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RESEARCH ARTICLE

Isolation and diagnostic of Clostridium histolyticum from wounds with sharp objects ,explosions and Splinters

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Abstract

A total of 75 samples (25 wound swabs with sharp objects , 25 wound swabs from explosions and 25 wound swabs from Splinters) of patients at many age group range from 15to 40 year old were collected from patients who attending the Samawah Teaching Hospital for pediatrics and Gynecology of AL-Muthanna governorates ; Through the period which extended from February 2014 to January 2015.

The isolation and identification methods of *Clostridium histolyticum* were followed upon the morphological, cultural and biochemical characteristics. The phenotypic results showed that the isolation percent percent of *clostridium histolyticum* were (56% , 84%, 68%) from wound swabs with sharp objects, explosions and Splinters respectively.

INTRODUCTION

Clostridium histolyticum, a Gram-positive, spore-forming anaerobic microorganism, was first isolated by Weinberg & Seguin from wounds of warfare in 1916 (1). Culture broth filtrates of C. histolyticum had lethal and hemolytic activities (2) caused by toxins differing in heat stability and susceptibility to oxidation (3). They also produced degenerative changes in various organs (4,5).

The clostridia are fermentative, oxidase-negative and catalase-negative. The strictness of anaerobic requirements varies among the species but they all prefer 10 per cent CO_2. Most clostridia require enriched media that include amino acids, carbohydrates, vitamins and blood or serum (6) .Optimum growth of most of the pathogenic species occurs at 37°C. There are over 80 *Clostridium* species of which about 11 are of veterinary importance. Most of the pathogenic species produce one or more exotoxins of varying potency (7).

Methods

Samples *:* A total of 75 wound swabs specimens were collected and examined from patients who suffered from wounds with sharp objects and as a result of exposure to explosions and Splinters which were referred to the Samawah Hospital.

Culture preparation and growth media

Prepare the obligate anaerobe cultures by swabbing from wounds into a tube containing 5 mL of reduced Enriched Thioglycollate Medium containing Vitamin K_1 and hemin. Mix well and adjust to a turbidity comparable to a 0.5 McFarland standard and the cultures were grown for (48-72)◦ h on blood agar plates containing 5% horse blood and the cultures were incubated in an anaerobic jar at 37C◦. After, grown colonies on blood agar were examined by gram stain (8).

API 20 A

The API 20 A test system consists of 20 microcupules containing substrates for carbohydrate utilization and enzymatic reactions. A suspension of each strain was prepared by adding pure cultures from an anaerobically incubated blood agar plate into the API Anaerobe suspension medium. The turbidity of the suspension was adjusted so as to be equal to or greater than the No. 3.0 McFarland standard. The suspension was inoculated into the microcupules under aerobic conditions. The test strips were incubated anaerobically in a culture jar at 37°C for 24 h. After adding the required reagents, the test strips were read visually based on color changes. The individual reactions were determined, numerical values were assigned to the positive and negative tests, a 7-digit profile number was generated, and the identification was determined by the API Analytical Profile Index (9).

Results and discussion

In this study, a total of 75 wound swabs specimens were collected and examined from patients who suffered from wounds. The results revealed that the percent of *clostridium histolyticum* were (56% , 84%, 68%) from wound swabs with sharp objects, explosions and Splinters respectively (Table 1) . Baradkar *et al* had shown cent percent correlation between the two (10) . Out of total 156 anaerobes isolated in our study, 48 were *Clostridium* spp. (30.8%). Rao *et al* had reported 56% *Clostridium* spp. in their study (11). These organisms were also isolated in the study by Rao *et al*. (11). Out of 48 patients in whom *Clostridium* spp. were isolated, they were the sole pathogen in 30 (62.5%) and *Clostridium* spp. along with non sporing anaerobes from 18 patients (37.5%). However, *Clostridium bifermentans, C. sporogenes* and *C. septicum* which were reported by other (10 ,12).

The total wound swabs with sharp objects according to the age groups were (21.42%,42.85%,28.57%,7.14%) respectively. The majority of cases occurring between the ages (20-24) years . While, the total Wound swabs from explosions according to the age groups were (9.52%,19.04%,42.85%,19.04%,9.52%) respectively. The majority of infections between the ages (25-29) years . So, most of the *Clostridium histolyticum* were also isolated from wound swabs from Splinters according to the age groups were (5.88%,29.4%, 35.2%,17.6%,11.7%) respectively . The majority of cases occurring between the ages (25-29) years (Table 2).

Table (1): Number and percent of isolation of *Clostridium histolyticum* from different clinical samples (n=75).

Clinical samples	No. of tested specimens	No. of +ve *Clostridium histolyticum*	%	No. of –ve samples	%
Wound swabs with sharp objects	25	14	56	11	44
Wound swabs from explosions	25	21	84	4	16
Wound swabs from Splinters	25	17	68	8	32
Total	75	52	69.3	23	30.6

Table (2): Age distribution of patients enrolled in the study of Wound swabs with sharp objects, Wound swabs from explosions and Wound swabs from Splinters .

Age (years)	Wound swabs with sharp objects				Wound swabs from explosions				Wound swabs from Splinters				Total	
	-ve samples		+ve samples		-ve samples		+ve samples		-ve samples		+ve samples			
	No.	%	No	%	No	%	No.	%	No.	%	No	%	No.	%
15-19	4	36.4	3	21.42	0	0	2	9.52	1	12.5	1	5.88	11	14.6

20-24	2	18.18	6	42.85	2	50	4	19.04	3	37.5	5	29.4	22	29.33
25-29	3	27.27	4	28.57	1	25	9	42.85	2	25	6	35.2	25	33.33
30-34	1	9.09	1	7.14	0	0	4	19.04	1	12.5	3	17.6	10	13.33
35-40	1	9.09	0	0	1	25	2	9.52	1	12.5	2	11.7	7	9.33
Total	11	100	14	100	4	100	21	100	8	100	17	68	75	100

Phenotypic characteristics of *Clostridium histolyticum*

Colonies Characteristics

Microscopic examination of 75 isolates grown on blood agar showed that 52 isolates appeared rods and positive for Gram's stain as shown in figure (1, 2**) .**

Clostridium histolyticum is a motile, gram-positive, facultative anaerobe and the colonies appear on blood agar small, rough, irregularly round, and are surrounded by a zone of weak hemolysis (13). These bacteria tend to clump in pairs or short chains and are rods of 3-5µm x 0.5-0.7µm. Cells are richly flagellate and very motile. *Clostridium histolyticum* produces large endospores and are asaccharolytic and proteolytic. This bacterium is anaerobic, however minimal growth may be obtained through aerobic culture (13).

The inhibition of growth by sugars, particularly under aerobic cultivation, does not seem to be due to a peroxide substance (14,15). The inhibition could not be antagonized by addition of fresh guinea pig serum. The combination of two growth-inhibiting factors, air and sugars, seems to be the cause for the particularly strong inhibition. Prevot (16) claimed that *C. histolyticum* is a strict anaerobic species, although this is contradictory to the well-accepted microaerophilism of this organism (17). This claim of Prevot, however, seems to be plausible when this organism is grown in the presence of sugars.

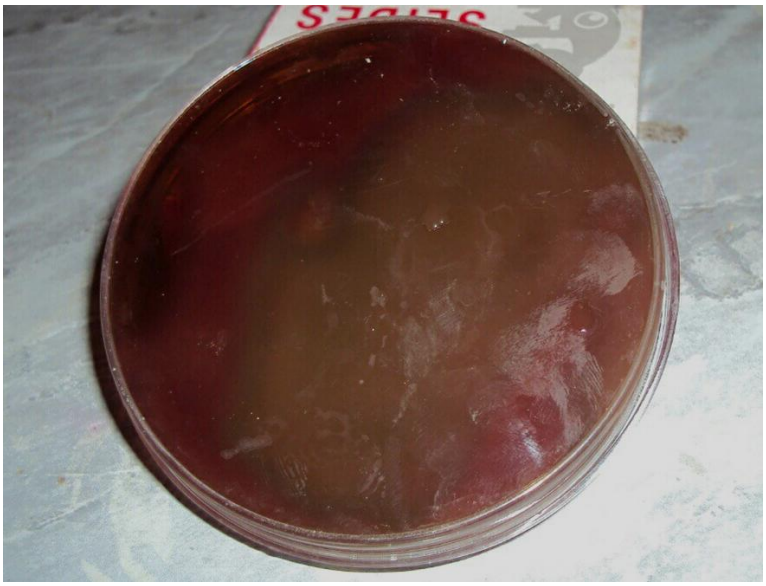

Figure (1): *Clostridium histolyticum* isolates on blood agar

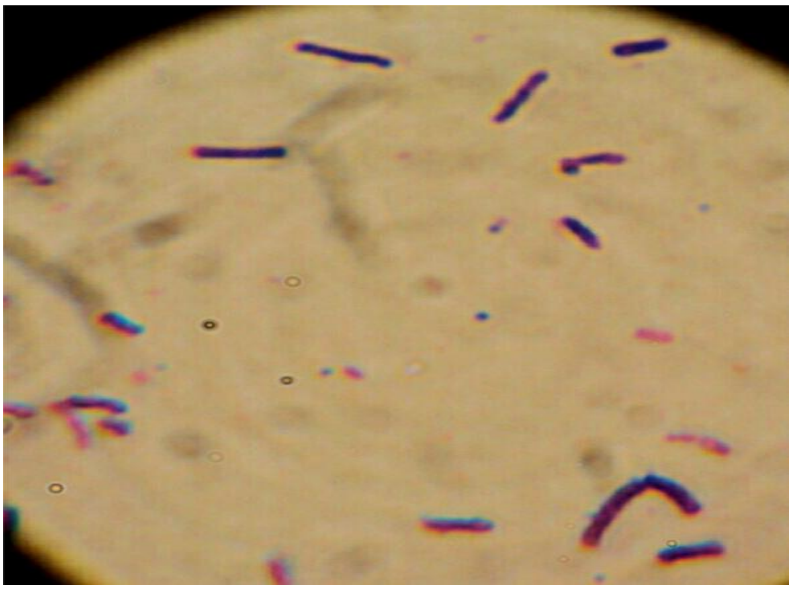

Figure (2): Gram's stain smear of *Clostridium histolyticum* (100 X) showing rods.

Biochemical characteristics (Api 20 A System)

Based on this system, a total of 52/75 (69.3%) of the total wound swabs with sharp objects 14(56) , wound swabs from explosions 21(84) and wound swabs from Splinters 17(68) were diagnosed as *Clostridium histolyticum* as shown in the figure (3).

The API 20A system has been extensively evaluated (18, 19, 20,21), 73% of the API results for 330 isolates were in agreement with the reference method (biochemical testing) when additional tests were routinely done. This is similar to the results of the present study in which API 20A correctly identified 71% of the isolates to the species level, including 21 of 25 Bacteroides isolates as well as 15 of 18 anaerobic rods, two of the most frequently isolated groups of anaerobes. API 20A gave disappointing results for the clostridia, identifying only 6 of 12 isolates correctly to the species level (22).

Recently, Meisel-Mikolajczyk and Dworczynski (23) chemically analyzed the sugar components of *C. histolyticum* , and demonstrated that they were comprised of glucose, xylose, rhamnose, mannose, ribose, and galactose. Since these sugars are strikingly consistent with those inhibitory for the growth of *C. histolyticum*, further investigation of this relationship might elucidate the mechanism of inhibition. Bulmash and Weaver (24) reported that the presence of glucose improved growth of this organism in a synthetic medium. Considering the finding that glucose is a cell component (23), we assume that this might happen in a synthetic medium which is otherwise extremely restricted in nutrition. However, Bulmash and Weaver did not give details.

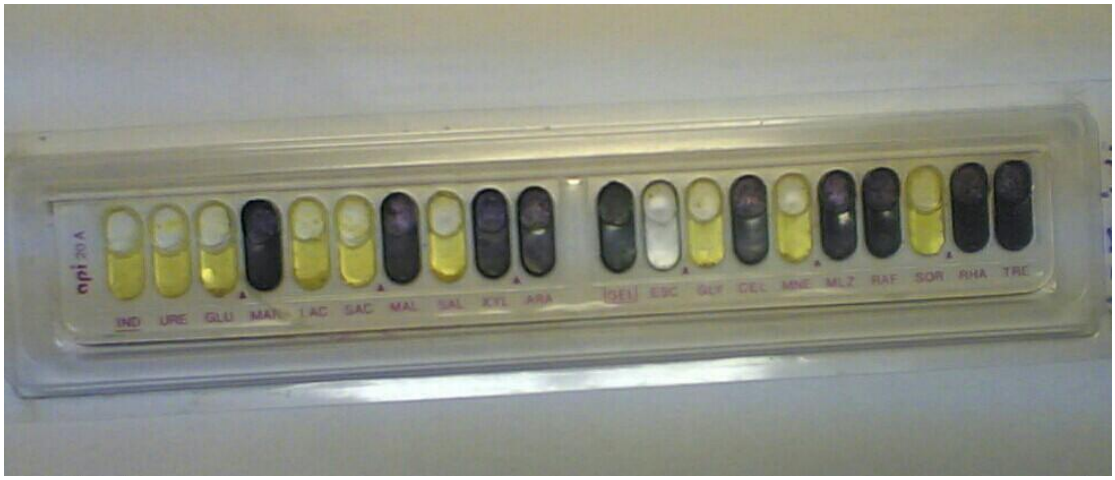

Figure (3): A standard Profile Identification (Api) anaerobic System of *Clostridium histolyticum* .

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