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Evaluation of nosocomial bacteria in Babylon province, Iraq

Farah Safaa Abdulhussein Alwan

Medical Laboratory Techniques Department, Al-Mustaqbal University College, 51001 Hillah, Babil, Iraq

Corresponding author email: farah.safaa@mustaqbal-college.edu.iq

Karrar Salih Mahdi

Medical Laboratory Techniques Department, Al-Mustaqbal University College, 51001 Hillah, Babil, Iraq

Email: karrar.salih@mustaqbal-college.edu.iq

Duha Mahdi Hussein

Medical Laboratory Techniques Department, Al-Mustaqbal University College, 51001 Hillah, Babil, Iraq

Email: dhuha.mahdi@mustaqbal-college.edu.iq

Abstract--This study was conducted to evaluate the occurrence of microbial contamination in the environment of some hospitals and to identify its source in Babil Governorate, during the period between 24/10/2021 to 16/12/2021, (133) samples were collected from different models of samples in Al-Hilla Surgical Hospital, maternity and Children Hospital, Al-Musayyib general Hospital, Marjan Teaching Hospital and Imam Al-Sadiq Hospital. The results of the study was showed the emergence of positive and negative bacteria in the hospital environment from a total of 186 isolates, where the numbers of negative bacteria were higher than positive bacteria (130, 56 isolates, respectively). Also results was revealed the widespread of gram-negative and gram-positive bacteria in emergency wards, especially in Hilla Teaching Hospital, Marjan Hospital, and Musayyib general Hospital, compared to other corridors, which amounted to (21, 9, 15 isolates), respectively.

Keywords--evaluation, nosocomial bacteria, hospital environment, Babylon province.

Introduction

The infections acquired from hospitals is one of the serious health problems that may affect patients, especially the elderly, the immunocompromised, premature babies, burn patients, intensive care patients, persons receiving long-term treatment, and patients being treated with certain medications such as broad spectrum antibiotics, immunosuppressants, cortisone, chemotherapy for cancer (McFee, 2009), and experts confirmed that negligence in applying infection control methods inside hospitals leads to a prolongation of stay in the hospital, that cause opening the way for the emergence of new types of bacterial infections that are resistant to antibiotics, and may raise death rates, and significantly affect the quality of medical work, in addition to increasing cost and habitation of hospital beds, and the statistics confirmed (Mehta *et al.*, 2014).

The incidence of hospitals infections in developed countries ranges between 5 to 10 percent of all admissions to hospitals and health institutions, and this percentage rise about 10 to 20 percent in developed countries, and the most common types of bacterial infections that infect patients while they are in hospitals are urinary and respiratory tract infection, blood poisoning and surgical wound infection (Haque *et al.*, 2018).

The recommendations of international health authorities indicated that more than a third of these cases can be prevented by following health rules and adhering to infection prevention guidelines (WHO, 2021). Types of infections acquired through hospitals before more than a century ago were characterized as a critical problem effect on quality of health care provided in hospitals, and 20% of these infections can be avoided (Haque *et al.*, 2018). The presence of high percentages of microorganisms in the air of the internal environment of hospitals is an increasing and worrying factor in relation to many acute diseases, infections and allergies caused by such microorganisms, these percentages also give an indication of the degree of cleanliness of the internal environment of hospitals that carry various types of microorganisms (Bonadonna *et al.*, 2017).

Fungal spores are considered one of the most important types of pathogens that can be transmitted through the external and internal air of the hospital environment, in addition to its transmission through visitors, patients and air conditioners (Belizario *et al.*, 2021). The evaluation and determination of the type, number and complexity of different types of bacteria present in hospital rooms and halls, especially sensitive units, such as surgical operating rooms is of great importance and concern at the global level (Monteiro *et al.*, 2022). Such an infection may have serious consequences in terms of an increase in the death rate, infection rate and length of stay of patients in hospitals, in addition to the increase the total cost (Khan *et al.*, 2017).

The movement of hospital staff between operating rooms and operating rooms and other parts of them without changing their clothes and shoes, as well as patients coming to the operating theaters without cleaning or shaving themselves together before entering the operating theaters are very important factors in the pollution of operating rooms and operating rooms. Surgical and subsequent development of hospital-acquired infections after various surgical operations

(Spagnolo *et al.*, 2013). A large proportion of hospital-acquired infections occur as a result of cross-contamination and transmission of microorganisms through the hands of health-care workers as a major source of microbial contamination (Suleyman *et al.*, 2018).

This study was aimed to

- Evaluation of the occurrence of microbial contamination in the environment of some hospitals in Babylon Governorate.
- Isolation of Gram-positive and Gram-negative bacteria from different hospital environments.

Materials and Methods

Equipment and Tools used

sequences	device name
1	Autoclave
2	Incubator
5	Balance
6	Refrigerator
8	Microscope
9	Hood
10	Petri dish
11	Berner
12	Loop
13	Slide
16	Spreader
17	Foial
18	Swabs
19	Cotton
20	Forceps

Culture media used

Nutrient agar

This medium was prepared according to the instructions of the supplied company, and sterilized by (autoclave) at a temperature of (121 C⁰) and a pressure of (15) pounds / inch 2 for a period of (15) minutes.

The solutions used

Gram stain

It was prepared according to what was mentioned in Buxton and Fraser (1977), as it consists of the following solutions:

- a. Crystal violet dye was prepared by dissolving 0.5 g of dye in 100 ml of distilled water.
- b. Iodine solution was prepared by dissolving 1 gm of iodine and 2 gm of potassium iodide in 300 ml of distilled water.
- c. Absolute alcohol .
- d. Sufranine dye was prepared by dissolving 0.05g of dye in 100ml of distilled water.

Specimen collection

During the study, (133) samples of wound models were collected for patients in some hospitals in Babil Governorate, for the period between 24/10/2021 to 16/12/2021. These included hospitals: Al-Hilla surgical hospital, maternity and children hospital, Al-Musayyib general hospital, Marjan teaching hospital, and Imam al-Sadiq hospital.

The samples were taken using sterile cotton swabs, and included taking samples from different wounds such as (operational wounds, including childbirth operations, gunshot wounds, war wounds, wounds of patients with diabetes and injuries of transport accidents (the swabs were transferred to the Microbiology Laboratory at the Technical Institute - Babylon / Community Health Department for the purpose of growing it on Nutrient agar medium on the same day.

Preparation of the culture medium

Nutrient agar culture medium was prepared by taking an balance and a filter paper, where the filter paper is placed on the scale. Then the powder was put of the culture medium on the filter paper and according to the company's instructions, 28 g / 1000 ml of distilled water has been weighed. The weighed powder was added to the distilled water placed in a conical flask (Flask). the culture medium melts were mixed well, then it is closed tightly with a cotton stopper and metal paper and it was put in an auto cleave device at a temperature of (121) ° C and a pressure of (15) pounds / inch 2 for (15) minutes. Then the culture medium was extracted and cooled to 45° C, then poured into Petri dishes and left until the culture medium solidified, and this process was carried out inside the hood device and near the fire (burner).

Culture of samples

After the culture medium solidified in Petri dishes, the samples were cultured from the swaps by passing the swap over the surface of the medium and in all directions, by diffusion method, and then marking the dishes (planting date - tank number - building name) then the dishes were placed in the incubator) at a temperature of 37 ° C for 24 hours, in an inverted form.

Bacteria purification:

On the next day, the bacterial colonies that appeared on the culture media were purified by taking a smear from each colony by (loop) and planting them on a new culture media by the planning method and incubated in the incubator at a

temperature of 37 ° C for 24 hours in an inverted manner (Kasim and Juma, 1992).

The study of phenotypic traits

After the N. agar medium was inoculated with the bacterial culture and incubated at 37C° for 24 hours, the phenotypic characteristics of the bacterial colonies were studied in terms of colony strength and color.

Study of microscopic features

After the bacteria were purified, a smear was taken from each dish (from each pure colony) and a bacterial membrane was prepared from the isolated colonies on a clean glass slide (slide) by (loop) after placing one or two drops of sterile distilled water and we spread it on the surface of the slide It was fixed by heat and then dyed with gram dye by the following steps:

- a substance (violet agent) was added for 1 minute.
- the slides were washed by distilled water.
- Gram's iodine was added for 1 minute.
- slides were washed by distilled water.
- alcohol or acetone was added for 10 seconds.
- the slides were washed by distilled water.
- Sufranine was added for 30 seconds - 1 minute.
- the slides were washed by distilled water.

After these steps, the slide was leaved to dry in the air, then a drop of Sidr oil was put (immersion oil) and examine it with a microscope using an oil lens (100 magnification units) to observe the interaction of cells with Gram stain (Kasim and Juma, 1992).

Results

Table (4-1) revealed the number of isolates of gram-negative and gram- positive bacteria in Hilla surgical hospital reached 11 in women's divisions, 9 in men's divisions, 21 in emergency, 12 in advisory, 11 hospital corridors, and 6 in burns unit, while it reached in Marjan hospital the number of isolations in women's divisions is 10, men's wards is 14, emergency is 9, and hospital corridors are 8. The number of isolations in the maternity and children's hospital has reached 7, in women's and children's wards 6, in the maternity halls is 9, while in Musayyib general Hospital the number of isolations in women's wards 7, men's lobbies 16, emergency 15, hospital corridors 4, and the number of isolations in Al- Sadeq hospital in consultation was 6 and hospital corridors 5.

Table (4-1): shows the number of isolates of Gram-negative and Gram-positive bacteria in a hospital environment

Hospital name	Women's lounges	men's lounges	Women's and children's lounges	preemie lobbies	maternity hall	Emergency	Advisory	hospital corridor	burn unit	Total
Hilla surgical	11	9	---	---	---	21	12	11	6	70
Marjan	10	14	---	---	---	9	---	8	---	41
Maternity and Children's	---	---	7	6	9	---	---	---	---	22
Musayyib general	7	16	---	---	---	15	---	4	---	42
Al- Sadeq	---	---	---	---	---	---	6	5	---	11

Table (4-2) shows the number of samples taken from Al-Hilla surgical hospital 32 samples, and the number of isolates was 70 divided between 26 positive bacteria and 44 Gram-negative bacteria, the number of samples in Marjan teaching hospital 26 samples, and 41 isolates divided between 13 positive and negative bacteria 13 of Gram stain 28, while 30 samples was taken from Musayyib general hospital, and the number of isolates 42 was divided between Gram-positive 9 and Gram-negative bacteria 33, and the number of samples was taken from the Maternity and children hospital 25 samples and the number of isolates 22 was divided between 7 positive bacteria and 15 Gram negative bacteria, while the number of samples in Imam Al-Sadiq hospital 20 samples and the number of isolates 11 was divided between Gram positive 1 and Gram negative 10 bacteria.

Table (4-2): shows the number of samples and the number of isolates of positive and negative bacteria for the hospital environment

Hospital name	Samples number	Isolates number	Gram positive bacteria	Gram negative bacteria
Hilla surgical	32	70	26	44
Marjan	26	41	13	28
Musayyib general	30	42	9	33
Maternity and Children's	25	22	7	15
Al- Sadeq	20	11	1	10
Total	133	186	56	130

Discussion

The results we obtained showed an increase in the prevalence of gram-positive bacteria in emergency lobbies of hospitals, with a significant superiority in the prevalence of gram-negative bacteria. A decrease in the prevalence of gram-positive bacteria in emergencies was noted in Hilla Surgical Hospital. These results are agreement with Hirai, 1991 was referred to the ability of gram-negative bacteria to survive in the presence of biological materials and water, but ends with water evaporation and removal of biological materials and water. Gram-

positive bacteria, which have ability to survive for several weeks in dry conditions, several references recommend and emphasize the need for good cleaning, disinfection and sterilization. I wanted to get rid of the various bacterial contamination in the hospitals (Rutala and Weber 2015).

Since the types of coliform bacteria are intestinal types, this leads to the confidence that food, water, and the intestines of hospitalized patients are the most important sources of contamination with them, and this is consistent with (Pena *et al.*, 2003; Ensayef *et al.*, 2009), where *E. coli* is the most important for coliform bacteria while coli bacteria, while *Pseudomonas aeruginosa* bacteria are usually disinfection solutions a major source of contamination with these bacteria, which are responsible for many opportunistic infections, they are usually found in most humid environments and differ in many characteristics such as the ability to survive and spread in hospital environments because they have many determinants of appearance in addition to their internal resistance to many of the live antibiotics and disinfectants, which made it a major factor in the lives of patients in hospitals, in addition to its responsibility for many epidemics that occur in emergency (Novak Babic *et al.*, 2020). The dependence and excessive use of antibiotics is an important reason for the emergence of many antimicrobial infections such as the resistance of *Staphylococcus aureus* to the antibiotic methicillin (Larsen *et al.*, 2022).

Conclusions

- The presence of gram-negative bacteria in the hospital environment is significantly higher than the gram-positive bacteria
- The spread of both gram-negative and gram-positive bacteria is concentrated in emergency divisions.

Recommendations

Conducting other studies on Gram-negative and Gram-positive bacteria, including determining other virulence factors, such as their ability to produce Hyaluronidase, Lipase, Deoxyribonuclease, Leukoidin, Collagenase, etc., and testing their multiple resistance to antibiotics and its effect on hemolysis patterns.

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