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Detection and Identification of pathogenic bacteria isolated from patients

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Abstract: The crucial item of this study is to isolate and identify the most dangerous pathogenic bacteria associated with various hospital patients. Shape, border, elevation, texture, and colour of bacterial colonies were necessiated for morphological characterization of the isolates. In the year 2022, researchers at Mansoura University Hospital gathered 120 samples from patients. Patients of varying sexes and ages(15-65 years) provided blood (40), urine (35), and faeces (45) for analysis -). The current findings showed that following incubation, 95 samples exhibited bacterial growth, whereas 25 samples did not. Except for strain 5, all of the colonies had a circular form. All colonies had an entire margin, raised elevation, creamy texture; and a color range from off white, pale yellow, and yellowish white, and all featured complete margins, elevated elevations, creamy textures, and unique colouring. In terms of cell shape, most isolates were found to be rods, while strains 7 and 8 were found to be a cocci shape; in terms of Gram strain, 3 strains were found to be Gram negative, while others strains were Gram positive; and in terms of motility, all strains were found to be motile , except for strains 3, 7, and 8.

keywords: Pathogenic Bacteria; Identification, Diseases, *E. coil*.

1. Introduction

The human body contains millions of microorganisms, some of which cause illness. However, the others are vital to human societies, including health benefits, food fermentation, sewage treatment, and the creation of fuel, enzymes, and other bioactive chemicals [1]. Microbes simulate biology and are utilized in biological warfare and bioterrorism [2]. The gut flora is part of the human microbiome. Hygiene measures target microbes, the main cause of many infectious diseases [3, 4]. Microorganisms differ, yet they all want to reproduce. Some of them change the world while doing it. Many infectious diseases are caused by bacteria [5].

An organism that may infect a host and cause sickness is called a pathogenic organism [6]. Only humans can become sick from a human infection. Pathogens are also referred to as infectious agents due to their ability to cause disease. Just like every other living thing, pathogens prioritize maintaining their own species and expanding it [7]. Pathogens may cause a wide variety of ailments, some more severe than others. Human bodies provide an ideal environment for the growth and proliferation of viruses due to their rich nutritional content [8]. The severity of an illness caused by a pathogen might vary greatly. Although some diseases are relatively harmless, others may be lethal, the typical cold, for example, is a very mild viral infection compared to the potentially lethal Ebola virus disease [9].

Many microorganisms have survived for thousands of years by their adapting to antimicrobials. They achieve this through DNA transfer or spontaneous mutation [10]. This mechanism allows certain germs to resist antibiotics, rendering them ineffective. These microorganisms gain multidrug resistance in several ways [11]. Multidrug-resistant bacteria withstand various drugs. Antimicrobial resistance is caused by antibiotic overuse, according to Cantón et al. [12]. Thus, either naturally resistant germs become more numerous than those that can be treated, or microorganisms acquire a resistance to the

drugs used to treat them.

This work mainly aims to study the isolation, purification and morphological characterization of some pathogenic human bacteria.

2. Materials and Methods

2.1. Collection of samples

In this study, a collection of 120 samples from patients of varying ages, genders, and ages ranging from 16 to 65 years in sterile counters, the samples were then placed in an incubator at a temperature of -4 degrees Celsius. The samples included 40 blood samples, 35 urine samples, and 45 stool samples were microbiologically evaluated.

2.2. Isolation and Purification of pathogenic bacteria

For the purpose of isolating potentially dangerous microorganisms, the nutrient agar medium was used. The medium was then chilled to a temperature of 50 degrees Celsius after it had been autoclaved at 121 degrees Celsius for twenty minutes. The pH of the medium was brought up to 7 with the help of a few adjustments. Glycerol stock was used to store the cultures at a temperature of -4 degrees Celsius for an extended period of time.

Incubations were performed on the surfaces of the NA plates using the obtained samples. After a period of 24 hours, morphologically distinct bacterial colonies were chosen from the bacterial cultures. These colonies were then streaked many times in order to get bacterial isolates. After that, all of the chosen isolates were subculture on nutrient agar slants, and ultimately, all of the purified bacteria were preserved at 4 °C for Short-term storage until they were further employed, and at 80 °C in nutrient broth with 50 percent (w/v) glycerol for Long-term storage.

2.3. Morphological characterization

Characterization of the isolates was performed in order to ascertain the morphology of the bacterial cells based on observable properties such as the shape of the cells, the color of the colonies, and the texture of the colonies. The standard gram staining procedure, as described by Cappuccino and Sherman [13], was used to determine this result.

2.3.1. Cell shape

According to Aneja et al. [14], the morphological properties of pure bacterial cultures were examined by the use of a microscope when the cultures were in the log phase.

2.3.2. Gram staining

After applying crystal violet all over the smear, it was left to stand for a quarter of a minute. The stain was removed by giving it a quick wash with a wash bottle filled with distilled water, and then the excess water was drained. After that, cover it with the iodine solution and let it sit for 30 seconds. After that, add 95% alcohol and stir it until there is no longer any violet hue. After rinsing the slide for a little while with water from a bottle of distilled water, the slide dried. The smear was treated with safranine in its most fundamental form for a period of twenty seconds. After giving the slide a gentle wash for a few seconds, drying it with bibulous paper, then letting it air-dry, adding a drop of cedar oil to it, and then examining it with an oil-immersion lens, the steps were repeated. According to Hucker and Conn [15], gram-positive bacteria have a purple appearance, whereas gram-negative bacteria have a pinkish-red appearance.

2.3.3. Motility test

Isolates were seeded in the center of semi-solid LB agar plates containing 0.2 percent agar, after which the plates were placed in an incubator at 30 degrees Celsius for 24 hours. After that, the diffusion of colonies was examined [16]. After 72 hours of development, endophytic bacterial culture may also be seen using microscopy with the use of a cavity slide in order to observe the movement of the bacteria [14].

3. Results and Discussion

3.1. Isolation of Human Pathogenic Bacteria

Throughout the study of the year 2022, a total of 120 patient samples were obtained from the Mansoura University Hospital. The clinical samples consisted of blood (40), urine (35), and stools (45), all of which were contained in sterile counters and incubated at a temperature of -4 degrees Celsius. The patients' ages ranged from 15 to 65 years, and their genders varied.

After the acquired isolates had been collected, purified, and isolated, the next step was to test them for their morphological, biochemical, and molecular identifications; the results of this screening are shown below.

In order to carry out the isolation procedure, a medium consisting of nutritional agar was used. Twenty microliters of samples were placed on the medium's surface, and it was then heated to 37 degrees Celsius for a period of one day (see Figure 1). After being incubated, 95 of the samples showed signs of bacterial growth, whilst 25 of the samples showed no signs of development.

Purification of Human Pathogenic Bacteria

After isolation, 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.

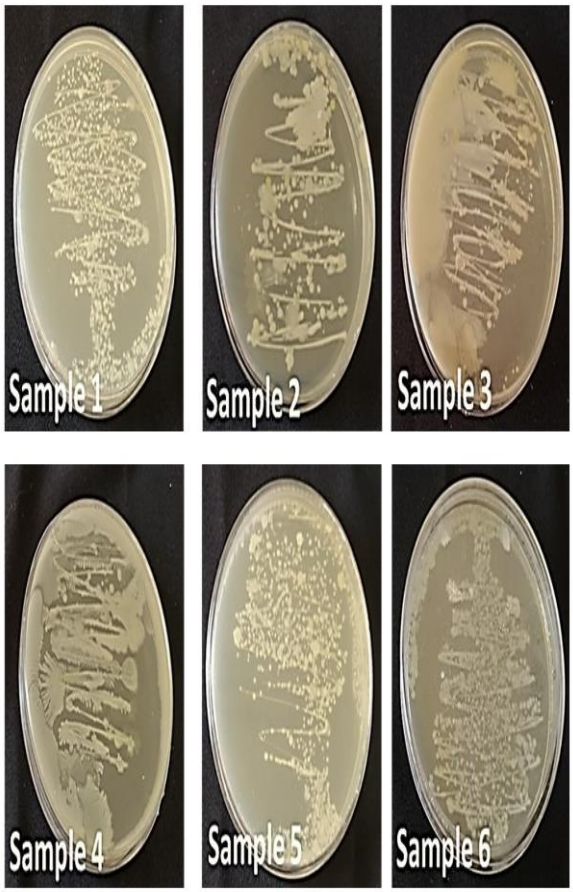

Figure 1. Isolation of human pathogenic bacteria (S1–S6) from urine, stool, and blood samples obtained from various hospital patients and cultured on L.B. agar medium.

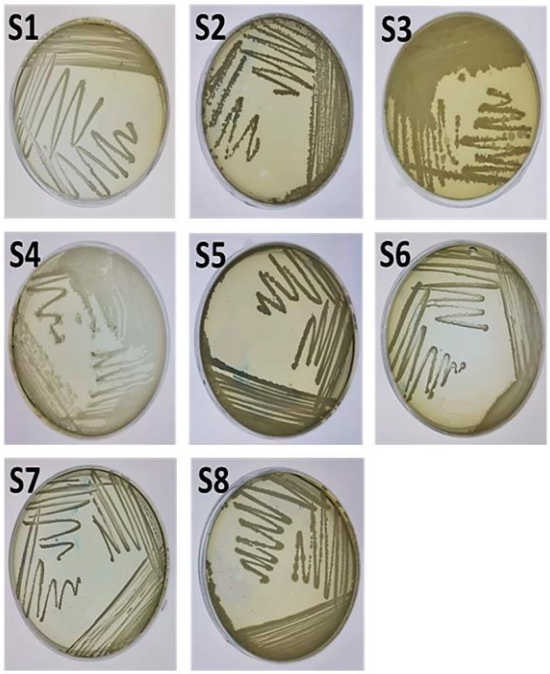

Figure 2. Purification of human pathogenic bacteria (S1-S8) from blood, urine, and stool samples obtained from various hospital patients.

Table 1. the number of human pathogenic bacteria identified from urine, stool, and blood samples collected from various 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		5	5	6

Morphological characterization of bacterial isolates

The bacterial isolates were characterized morphologically according to colony shape, margin, elevation, texture, pigmentation. The colonies shape was circular except for two irregular shapes. All colonies had an entire margin, raised elevation, creamy texture; and a color range from off white, pale yellow, yellowish white and red as shown in Table 2.

The bacterial isolates were scanned microscopically according to cell shape, whereas most isolates were rod shape (11 isolates) and five were cocci. Gram stain revealed 7 strains to be Gram positive and 9 strains Gram negative. The motility test

revealed 8 motile strains and 8 non-motile strains (Table 3 and Figure 3).

These strains were found only in hospitalized individuals. The colonies morphology of the different isolates was distinct. There is a wide range of bacteria, both Gram-negative and positive [17]. This bacterium belongs to the ESKAPE genus, which was only recently identified as the leading cause of the world's infectious disease crisis among humans (Boucher et al., 2009; Rice, 2010). Since it is impossible to totally avoid the selection of resistant bacteria, it is vital to create new antibiotics throughout time. This indicates that the antibiotic resistance problem becomes worse with time because each time an antibiotic is used, it helps the few bacteria that have a resistance gene against it to survive. This signifies that the resistance gene has spread throughout the body and the surrounding environment, and that a greater number of bacteria are now resistant to treatment with the antibiotic in question [18].

Table 2. Some pathogenic bacteria isolated from urine, stool, and blood samples, where collected from various hospital patients exhibit the following morphological characteristics.

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

Table 3. Microscopic characteristics of isolated bacteria from human blood, stool, and urine samples of various genders and ages.

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

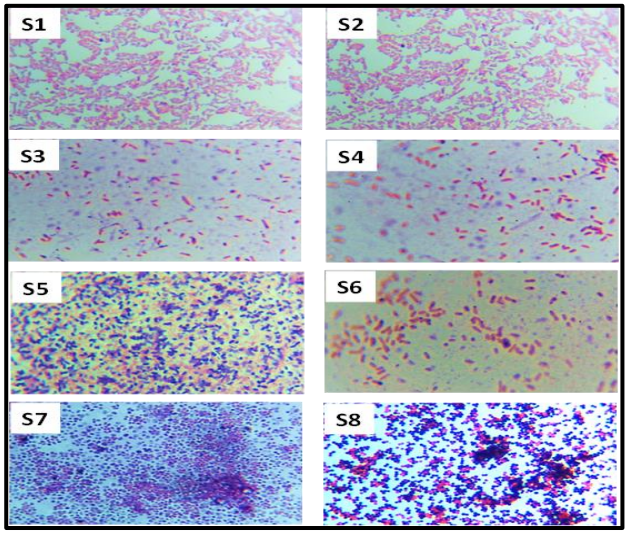

Figure 3. Gram-stained of human pathogenic bacteria (S1–S8).

4. Conclusion

In the current study, a total of 120 MUH clinical samples were gathered in the year 2022. Patients of varying sexes and ages (15-65 years) provided blood (40), urine (35), and stool (45) for analysis (-65). After incubation, 95 samples showed bacterial growth, whereas 25 did not. The colonies ranged in hue from off-white to light yellow and yellowish white to orange, and all featured complete margins, elevated elevations, creamy textures, and unique colorings. The samples indicated that most strains are Gram negative, while strains 3 and 6 are Gram - positive; all of the isolates were rod-shaped except for strain 7 and 8, which was cocci form; Gram positive.

4. References

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