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Investigation of clostridium perfringens in contaminated lobbies and surgical theater and showing their resistant to metronidazole

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***Abstract*---**Objective: The study aimed to isolation and diagnosis *Clostridium perfringens* from Al-Rammathia general hospital environment (lobbies and operating theaters) and determining the percentages of *Clostrdium perfringens* contamination in lobbies and operating theaters and comparing them with each other. Methodology: Collected in the current study (188) sample of the hospital environment, which included walls and flooring tools and beds for lobbies and operations room, in Al-Rumaitha General hospital and for a period ranging from October (2021) until April(2022)these samples were taken by sterile cotton swabs and used to transport them to the microbiology laboratory at the College of Science / University of Muthanna in media of *Clostridium perfringens* broth to perpetuate them, then the medium containing the sample was boiled in a water bath at 100 degrees Celsius for 20 minutes and incubated anaerobic using Anaerobic jar and gas-generating bags (Gas pak). Which provides the anaerobic conditions necessary for bacterial growth through oxygen reduction inside the container's atmosphere at a temperature of 37 for a period ranging from 24-48 hours. It was then re-implanted on the final media for purification before being used in diagnostic testing. Result: The diagnosis of bacterial isolates on the cultures media was carried out according to their phenotypic characteristics, as they gave on the Chrom agar *Clostridium perfringens*, a circular, convex, and glossy oranges colonies, It was also shown through a microscopic examination of the isolates that they are a gram positive, bacillus-shaped with flat ends, which may be slightly swollen due to the presence of the spore, and are arranged in the form of single or double, As for the biochemical tests, all isolates were positive for methyl red and litmus milk reaction, while they were negative for oxidase catalase, motility, indole test, voges-proskauer,

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The results of the current study showed that the total percentage of *Clostridium perfringens* isolates reached isolate with a ratio of (60%) out of 188 samples collected from different clinical sources, including hospital environment (lobbies and operating theaters) the lobbies and operating theaters included surgical tools, wall, beds, Tables, Floors. The results showed that the highest percentage of *Clostridium perfringens* isolate were among the surgical theater sample which reached 36.4% Table (2), while the percentage of isolates were taken from lobbies sample reached 31.3% Table (1). Conclusion: The results of the study showed that infection with *Clostridium perfringes* was more in the sample isolation of the operation theater compared with samples isolation of lobbies of the hospital environment.

***Keywords*---**Chromogenic agar, *Clostridium perfringens,* Contamination percentage, Antibiotic resistant.

Introduction

The hospital environment, objects/instruments, and healthcare staff can all be colonized by various microbial agents. Direct contact with infected and/or colonized patients, as well as goods, can cause transmission, leading in morbidity and mortality [1] [2]. High levels of germs in the hospital's indoor environment are becoming an increasing topic of concern. This has been associated to a number of acute diseases, infections, and allergies caused by microorganisms.[3]

Contamination of wounds and burns with bacteria is one of the most serious difficulties and challenges faced by people living with, and contamination with anaerobic bacteria, particularly *Clostridium perfringens,* is one of the signs that poses a threat to infected people's life. *Clostridium perfringens*, originally *Clostridium welchii*, is a non-motile, gram-positive, obligate anaerobic, encapsulated, spore-forming bacteria.[4] *Clostridium perfringens* is a normal flora found in humans and animals' intestines, and soil and marine sediments provide a natural habitat for these bacteria. [5] They're opportunistic microorganisms that cause wound infection, gas gangrene, contaminated wounds, and anaerobic cellulitis, as well as burn infection, bacteremia, and septicemia. They're opportunistic microorganisms that cause wound infection, gas gangrene, contaminated wounds, and anaerobic cellulitis, as well as burn infection, bacteremia, and septicemia.[6] Bacteria's pathogenicity is largely owing to their ability to create a wide range of enzymes and poisons, as well as capsules and spores.[7] The production of toxins differs between strains of C. perfringens and its the basis of the categorization system that relies on the production of four of the main toxins (alpha, beta, epsilon, iota), which divides the strains of C. perfringens into toxicity patterns from A to E.[8][9] In addition to toxin synthesis, *Clostridium perfringens* can create spores. These dormant spores can tolerate moist heat, cold, UV radiation, desiccation, and a range of other conditions [10].

Materials & Methods

Collection and Culturing of Samples

Collected in the current study (188) sample of the hospital environment, which included walls and flooring tools and beds for lobbies and operations room, in Al-Rumaitha General hospital and for a period ranging from October (2021) until April(2022)these samples were taken by sterile cotton swabs and used to transport them to the microbiology laboratory at the College of Science / University of Muthanna in media of Clostridium perfringens broth to perpetuate them, then the medium containing the sample was boiled in a water bath at 100 degrees Celsius for 20 minutes and incubated anaerobic using Anaerobic jar and gas-generating bags (Gas pak). Which provides the anaerobic conditions necessary for bacterial growth through oxygen reduction inside the container's atmosphere at a temperature of 37 for a period ranging from 24-48 hours. It was then re-implanted on the final media for purification before being used in diagnostic testing.

Diganosis of *Clostridium perfringens*

The bacteria diagnosed according to the method indicated by studying the shape of bacterial colonies on solid nutrient media, Chrom agar and blood agar, depending on the biochemical tests mentioned by [11] The colonies in terms of colony size, diameter, height, color and shape of its edge. The diagnosis included making smears from the novae on clean glass slides stained with a cream dye. After that, the shape, color and arrangement of the stained cells were observed by light microscopy using an oil lens for the purpose of diagnosing the bacteria as negative or positive for the gram stain[12].

Detection of Some Virulence Factors of *Clostridium perfringens*:

Capsule Production

The presence of the capsule was detected by placing a drop of India ink stain on the surface of a clean glass slide, then using the loop, a pure colony was taken and placed on the glass slide, mixed with the stain, and after the slide was left to dry, it was examined by optical microscopy using an oil lens (100X). [13]

Spore formation

The presence of the spore was detected using malachite green stain and safranin stain, by placing a pure colony on a glass slide, brushing it on, and then fixing it with a flame, and then putting a malachite green stain (7.5 percent) on the slide, leaving it for 10 minutes, then washing it with sterile distilled water and submerging it in safranin and leaving it for one minute, then washing it with water[14]

Antibiotic sensitivity test

The antibiotic sensitivity test for bacterial isolates was carried out using the Kirby-Bauer disk diffusion method, as described by J. G. Cappuccino, et al [15].by preparing a bacterial trap and comparing its turbidity with a McFarland Standard tube (0.5) equivalent to $1.5x\ 10^8$ cells/ mL, and then spreading the suspension onto the Columbia base agar medium with a sterile cotton swab. The dishes were allowed to dry for 5 minutes at room temperature after being planned in different directions to ensure uniform stickiness, and then antibiotic tablets were dispersed at 5 tablets per dish using sterile forceps and incubated for 18 to 24 hours at 37°C in anaerobic conditions. The diameter of the inhibitory zone around the disks was measured, and the results were compared to a global standard tables in CLSI [16]

Results and Discussion

The diagnosis of bacterial isolates on the cultures media was carried out according to their phenotypic characteristics, as they gave on the Chrom agar *Clostridium perfringens* , a circular, convex, and glossy oranges colonies, It was also shown through a microscopic examination of the isolates that they are a gram positive, bacillus-shaped with flat ends, which may be slightly swollen due to the presence of the spore, and are arranged in the form of single or double, [17]. As for the biochemical tests, all isolates were positive for methyl red and litmus milk reaction, while they were negative for oxidase, catalase, , indole test, voges-proskauer , [18][19]. The results of the current study showed that the total percentage of *Clostridium perfringens* isolates reached isolate with a ratio of (60%) out of 188 samples collected from different clinical sources, including hospital environment (lobbies and operating theaters) the lobbies and operating theaters included surgical tools, wall, beds, Tables, Floors.

The results showed that the highest percentage of *Clostridium perfringens* isolate were among the surgical theater sample which reached 36.4% Table (2), while the percentage of isolates were taken from lobbies sample reached 31.3% Table (1),

Table (1): The numbers and percentages of *Clostridium prefringenes* isolated from the hospital lobby

Lobby	Source of isolates					*P value*	*Pollution of lobby*
	Medical devices and tools	Walls	Tables	Floors	Beds		
Esotericism lobby							
Yes, *n (%)*	1 (10.0%)	5 (50.0%)	3 (27.3%)	4(33.3%)	3 (33.3%)	0.421 ¥ NS	16 (30.8%)
No, *n (%)*	9 (90.0%)	5 (50.0%)	8 (72.7%)	8 (66.7%)	6 (66.7%)		36 (69.2%)
Surgical lobby							

Yes, *n* (%)	2 (25.0%)	4 (40.0%)	5 (50.0%)	3 (33.3%)	2 (20.0%)	0.651 ¥ NS	16 (34.0%)
No, *n* (%)	6 (75.0%)	6 (60.0%)	5 (50.0%)	6 (66.7%)	8 (80.0%)		31 (66.0%)
Emergency Lobby							
Yes, *n* (%)	1 (11.1%)	4 (36.4%)	2 (20.0%)	4 (50.0%)	2 (28.6%)	0.574 ¥ NS	13 (28.9%)
No, *n* (%)	8 (88.9%)	7 (63.6%)	8 (80.0%)	4 (50.0%)	5 (71.4%)		32 (71.1%)
Total p value	0.871 ¥ NS	0.798 ¥ NS	0.324 ¥ NS	0.711 ¥ NS	0.802 ¥ NS		0.781 ¥ NS
The percentage of pollution of each source							
Yes, *n* (%)	4 (14.8%)	13 (41.9%)	10 (32.3%)	11 (37.9%)	7 (26.9%)	0.271 ¥ NS	45 (31.3%)
No, *n* (%)	23 (85.2%)	18 (58.1%)	21 (67.7%)	18 (62.1%)	19 (73.1%)		99 (68.7%)

Table (2): The numbers and percentages of *Clostridium prefringenes* isolated from the hospital operating theater

Operating theater	Source of isolates					*P value*	*Pollution of operating theater*
	Medical devices and tools	Walls	Tables	Floors	Beds		
Surgical operating theater							
Yes, *n (%)*	2 (22.2%)	5 (50.0%)	2 (25.0%)	4 (40.0%)	3 (42.9%)	0.697 ¥ NS	16 (36.4%)
No, *n (%)*	7 (77.8%)	5 (50.0%)	6 (75.0%)	6 (60.0%)	4 (57.1%)		28 (63.6%)

The reason for hight percentage of *Clostridium perfringes* isolate from surgical theater sample is due to the This is due to neglect in leaving the doors of the surgery rooms open after sterilization, which allows air currents laden with various bacteria to enter, contaminating the atmosphere around the operation room. In this case, hospitals are considered an environment that contains a large number of germs as a result of visitors entering with various diseases, knowing that *Clostridium perfringens* bacteria can live for several weeks in a dry environment such as beds, medical tools, and some fabrics due to their ability to produce spores that can withstand unfavorable conditions.[20]

Medical personnel are also an external source of contamination in operating rooms, and their movement between operating rooms and other parts of the hospital without changing their clothes and shoes is a risk. Furthermore, patients' failure to clean or shave properly before entering the operating room is a major contributor to the pollution of operating rooms and the spread of infections acquired in hospitals following various surgeries [21]

the population density surrounding the hospital plays a major role in the increasing contamination, as the districts and subdistricts are an open environment with more ventilation than the center and neighboring buildings, which are a barrier to good ventilation, in addition to surgical medical tools and their role in the proportion of contamination, as the better the sterilization, the less contamination.[22]

It was observed through the result of sensitivity test for antibiotics, that isolates of *Clostridium perfringes* showed high resistant to metronidazole and the resistant percentage (100%) for each of them .The cause for isolates' resistance to metronidazole is due to 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 enzymes involved in DNA repair.[23]

Conclusion

The results of the study showed that infection with *Clostridium perfringes* was more in the sample isolation of the operation theater compared with samples isolation of lobbies of the hospital environment

Ethical approval

The study was approved by the administration and officials of Al-Rammathia 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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