Research Paper

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Bacteriological Study of Clinically Suspected Cases of Gas Gangrene.

Medical Science

KEYWORDS : Gas gangrene, anaerobic culture, clostridium spp.

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ABSTRACT

Objective: To study bacterial isolates from clinically suspected cases of gas gangrene.

Methods: Study period was from January 2012 to July 2012. Samples were collected from 50 clinically suspected cases of gas gangrene. Immediately Gram stain, aerobic culture and inoculation into Robertson's cooked meat (RCM) medium were done. Anaerobic culture was done from inoculated RCM onto CDC blood agar with gaspack system for 48 hours. Colony morphology, Gram staining and aerotolerance tests were performed.

Results: There were total 16 smear positive cases (32%). Out of which 12 specimens showed anaerobes in form of 22 obligate anaerobe isolates. 21(95.45%) were suggestive of Clostridium species and 1(5.55%) was suggestive of Fusobacterium spp. In the remaining Klebsiella spp. followed by Enterococcus spp. were common isolates.

Conclusion: Initial conventional steps for identification of anaerobes helps clinicians in institution of specific therapy and eventually better patient management in clinical emergency like gas gangrene.

Introduction

Gas gangrene is an infectious disease emergency, characterized by rapidly spreading, edematous myonecrosis. It is usually associated with invasion of tissue, especially by Clostridium spp. alone or along with various anaerobic and aerobic bacteria. The disease is characterized by rapid onset of myonecrosis with muscle swelling, severe pain, gas production, and sepsis. Causative factors for this disease are traumatic injuries (most common) followed by surgery, intramuscular injection, I.V. drug abuse. Diagnosis of gas gangrene is difficult because of various bacterial and nonbacterial lesions simulate it [1, 2, 3].

Apart from the rare cases of anaerobic streptococcal infection, the microorganisms of prime importance in gas gangrene are obligately anaerobic spore-bearing bacilli; that is, they are Clostridium species. The Clostridia comprise a very large and diverse group of essentially saprophytic organisms, which are widely distributed in nature-in soil, in dust, in water, and in the alimentary tract of most animals. Their large numbers and wide distribution thus render them one of the commonest types of bacteria to be found in wounds; indeed, in most parts of the world any large wound is likely to contain clostridia. Fortunately only a very few species are generally accepted as pathogenic, and still fewer are capable of causing gas gangrene in man [3,4].

Materials and methods

A total of 50 clinically suspected cases of gas gangrene admitted at our institution during the period of January 2012 to July 2012.

Specimens were collected from deep site of the wound with great care to avoid contamination and normal flora. All specimens were processed immediately for Gram stain, aerobic culture and inoculation into Robertson's cooked meat medium (RCM).

On examination of Gram stained smear following features were noted

- Presence or absence of inflammatory cells
- Presence of organism and their gram reaction
- Size, shape, arrangement and relative number of organisms
- Presence of spore, their shape, position in cell

Inoculated RCM were incubated at 37ºC for 48 hrs. After that change in meat particles and in fluid of the RCM was noted. Gram stained smear were made from RCM and observed. Primary isolation was done by using freshly prepared CDC blood agar which is a non-selective media for all types of anaerobes [3].

All inoculated CDC blood agar plates were immediately put into the anaerobic jar (3.5 L polycarbonate jar) with a Anaeropack and anaerobic indicator tablet for production of anaerobic environment and its confirmation. The plates were incubated at 37ºC and examined after the 48 hours. The color change of anaerobic indicator tablet was confirmed every time before examination of plates.

Colonies on CDC blood agar were noted under the dissecting microscope as number of anaerobes has distinctive colonial features. It is also a valuable aid during the subculture of colonies to obtain pure culture isolates [3] Gram stained smear of each colonies were performed to observe Gram stain reaction, morphology and spores. Each colony type from the anaerobic blood agar is sub cultured to an aerobic blood agar plate and incubated overnight.

Aerobic culture was done onto Nutrient agar, Mac conkey agar and Blood agar. All aerobic isolates were confirmed by routine conventional biochemical tests.

Results

Following are the data obtained from the study.

Table – 1: Primary Causative Event:

Causative Factor	Number & Percentage
Road traffic accident	23 (46 %)
Other Traumatic Injury	11 (22%)
Post Surgical	04 (08%)
Electric Burn	04 (08%)
Intramuscular Injection	02 (04%)
No History of Trauma	06 (12%)
Total	50

Table 1 show that the Road traffic accident is the leading causative agent.

Table -2: Age Group Wise Distribution of positive cases:

Age Group	No. of cases	Percentage
10-20	03	18.75 %
21-30	03	18.75%
31-40	05	31.25%
41-50	02	12.5%
51-60	02	12.5%
61-70	01	6.25%
Total	16	100%

Table 2 shows that relatively younger age group (10-40 years) is affected predominantly.

Table-3: Sex Wise Distribution:

Sex	No. of cases	Percentage
Male	12	75 %
Female	04	25%
Total	16	100%

Table -3 shows that majority of affected patients were male.

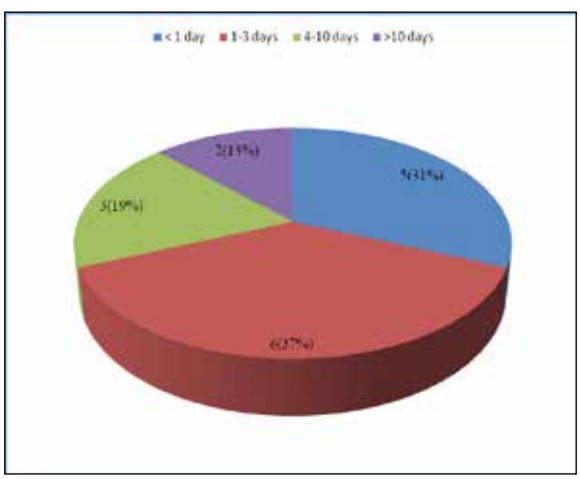

Fig-1: Incubation period in positive reported cases

Table-4: Positive cases by Direct
Microscopy and Anaerobic Culture

	Culture Negative	Culture Positive	Total
Smear Negative	30	4	34
Smear Positive	4	12	16
Total	34	16	50

- There were total 16 smear positive cases (32%). Out of which 12 specimens showed presence of Clostridium species in anaerobic culture.
- This 12 culture positive specimen had total 22 isolates of obligate anaerobes.
- Out of 22 obligate anaerobe 21(95.45%) were suggestive of Clostridium species and 1(5.55%) was suggestive of Fusobacterium spp.
- Out of 16 culture positive cases 15(93.75%) were mixed with facultative anaerobes and only one (6.25%) case showed presence of pure growth of Obligate anaerobe.
- There were 79 isolates obtained by Aerobic culture and their distribution is as follows.

Klebsiella species	19
Enterococcus species	14
Staphylococcus aureus	12
Acinetobacter species	09
Escherichia coli	07
Pseudomonas species	06
Coagulase negative Staphylococci	05
Proteus species	04
Streptococcus species	02
Citrobacter species	01
	79

Discussion

Gas gangrene, once considered as a "Disease of war", is now seen in urban civilian set up where road traffic accidents have caused surge in number of cases [1, 2]. This study shows that younger age group (10-40 years) is the major affected group. Also striking feature is that almost 75% affected patients were male. This may be due to their outdoor activities.

Electric burn and Intramuscular injections are also appearing to be risk factors. Many clinically suspected patients showed foul smelling discharge and blackening of muscles but it was not exclusively related to gas gangrene. Also crepitation of affected site was seen in 7 (43.75%) positive cases. Crepitation was also observed in 5 negative reported cases. So, combination of gas in tissues and necrotizing myositis does not always indicate a clostridial infection. Bessman and Wagner reported 49 cases of gas gangrene with myositis and soft tissue gas of which only one was caused by clostridial organisms [5].

The incubation period of gas gangrene is usually short, almost always less than 3 days, and in the majority of cases less than 24 hr [4]. In present study also incubation period in 68% cases was <3 days.

Gas gangrene in most cases is a polymicrobial infection [2, 3, 4] .In this study also, except one positive case all other showed presence of facultative anaerobes. There were total 101 bacterial isolates out of which 22 (21.78%) were obligate anaerobes out which 21 isolates were of Clostridium spp. So this study is in accordance with other study which shows that the Clostridium spp. are the major causative agents of Gas Gangrene [6].

Table 5: Presumptive identification of clostridium species [3, 4, 7]

CDC Colony morpholgy	Gram Stain of colonies	Presumptive identification
Rough , irregular, flat, rhizoid or spreading colonies	Small gram positive bacilli with oval, nonbulging, subterminal spores	C. septicum
Gray to yellow, circular, glossy, dome shaped, entire.(May show double zone of beta hemolysis)	Gram variable rods with blunt ends occurring singly or in pairs; spores seldom seen	C.perfringens
Large yellowish colonies with irregular edge	Gram positive bacilli with oval, slightly bulging, subterminal spores	C.sordellii
Gray, circular, markedly irregular to rhizoid margins resembling a "Medusa head"	Gram positive bacilli with oval, subterminal spores	C.sporogens
Grey-white; circular to slightly irregular, semiopaque, nonhemolytic	Gram variable straight or curved rods with terminal round spores	C.ramosum

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Fig.2.1 Target hemolysis of C.perfringens on CDC blood agar

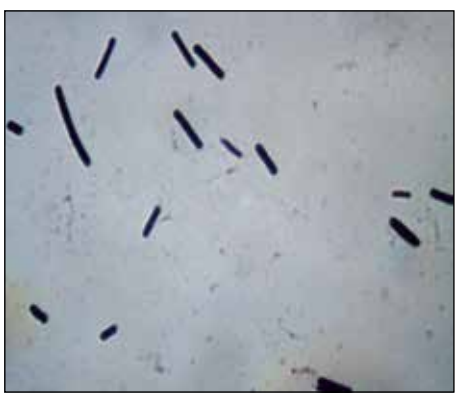

Fig.2.2 Clostridium perfringens in Gram stain

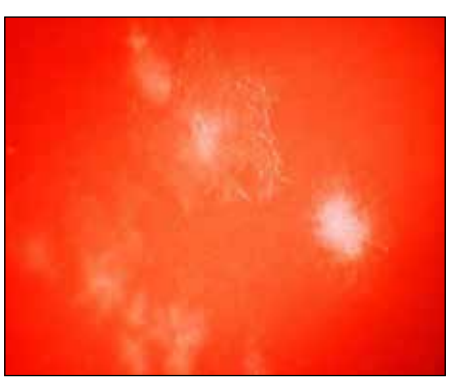

Fig.3.1 Colony of C.septicum on CDC blood agar

Fig.3.2 Gram stain appearance of C.septicum

Fig.4.1 Colony of C.sordellii on CDC

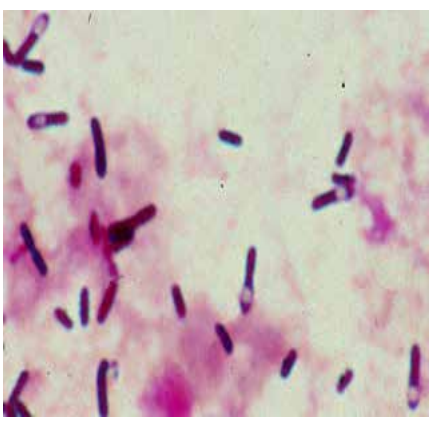

Fig.4.2 Gram stain appearance of C.sordellii

There were total 79 aerobic bacteria isolated. Among them 46 (58.22%) were Gram negative bacilli and 33 (41.78%) were Gram positive cocci. Klebsiella species (24%) were the major isolated organism followed by enterococcus species (17.8%). Aerobes, usually members of Enterobacteriaceae, S.aureus and Streptococcus spp. are identified commonly along with the causative agents of clinical gas gangrene [8].

Conclusion

Gas gangrene is a clinical emergency and needs to be diagnosed and treated immediately. In this study following conclusions were drawn.

- Gas gangrene in urban civilian set up is usually associated with road traffic accidents, traumatic injuries, post surgery etc. Maximum patients were young male.
- Clinical history and wound examination is essential to support laboratory investigation.
- As incubation period in true gas gangrene is very short, sample should be collected immediately with taking care not to contaminate it with saprophyte and minimal contact with atmospheric oxygen to isolate the pathogenic anaerobes.
- Clostridium species are the major culprit in this disease. They can be identified by anaerobic culture and aerotolerance test. This study has shown that the isolation of anaerobes can be achieved by a practical, simple and relatively inexpensive system that permits presumptive identification of these bacteria routinely in the laboratory
- Species identification of anaerobes requires exhausting biochemical and culture tests. In spite of this, patient history, clinical evaluation along with initial conventional steps for anaerobes identification gives important clue to support diagnosis of clinical emergency like gas gangrene which will guide clinician to take specific management steps and in turn save the patient's life!

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