



Isolation and diagnosis of *Clostridium perfringens* from lobbies and surgical theaters of hospitals and Showing their Sensitivity to antibiotics

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Abstract

The study included taking 100 samples from different sources, including the hospital environment samples (lobbies and operating theaters), in Al-Khader General Hospital and in the city of Iraq for the period from October 2021 to April 2022. The results of isolation and diagnosis showed the growth of 27 isolates that are positive for *Clostridium perfringens*, distributed between 18 isolates (25.7%) from pollution of different lobby hospital, 9 isolates (30.0%) from different operating theaters, Microscopical, cultural, and biochemical examinations were used to diagnose these isolates in addition to the Api 20A method. A variety of antibiotic kinds were examined to see how sensitive the isolates were, and All bacterial isolates tested positive for high sensitivity to imipenem when the isolates' resistance to various antibiotics was assessed. Streptomycin sensitivity was (96.66%), Ampicillin sensitivity was (13.3%), and Erythromycin sensitivity was (86.66%), while metronidazole resistance was 100% in all isolates.

Keywords:

Chromogenic agar, Clostridium perfringens, Contamination percentage, Antibiotic sensitivity.

Objective: The study's objectives included isolating and diagnosing *Clostridium perfringens* from the Al-Khader general hospital's environment (lobbies and operating rooms), calculating the percentages of *Clostridium perfringens* contamination in the two areas, and comparing those percentages.

Introduction:

Different microbial agents have the potential to colonize the hospital setting, objects/instruments, and medical personnel. Direct contact with ill or colonized people or objects can spread an infection, increasing morbidity and mortality [1][2]. High concentrations of germs in the hospital's indoor environment are a growing source of worry. This has been linked to a range of infectious disorders, allergies, and acute illnesses brought on by microbes. [3]

Originally known as *Clostridium welchii*, *Clostridium perfringens* is a gram-positive, non-motile, obligatory anaerobic, encapsulated, spore-forming bacteria. [4] In the intestines of both people and animals, *Clostridium perfringens* is a common flora, and it naturally inhabits

soil and marine sediments [5]. The categorization system that divides the strains of *Clostridium perfringens* into toxicity patterns from [A to E] is based on the production of four main toxins (alpha- beta- epsilon- and iota), which vary between *Clostridium perfringens* strains. [6][7]

Clostridium perfringens infection is a serious and widespread problem that threatens public health as it is widespread in nature and has the ability to resist difficult environmental conditions, as well as being one of the main pathogens of invasive cancarina responsible for causing many diseases such as wounds and burns, and anaerobic cellulitis, Uterine infections and diarrhea associated with taking anti-life, including strains that cause food poisoning[8]

Clostridium perfringens can also produce spores in addition to producing toxins. The moist heat, cold, UV rays, desiccation, and a variety of other circumstances are all tolerated by these latent spores. [9]

Materials & Methods:

Collection and Culturing of Samples

In the current study, a total of 100 hospital environment samples from Al-Khader General hospital were collected. These samples were taken using sterile 1 cotton swabs and transported to the microbiology lab at the College of Science / University of Al-Muthanna in media of *Clostridium perfringens* broth to perpetuate them (Gas pak). It maintains the anaerobic conditions required for bacterial growth through oxygen reduction inside the container's environment at a temperature of 37 for a period of time.

then, the sample's medium was heated to a temperature of 100 degrees Celsius in a water bath for 20 minutes, and anaerobic incubation was performed using an anaerobic jar and gas-generating bags (Gas pak). which, for a duration of between 24 and 48 hours, at a temperature of 37, creates the anaerobic conditions required for bacterial growth by oxygen reduction inside the container's atmosphere. Prior to use in diagnostic tests, it was purified and re-implanted on the final media.

Identification of *Clostridium perfringens*

Identification of *Clostridium perfringens* Isolates were identified based on a microscopy examination and biochemical tests, according to C. M. Kelly et al., P.M. The diagnosis of bacterial isolates was also confirmed using the Api 20A diagnostic method.* *Phenotypical examinations*

The study included the characteristics of typical colonies of shape, texture, and size on solid media used in isolation, as well as conducting swabs of bacteria on a clean slide and then stained with Karem dye to observe the shape and clusters of cells, while malachite green was used to observe the shape and location of the spore inside the cell[10]

***Biochemical test**

The bacteria were diagnosed based on a number of biochemical tests, including catalase production, oxidase production, indole test, and voges-proskauer reagent [11]

Antibiotic sensitivity test:

According to J. G. Cappuccino et al.¹⁰, the bacterial isolates were tested for antibiotic sensitivity using the Kirby-Bauer disk diffusion method by creating a bacterial trap and comparing its turbidity to a McFarland standard tube [0.5] equivalent to 1.5×10^8 cells/mL^[12], using a clean cotton swab, spreading the suspension onto the Columbia base agar medium. To ensure equal stickiness, the plates were produced by planning in different directions. They were then allowed to dry for 5 minutes at room temperature., and then distributed 5 antibiotic tablets per dish using sterile forceps. The dishes were then incubated for 18 to 24 hours at 37°C under anaerobic conditions. Results obtained by measuring the diameter of the disk-encircling inhibitory zone were then compared to the worldwide standard tables in CLSI.^[13]

Results & discussion

According to. their phenotypic characteristics, which included the fact that they produced circular, convex, and glossy orange colonies of *Clostridium perfringens* on Chrom agar, the identification of bacterial isolates on the culturing media was made. The isolates' gram-positive, bacillus-shaped, flat ends—which may be somewhat inflated due to the presence of the spore—and single or double arrangement were. also demonstrated through microscopic inspection of the samples [14]. Regarding the biochemical testing , all isolates passed the methyl red and litmus milk reactions but failed the oxidase, catalase, indole test, voges-proskauer, and oxidase tests [15][16]

The results of the current study showed that the total percentage of *Clostridium perfringens* isolates reached isolate with a ratio of (45%) out of 100 samples collected from different sources, including lobbies, operating theaters , beds patients, wall, tables, floors from the hospital environment (Table 1),

The percentages of each lobby's pollution in Al-Khader General Hospital were displayed in a table (1). The current findings show that although there are somewhat higher percentages of intensive care and resuscitation lobby than emergency lobby 8 (25.8%) against 10 (25.6%), overall there are no statistically significant differences in the proportion of pollution between the two lobby types. The current data indicate that there is a higher frequency of pollution in floors from both types of lobby when compared to other sources of isolates, although the difference is not statistically significant ($p > 0.05$).

Table (1): The numbers and percentages of *Clostridium perfringens* isolated from the lobby of Al-Khader General hospital.

Pollution of lobby	P value	Source of isolates					Lobby
		Beds	Floors	Tables	Walls	Medical devices and tools	
Emergency Lobby							
10 (25.6%)	0.628 ¥ NS	1 (16.7%)	4 (44.5%)	2 (28.6%)	2 (20.0%)	1 (14.3%)	Yes, n (%)
29 (74.4%)		5 (83.3%)	5 (55.5%)	5 (71.4%)	8 (80.0%)	6 (85.7%)	No, n (%)

Intensive care and resuscitation Lobby							
8 (25.8%)	0.160 ¥ NS	0 (0.0%)	4 (50.0%)	1 (20.0%)	3 (37.5%)	0 (0.0%)	Yes, <i>n</i> (%)
23 (74.2%)		5 (100.0%)	4 (50.0%)	4 (80.0%)	5 (62.5%)	5 (100.0%)	No, <i>n</i> (%)
0.987 ¥ NS		0.338 ¥ NS	0.819 ¥ NS	0.735 ¥ NS	0.410 ¥ NS	0.377 ¥ NS	Total p value
The percentage of pollution of each source							
18 (25.7%)	0.108 ¥ NS	1 (9.1%)	8 (47.1%)	3 (25.0%)	5 (27.8%)	1 (8.3%)	Yes, <i>n</i> (%)
52 (74.3%)		10 (90.9%)	9 (52.9%)	9 (75.0%)	13 (72.2%)	11 (91.7%)	No, <i>n</i> (%)

Table (2): The numbers and percentages of *Clostridium perfringens* isolated from the operating theater of Al-Khader General Hospital

Pollution of operating theater	P value	Source of isolates					Operating theater
		Beds	Table	Floors	Walls	Medical devices and tools	
Surgical operating theater							
9 (30.0%)	0.919 ¥ NS	1 (20.0%)	2 (28.6%)	2 (40.0%)	3 (37. 5%)	1 (20.0%)	Yes, n (%)
21 (70.0%)		4 (80.0%)	5 (71.4%)	3 (60.0%)	5 (62.5%)	4 (80.0%)	No, n (%)

This is due to negligence in leaving the doors of the surgery rooms open after sterilization, which allows air currents laden with various bacteria to enter and contaminate the environment around the operation room. This is the cause of the high percentage of *Clostridium perfringens* isolate from surgical theater sample. Knowing that *Clostridium perfringens* can live for several weeks in a dry environment like beds, medical equipment, and some fabrics because they are able to produce spores that can withstand unfavorable conditions, hospitals are in this case considered an environment that contains a large number of germs as a result of visitors entering with various diseases.[17]

The results of an antibiotic sensitivity test revealed that *Clostridium perfringens* isolates had a high sensitivity to imipenem (100%) and that the ratio of their sensitivity to streptomycin had reached (96.66%). It is a bactericidal antibiotic that binds to the ribosome's 30S subunit and prevents protein synthesis. According to the table (2).

Table 2: sensitivity test of *Clostridium perfringens* isolates.

		<i>Clostridium perfringens</i> samples				Character	Antibiotics
Resistance		Intermediate		Sensitive			
%	No	%	No	%	No		
—	—	—	—	100%	30	IPM	Imipenem
96.66%	29	3.33%	1	—	—	S	Streptomycin
86.66%	26	13.33%	3	—	—	E	Erythromycin
3.33%	1	66.66%	20	20%	6	Am	Ampicillin
100%	30	—	—	—	—	MET	Metronidazole

While the isolates' sensitivity to the antibiotic Erythromycin was (86.66%), this medication binds to the 50S subunit of the ribosome, which blocks the transfer of peptides and protein-making machinery and halts translation [18]. All of the isolates were resistant to metronidazole, with a 100% resistance rate for each drug, and none of them demonstrated sensitivity to either drug.

Metronidazole resistance in isolates is caused by a decrease in the drug's permeability or an increase in its flow, a drop in the drug's activation, and an increase in the activity of DNA repair enzymes [19]. The results of the sensitivity test demonstrated that imipenem was considered an effective treatment for *Clostridium perfringens*, which belongs to the class of carbapenems that have a broad impact on both gram positive and gram negative bacteria. The reason for these isolates' high sensitivity to imipenem is that they are resistant to the bacterial enzyme beta-lactamase, which has a lethal effect on bacteria. [20]

Conclusion

According to the study's findings, the isolation of the operating room was more conducive to *Clostridium perfringens* infection than the isolation of the hospital's lobbies. All bacterial samples isolates shown great sensitivity to imipenem (100%) and high resistance to metronidazole (100%) respectively.

Ethical approval: The study was approved by the administration and officials of Al-Khader Teaching Hospital at Al-Muthanna province/ Iraq. Patient consents were also taken to obtain hospital environment samples to be cultured in the laboratory.

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