Sterile Sringes and for samples

Introduction: -

Bacteria *Clostridium Perfringens* widespread in nature , it exist in soil , dust streets , feces , food varied , sewage , and it is the intestinal tract of humans and animals like a natural environment for it[1,2], explained[3] *CL.perfringens* may be isolated from the atmosphere surgical wards and that this is due to several reasons, the most important , poor ventilation systems , that the atmosphere of these bacteria in the hospital environment and in the surgery and burns wards where lying patients with burns and wounds are cases in which an alarming and to call the attention of the competent authorities as this caused when bacteria presence in the position of the burn wound and the death of a local of that area , a so-called soft Balcankrina (Gas gangerena)[4,5].

These are cases of fatal cases , after the growth of certain types of clostridium anaerobic muscles in the body tissue of various liberated toxins external transmitted within these tissues at high speed and deliberately emergence of this case on the complex interactions that occur between bacteria and their toxins enhanced on the one hand and between the tissues and organs of infected on the

other hand[6], the most important toxins that cause the generation of disease gangrene gas is toxin Alpha (toxin א) or enzyme (Lecithinas)[7] , who works on the separation Lecithinas and Alasvenkomaalan of fatty protein that enter in installation cell membrane for most of the cells, tissue and blood cells[8] .

Hence the disease gangrene gaseous begin by this enzyme where smashes red

these tubes anaerobically for (6-16 hours) and degree º 34 m , to be a fatty layer floating above the surface of the solution indicates a positive test [11].

4-Detection of toxicity CL-perfringens isolates

A) Generating toxins : - Toxicity test of *CL.perfringens*

The method followed by Yamamoto *et.a*l.,(1972) , which includes the development of clostridium isolates water in the center of cooked meat genitive maltose sugar by 20 % (1 % as materials for stimulating the production of toxins by most isolates of Clostridium perferinges where inoculated on blood glucose 1% and incubate for 24 hours in º 34 Cand meat and maltose and incubate anaerobically for 24-48 hours and the degree º 37 C [12] .

B) Extract of toxins from grown bacteria: -

To separate the bacteria from its liquid culture by using centrifuge in 6000 r / min for half an hour warmly º 24 m , taking the liquid supernatant that containing toxins and kept in sterile tubes in º 4 until testing that does not exceed a period of storage for two days [13].

C) detection of toxicity : -

According to the method followed by (Yamamoto et.al., 1972) take 0.5 ml of leaky implanted bacteria containing toxins in the vein of the mouse white and note the results starting from the period of the lap for 24 hours of doom animal laboratory.

Results & Discussion: -

collected from the environment that rotate the swab in place of the combination , quoted after the samples to the laboratory within 5 minutes to cultured the samples onThyoglyco late medium and cooked meat medium, incubated at 37 C for 18-24 hours under anaerobic conditions , then transplanted on the Clocose - blood agar). (Added glucose as an energy source and as a reducing agent) were incubated under anaerobic conditions using anaerobic jar Badtfrigha and filled with nitrogen twice - after incubation 24 hours at a temperature 37 º and is tested colonies typical of *CL.perfringens* and re-planting on the same center for refining and then operate tests confirmatory on those colonies for the purpose of diagnosis .

2- Diagnosis: -

The diagnosis was based on the phenotypic characteristics of the bacteria, such as the form of bacteria , fermentation weathers in pipes , the nature of the fermentable sugars nature pigmentation with Cram stain inaddition to the Biochemical tests including test nitrate reductase , generating sulfide hydrogen , t hydrolyzed starch , either serological tests mouth made using (Kits) Private equipped with the company (Difco)[10].

3- Detection of enzyme Lecethinase : -

For the detection of this enzyme and known its effectiveness and the amount of toxic Alpha (Lecithinaso) followed the method of (Smith & Aebuthno, 1974) by adding 0.5 ml of a leaky implanted clostridium perfrenges developing in the middle of Water meat containing maltose to Alananwib containing 5 ml of egg yolk , incubated

patients with burns and wounds in gauze and tools that are completely sterile calls for caution and take effective preventive ways , was sterilized tools, Surgical and other supplies using the oven heat to 160 for three hours or 180 heat for two hours, and that any reduction of the period of time required by the bailiffs or gives workers the opportunity casuals as invading bacteria gas gangrene remain alive [14].

Lidwell & noble, (2002) showed that the method of ventilation have a role in the pollution of atmosphere hospitals as the open doors and windows in the wards or when crashes central ventilation and the use of exhausted fan air allowed into the outside air and dust into the hall or lounges and repeat contaminated with bacteria soft gangrene despite of continuous sterilization of tools and workbenches and floor hall[15].

quantities , but not because of Clostridium but there are (17) bacteria satisfying her ability to bring this phenomenon and the most common are (Enterococcus) and is one of the serious injuries that lead to the destruction of In order to give the patient the correct treatment must differentiate between the two above cases , especially when given (Hyperbaric Oxygen Therapy)[16].

The results of this study showed that the rate of contamination with bacteria *Cl-perfringens* in the burns wards were (32%) which is higher than the rate recorded in the surgery wards , which was (24%) and the highest rate of pollution in the burns wards were in floored (50%), followed by burns of the patients (40%) in the gene less pollution (20%) patients were in dresses as well as the tools and gauze table (1) . But in the surgery wards was the highest percentage (40%) in patients followed in the flooring and inflamed wounds of patients as the ratio of the two pollution (30 %) .

Lower rate (10%) patients were in dresses Table (2) . The presence of the bacteria *Cl.perfringens* was expected in infections at the wounds that show symptoms of gangrene can be present in the vicinity of patients , especially

It was clear from our study that there is a 16 patients with burns and wounds and exhibiting symptoms gas gangrene, but the swabs wounds or pus taken from places accident did not give a positive result for the growth of bacteria, *Cl-perfringens* or any other type of bacteria Clostridium that cause gangrene gaseous and interpreted ((skiles 1988), this case, the presence of injuries generated by the gases in large

Table (1) the percentage of contamination with bacteria (*CL.Perfringens*) in the burns wards

No.	Site	number of samples	the number of positive result for *CL.Perfringens*	%
1	floor	10	5	50
2	Beds of patients	10	3	30
3	dresses patients	10	2	20
4	tools and gauze	10	2	20
5	burns patients fiery	10	4	40
6	Total	50	16	32

Table (2) the percentage of contamination with bacteria (*CL.Perfringens*) in surgery wards

No.	Site	number of samples	the number of positive result for *CL.Perfringens* growth	%
1	floor	10	3	30
2	Beds of patients	10	4	40
3	dresses patients	10	1	10
4	tools and gauze	10	2	20
5	surgery patients fiery	10	3	30
6	Total	50	12	24

from patients , was the type (A), either distribution was (11) isolation rate (39.28%) belonging to the type of serum (A) and (6) isolation (21.4%) belong to the type (B) , (6) the isolation rate (21.4%) belong to the type serum (C) (4) the isolation of a rate (14.2%) belong to the type serum (E) table (4) is thus clear that the highest frequency was the type serous type (A) and this demonstrates the high potential for contamination of

The total isolates of (*CL.Perfringens*) in this study was (28), (7) of them were isolated from burns and wounds of patients who showed symptoms of gangrene and (21) from the floor and the isolation of patients and family dresses gauze and surgical tools in wards burns and when the classification of these isolates were serologically found it back to the four types of serological which (A, B, C, E) and that all isolates were isolated

that cause was high (100 %) compared with isolates the illness environment that the percentage of toxicity in which overall (39.28 %) may be due to the growth of bacteria inside the fabric will be activated for secretion of toxins and crises[18]and came our results this confirmed the results of the[19] that isolates the wounds are more toxic than isolates the ocean and to consider the compatibility positive generate Lecithinase with toxicity in mouse White was compatibility great and it turned out that all isolates were negative in the examination of toxicity it is generating enzyme Lecithinase(toxin alpha) and that there two isolates were very toxic for White mice and the period destruction animals were (15 hours) is that it is not generating enzyme Lecithinase, suggesting that there are other poisons such as toxins Beta and Epsilon and Iota her der in promoting Se isolates *clostridium prefringenc*[20]), and was the most species serological toxicity are isolates of group (A) followed by (B), (C), (E) , depending on the time period for the destruction of mouse White extended between two to six hours and did not depend on the thickness of the fat layer as an indication of the effectiveness of toxinAlpha enzymatic as the thickness of the fat layer for some isolates of sero-group equal to (6) cm , but the period of the loss was (5) hours while the thickness of the fat layer isolates the other is equal to (3) cm but destruction lasted only two hours , and the results of this study wounds in the hospital , said (Bodian, 1989) that the serotype (A) is primarily responsible for the cases of gangrene gas as the (80%) of cases gas gangrenewounds dating back to this serotype[17] .

As for the detection of enzyme Lecethinase (toxinAlpha) has produced (12) isolation of isolates *clostridium prefringens* enzyme Lecithinase at the center of the egg yolk adoption measure the thickness of the fat layer floating above the grown -containing solution egg yolk differed thickness of the fat layer in the readings positive in test tubes as between (2-6 cm) through (6) hours , all isolates were isolated from wounds and burns patients were producing at a rate of alpha toxin (100%) , while the (9) isolates (42.85%) of the total (21) was isolated environmental producing poison Alpha table (3) showed the results of toxicity test isolates in mouse white laboratory that isolates the toxic led to the destruction of the animal after 15 hours, and most isolates toxicity are isolates of group (A) and aggregates (E, C, B) the adoption of the time period for the destruction of the animal , the isolates Group (A) taken from burns and wounds of patients infected with toxic by (100%) and taken from burns patients by (39.28 %) , while the percentage of the total toxicity isolates *clostridium prefringens* isolated in the study were (65.14 %) Table (4) . Looking at the isolates wounds and burns alone , we find

clostridium prefringenc whether in patients or in their surroundings as the presence of these bacteria, such fury patients with burns and wounds who are suffering from a lack of immune defenses and immune barrier , especially a first (skin) is a situation that calls attention to it could lead to enormous health disasters

believes with the results of a study (Hinton) in that there is a consensus in a positive generate Lecithinase with screening toxicity in mouse white while he[21]that there was no consensus between the two cases in our study and in this , the results of our study indicate the seriousness of the presence isolates

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Table (3) susceptibility bacterial isolates (*CL.perfringens*) to produce Alpha toxin (enzyme Lecithinase) in terms of the measurement of the thickness of the fat layer floating on the center of the egg yolk

Reference	Number of isolates	Number of isolates producing toxin	Source percentage for the production of toxin	fatty layer thickness
Wounds and burns patients fiery	7	7	100%	3-6cm
Floors	8	4	50%	2-4cm
Patients dresses and beds	10	3	30%	6cm
Tools and gauze	3	2	66.6%	5cm
Total	28	16	57.14%	

Table (4) toxicity to bacteria aggregates serological (*CL.perfringens*) in terms of the destruction of isolated mouse White Prach bacteria injected into a vein Quraini

Reference	Number of isolates	Type	No. of isolated toxic	%
Wounds and burns patients	7	A	7	100
Floors	8	1E+6B+1A	5	62.5
Patients dresses and beds	10	6C+4A	4	40
Tools and gauze	3	2E+1A	2	66.6
Total	28		18	65.14

References

8- Stevens DL , Bryant AE. The Role of Clostridiual toxin in the pathogenesis of gas gangrene. Clin Inf Dis 2002; 35:p593-5100.

9- Eaton JT, Naylor CE, Howells AM etal. Crystal structure of the C. prefringens alph-toxin with action site closed by a flexible loop region.Jmol Biol 2002; 319:(2):275-281.

10-Colle,J.G.Fraser ,A.G. Marmion , B.P. and simmon , A.S.(1996) practical medical microbiology . chunchill Living stone .

11-Jacke, M.D.(2000). Comparative study about lecithinase in Clostridium sp. Chilli stone.

12-Yamomoto , R. Lawburry ,.; Hafman , D. (1972). Text books of medical microbiology , pathogericity and diseases managent , 2 th .ed. Lippincott, wialliams & willikins .

13-Nord , F ; Janes , R.; PFaller , R.(1990) . how best to test for toxicity of bacteria , CAP in the News CAP to day. Cover story , P :11 – 14.

14-Albert , D.K. (2001) . Methods of sterilization with abstract of CL.perifringens, stuation in the fedral republic of Germany , J.INF.med., 18 ; 6 – 21 .

15-Lidwell , F.D. and Noble , R.(2002). Methods of controlling on contamination , frequencies from the SENTRY surveillance program (united states and Canada , (2000) 4 z : 111- 115 .

16-Skiles , p .(1988) . predictive factors for an erobic infections caused by gram positive and gram negative bacteria, Enferm. Infect. 11: 2-9.

17-Bodian , M.(1989) . Serological study about CL.perfringens in the surgical unit.surg.Infect.6i657- 675 .

1-Stevens DL. The pathogenesis of Clostridial myonecrosis. Int Microbiol 2000,Oct;290:4997-502.

2-Louise B. Hawley, (2000). High – yield microbiology and infectious diseases. Printed in the United States of America. med. 9: 49. Lippincott, Wialliams & Wilkins.

3-Lowburry , S.D . and Lilly , J.M. (2003) . Biochemical test for identifaction of bacteria and other microorganisms . 2th . ed . Churchill living stone .

4- Bakker, DJ. Clostridial myonecrosis. In: Bakker DJ, Cramer FC eds. Hyperbaric Surgery: Perioperative care. Falstaff, AZ,Best Publ company, 2002, 283-316.

5- Bryant, A. E., Chen, R. Y., Nagata, Y., Wang, Y., Lee, C. H., Finegold, S., Guth, P. H. & Stevens, D. L. (2000b). Clostridial gas gangrene . II. Phospholipase C-induced activation of platelet gpIIbIIIa mediates vascular occlusion and myonecrosis in *Clostridium perfringens* gas gangrene. J Infect Dis 182, 808±815.

6- Lucey BP, Hutchins GM. William H. Welech and the discovery of *Bacillus Welchii* Arch Path Lab Med 2004; 128(10):1193-95.

7- Hofman, P., Le Negrate, G., Mograbi, B., Hofman, V., B., Hofman, V., Brest, P., Allinana- Schmid, A., Flatau, G., Boquet, P. & Rossi, B. (2000). Escherichia coli
cytotoxic necrotizing factor-1 (CNF-1) increases the adherence to epithelia and the
oxidative burst of human polymorphonuclear leukocytes but decreases bacteria phagocytosis. J. Leukoc Biol 68, 522±528.

18-Martin , f , Doern, M.J. Sukop.R(1990). Bacterial pathogens isoletes from patients
with general infections. Enferm. Infect. Microbral. Clini. 14 (9) : 20- 29 .
19-Denser , R.B.(1997). Detection of X.Toxin in some an erobic bacteria . J,med . Assoc. thai , 11. 5 – 12 .
20-Hinton, M.D. (2002) . Risk Factor of an erobic Nosocomial infection . J .Grit . car . med . 153 : 185 – 62 .
21-Mishra , D. (1998). Study about CL.perfringens toxicity and pathogenicity. J .med. 4 : 5- 11 .

دراسة بكتيرية بالكانكريا الغازية لمرضى الجروح والحروق

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الخلاصـــة : -

اظهرت نتائج دراستنا هذة ان نسبة التلوث ببكتريا الكلوستريديم برفرنجيز في ردهات الحروق كانت(32 %) من اصل (50) عزلة وان اعلى نسبة للتلوث كانت في ارضيات هذه الردهات (50 %) تليها حروق المرضى الملتهبة (40 %) ، اما في ردهات الجراحة فكانت نسبة التلوث (24 %) واعلى نسبة للتلوث فيها كانت في اسرة المرضى (40 %) تليها الارضيات (30 %) وجروح المرضى الملتهبة (30 %) ايضا . تم عزل (28) عزلة لهذه البكتريا صنفت الى اربعة مجاميع مصلية هي المجموعة (A) بنسبة (39,28 %) و B بنسبة (21,4 %) و C بنسبة (21,4 %) و E بنسبة (14,2 %) وان جميع العزلات المعزولة من المرضى والبالغ عددها (7) عزلات انتجت انزيم اللسثنيز أي نسبة (100 %) في حين ان (9) عزلات من مجموع (21) عزلة عزلت من بيئة المستشفى (ردهات الجراحة والحروق) كانت منتجة لهذاالانزيم اعلاه أي بنسبة (42,85 %) واظهرت نتائج اختبار السمية في الفئران المختبرية ان النسبة المئوية الاجمالية للسمية التي ابدتها العزلات البكتيرية كانت (65,14 %) وان كافة عزلات الحروق والجروح كانت سامة أي بنسبة (100 %) في حين ان (28) عزلة من عزلات المحيط كانت سامة ايضاٍ بنسبة (39,28%) ، وان اشد العزلات سمية هي المجموعة A تليها B . C . E وان هنالك توافق كبير بين انتاجية هذه العزلات للسم الفا (انزيم اللسثنيز) مع السمية في الفأر الأبيض .

مفاتيح الكلمات: الكانكريا الغازية, داء المبيضات.

Biology Classification QR 75- 99.5