MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Isolation and Identification of Major Pathogenic Bacteria from different hospital patients

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Received: 15/10/2022
Accepted: 24/10/2022

AbstractThe present study aimed to isolation and identification of major pathogenic bacteria from different hospital patients. The bacterial isolates were characterized morphologically according to colony shape, margin, elevation, texture, pigmentation. A total of 120 clinical samples were collected during the 2021 year from Mansoura University Hospital. The clinical samples included blood (40), urine (35), and stool (45) from different patients with different genders and ages ranging from (16-55 years). In the present result, 95 samples had bacterial growth after incubation, on the other side 25 samples did not indicate any growth. all the colony's shape was circular except strain 5 was irregular in shape. All colonies had an entire margin, raised elevation, creamy texture; and different color (off-white, pale yellow, yellowish – white, and orange) of pigmentation. The examined microscopically for cell shape, which revealed that the majority of isolates were rod shaped except strain 3 were cocci shape; Gram strain whereas the samples showed that majority strains are Gram negative but strain 3and 6 are Gram – positive and motility whereas the samples showed all strains that could motile except strains (2,3 and 8) are non-motile

keywords: Pathogenic Bacteria; Isolation, Identification, Deseases, *E. coil*, .

1. Introduction

The human body is a home to millions of these microbes, some of them responsible for invading our bodies and making us sick. Some others are important for our health (1), In a number of ways, they are essential to human culture and health, including food fermentation, sewage treatment, and the creation of fuel, enzymes, and other bioactive materials. (2). In addition to being used in biological warfare and bioterrorism, microbes are crucial model organisms in biology (3; 4). The important gut flora is one of the bacteria that make up the human microbiota in the body. Microbes are the primary cause of many infectious illnesses, making them the focus of hygiene precautions (5; 6). There are a wide variety of microorganisms, but they are all interested in one thing: reproducing. It just so happens that some of them make an impact on the world around them while they go about that task. Microorganisms act as the causative agents (pathogens) of several infectious diseases (7).

A pathogenic organism is an organism which can cause diseases in a host (9). A human pathogen can cause illness in humans. Because they induce illness, pathogens are also known as infectious agents. Pathogens emphasise survival and reproduction, just like any other creature (8). Different illnesses, some more serious than others, can be brought on by pathogens. Given the abundance of nutrients in human bodies, pathogens may thrive and proliferate there (9). Pathogen-induced infections can range in severity. While some illnesses may be minor, others can be fatal, For instance, compared to the fatal Ebola virus illness, the common cold is a moderate viral infection (Fung et al., 2020).

The capacity of many microbes to adapt to antimicrobial treatments has allowed them to persist for thousands of years. They do this either through DNA transfer or spontaneous mutation (10). This method enables certain bacteria to fight off the effects of various

antibiotics, leaving them useless. These microorganisms generate multidrug resistance in a number of ways. (11). Bacteria are multidrug resistant, meaning they are resistant to multiple different antibiotics. According to (12), the misuse of antibiotics is the major source of antimicrobial resistance. As a result, either specific types of germs that are naturally resistant to antimicrobials become considerably more common than the ones that are readily eradicated with treatment, or microorganisms develop a defence against the medications used to treat them.

This work mainly aims to isolation and identification of major pathogenic bacteria from different hospital patients

2. Materials and Methods

2.1. Collection of samples

This study deals with the isolation and characterization of bacteria from blood, urine, and stool of hospitals patients. 120 samples (40 blood, 35 urines, and 45 stool samples) were first collected from different patients with different genders and ages ranging from (16 – 65 years) in sterile counters then incubated at -4 ºC.

2.2. Isolation and Purification of pathogenic bacteria

The nutrient agar medium was used for the isolation of pathogenic bacteria. The medium was sterilized by autoclaving at 121℃ for 20 min and cooled to 50 ℃. The pH of the medium was adjusted at pH=7. Glycerol stock was used for storing the cultures for a longer time at refrigerated conditions.(℃ 4-)

The collected samples were inoculated on the surfaces of the NA plates. After 24 hrs from the bacterial cultures, morphologically different bacterial colonies were selected and are repeatedly streaked to achieve bacterial isolates. All selected isolates were subculture in nutrient agar slants and finally, all the purified bacteria were maintained at 4 °C for Short-term storage till further used, −80°C in nutrient broth with 50% (w/v) glycerol for Long-term storage.

2.3. Morphological characterization of isolates

The isolates were characterized to determine the morphology of the bacterial cells upon noticeable characteristics such as cell shape, colony color, and texture. This was determined by the traditional gram staining method described by (13) .

2.3.1. Cell shape

The purified cultures, at the log phase, were observed microscopically for the cell morphological characteristics according to (14).

2.3.2. Gram staining

The smear was covered with crystal violet and let to stand for 30 seconds. The stain was washed out briefly using a wash bottle of distilled water, excess water was drained. Then covered with iodine solution and let it stand for 30 seconds, added 95% alcohol till no violet color comes off. For a brief while, distilled water from a bottle was used to rinse the slide.. The smear was covered with basic safranine for 20 seconds. The slide was washed gently for a few seconds, dried with bibulous paper, and air-dried, and a drop of cedar oil was added then examined using the oil-immersion lens. Gram-positive bacteria appear violet and gram-negative bacteria appear pinkish-red (Hucker and Conn, 1923) .

2.3.3. Motility test

Isolates were inoculated on the middle of semi-solid LB agar plates (0.2% agar) then incubated at 30°C for 24 hrs then diffusion of colonies can be observed (Elbeltagy et al., 2000). Also microscopically using cavity slide can be used for observing the motility of endophytic bacterial culture after 72 hrs of growth (14).

3. Results and Discussion

3.1. Isolation of Human Pathogenic Bacteria

A total of 120 clinical samples were collected during the 2021 year from Mansoura University Hospital. The clinical samples included blood (40), urine (35), and stool (45) from different patients with different genders and ages ranging from (16-55 years) in sterile counters then incubated at -4ºC. First the obtained isolates were isolated, purified then screened for their morphological, biochemical, and molecular identification; as results showed below.

As indicated in Figure 1, the isolation was carried out using a nutrient agar medium by inoculating 20 µl of samples on the surface of

the medium and incubating at 37 oC for 24 hours.95 samples had bacterial growth after incubation, on the other side 25 samples did not indicate any growth.

3.2. Purification of Human Pathogenic Bacteria

After isolate, the growth colonies were purified according to the difference in shape and pigmentation. A total of 8 pathogenic bacteria were purified from the 120 samples as lows ,4 bacterial strains were isolated from 40 blood samples, 5 strains from 45 urine samples, and 3 isolates from 35 stool samples as shown in Table 1 and Figure 2.

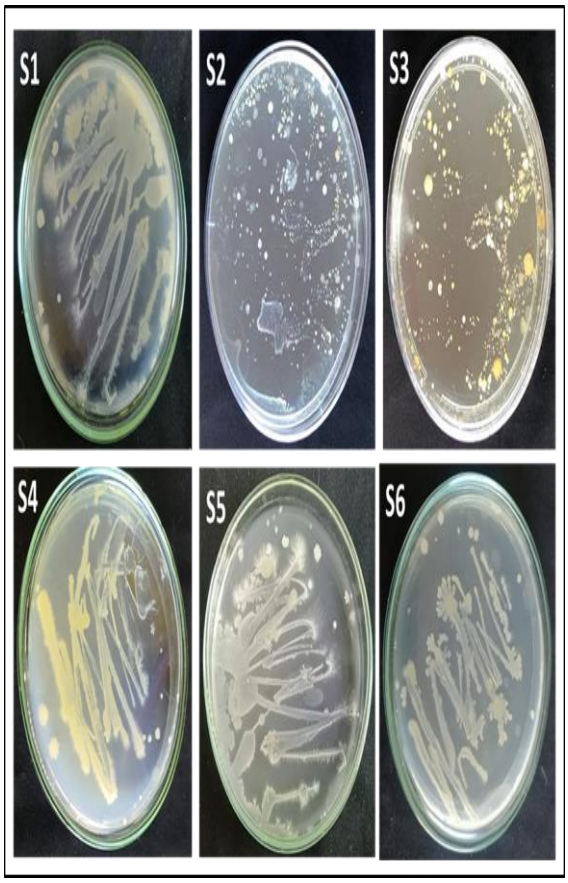

Fig 1. Isolation of human pathogenic bacteria (S1-S6) from urine, stool, and blood samples on L.B agar medium collected from different hospital patients.

3.3. Morphological characterization of bacterial isolates

The bacterial isolates were characterized morphologically according to colony shape, margin, elevation, texture, pigmentation where all the colony's shape was circular except strain 5 was irregular in shape. As demonstrated in Table 2, all colonies had an entire margin, raised elevation, creamy texture; and different color (off-white, pale yellow, yellowish – white, and orange) of pigmentation as shown in Table 2.

They were then examined microscopically for cell shape, which revealed that most isolates were rod –shaped except strain 3 were cocci shape; Gram strain whereas the samples showed that majority strains are Gram negative but strain 3and 6 are Gram – positive and motility whereas the samples showed all strains that could motile except strains (2,3 and 8) are non-motile as shown in the Table 2 and Figure 2.

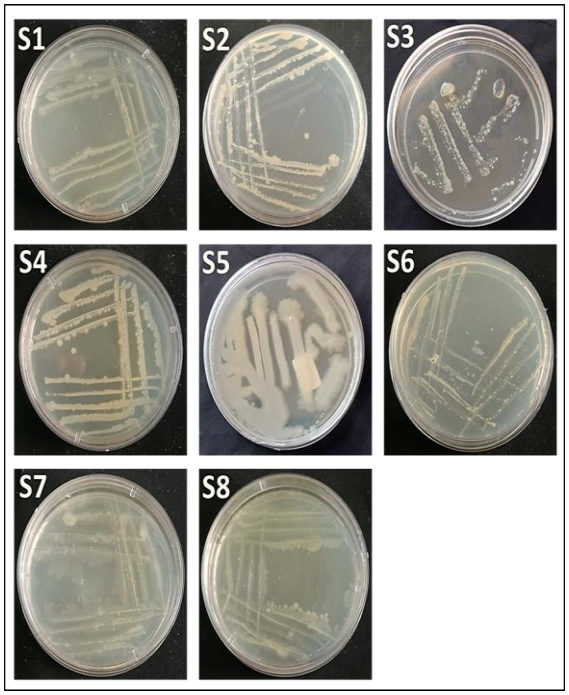

Fig 2. Purification of human pathogenic bacteria (S1-S8) from urine, stool and blood samples collected from different hospital patients

Table 1. The number of human pathogenic bacteria isolated from urine, stool and blood samples collected from different hospital patients.

Isolates Serial	Code	Patients' Samples		
		Blood	Stool	Urine
Strain1	S1	-	+	+
Strain2	S2	-	+	+
Strain3	S3	+	-	+
Strain4	S4	-	+	-
Strain5	S5	-	+	+
Strain6	S6	+	-	-
Strain7	S7	+	+	-
Strain8	S8	+	+	+
Total		4	6	5

Table 2. Morphological characteristic of human pathogenic bacteria isolated from urine, stool and blood samples collected from different hospital patients.

Isolates serials	Characteristics				
	Colony shape	**Margin**	**Elevation**	**Texture**	**Pigmentation**
Strain 1	Circular	Entire	Raised	Creamy	Yellowish white
Strain 2	Circular	Entire	Raised	Creamy	Yellowish white
Strain 3	Irregular	Entire	Raised	Shiny Creamy	Off-white
Strain 4	Circular	Entire	Raised	Shiny Creamy	Off-white
Strain 5	Irregular	Entire	Raised	Creamy	Off-white
Strain 6	Circular	Entire	Raised	Creamy	Yellowish white
Strain 7	Circular	Entire	Raised	Shiny Creamy	Off-white
Strain 8	Circular	Entire	Raised	Creamy	Yellowish white

Table 3. Microscopic characteristic of isolated bacteria from blood, stool and urine samples collected from human with different genders and ages.

Isolates serial	Gram stain	Shape	Motility
Strain 1	-	Rod	Motile
Strain 2	-	Rod	non-motile
Strain 3	+	Cocci	non-motile
Strain 4	-	Rod	Motile
Strain 5	-	Rod	Motile
Strain 6	+	Rod	Motile
Strain 7	-	Rod	Motile
Strain 8	+	Rod	Non-motile

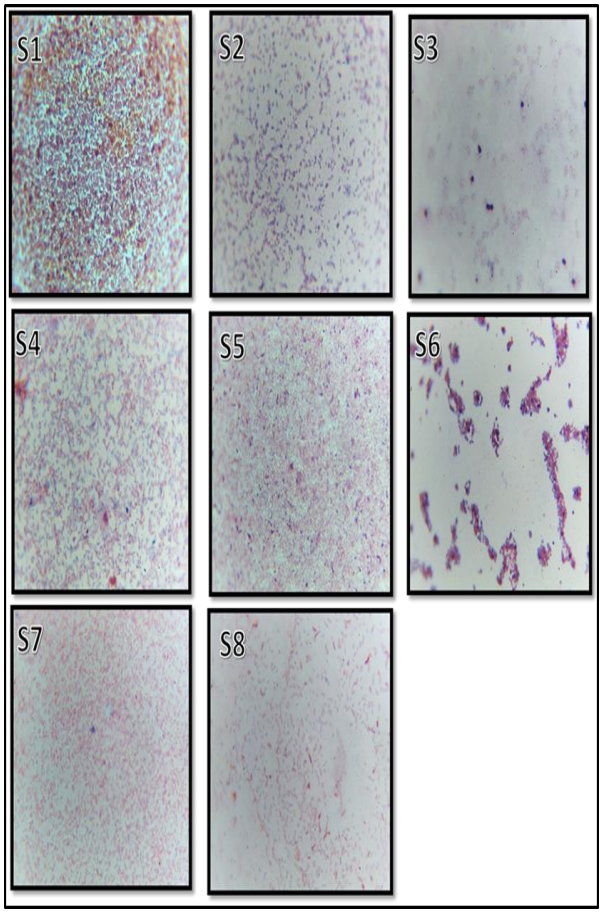

Fig 3. Gram stain of human pathogenic bacteria (S1-S8) from urine, stool and blood samples collected from different hospital patients.

4. Conclusion

In the present results, a total of 120 clinical samples were collected during the 2021 year from MUH. The clinical samples included blood (40), urine (35), and stool (45) from different patients with different genders and ages ranging from (16-55 years). 95 samples had bacterial growth after incubation, on the other side 25 samples did not indicate any growth. All colonies had an entire margin, raised elevation, creamy texture; and different color (off-white, pale yellow, yellowish – white, and orange) of pigmentation. the majority of isolates were rod –shaped except strain 3 were cocci shape; Gram strain whereas the samples showed that majority strains are Gram negative but strain 3 and 6 are Gram – positive.

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