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Identification of Pathogen Microorganisms *via* Morphological, Cultural and Biochemical Properties

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ABSTRACT

In many distinct areas of microbiology, the ability to identify microorganisms has important application. As an example, could be the identification of microorganisms helps us characterize biodiversity. In some branch of microbiology that investigates pathogenic microorganisms, the primary focus is to isolate, identify, and study microorganisms responsible for infectious disease.

This work is an important step towards the identification of pathogen microorganisms, which is essential for scientists involved in many areas of applied research and industry which ranges from clinical microbiology to food production. There are many criteria for the division of the abundance of methods used in the area of the identification of microorganism; however, generally they can be assigned to direct and indirect techniques. In recent years, more attention has been placed on the explore for modern, quick methods to the identification of microorganisms along with antibiotic drugs and their metabolites. The detection and identification of the germs responsible for the infectious disease is also an essential factor in the implementation of appropriate antibiotic therapy.

We propose in this study various identification methods of microorganisms, including culture and selective media as well as biochemical identification techniques.

Keywords: Pathogens; Bacteria; Fungi; Identification methods; Clinical diagnostics

INTRODUCTION

In many distinct areas of microbiology, the ability to identify microorganisms has important application. As an example, could be the identification of microorganisms helps us characterize biodiversity. In some branch of microbiology that investigates pathogenic microorganisms, the primary focus is to isolate, identify, and study microorganisms responsible for infectious disease. The identification of bacteria was carried out in accordance with Bergey's Manual. Traditional methods involve culturing microbes using a range of nonselective and selective methods, followed by biochemical confirmation among others [1].

Bacteria are identified routinely by morphological and biochemical tests. The identification is required so as to cure the illness or the infection caused due to the bacteria by using appropriate antibiotics. Identification also holds significance for epidemiological purposes [2]. The specific atmospheric conditions are important for isolating, detecting and identifying bacteria and fungi. Other important growth assessments include the incubation temperature, nutrients required, and resistance to antibiotics.

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In recent years, more attention has been placed on the explore for modern, quick methods to the identification of microorganisms along with antibiotic drugs and their metabolites. The detection and identification of the germs responsible for the infectious disease is also an essential factor in the implementation of appropriate antibiotic therapy.

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The emergence and spread of antibiotic resistance among pathogenic bacteria has been a growing problem for public health in recent decades. There are challenges in the combat of bacterial and fungi infections and accompanied diseases and the current shortage of effective drugs, lack of successful prevention measures [3]. It is becoming increasingly recognized that not only antibiotic resistance genes encountered in clinical pathogens are of relevance, but rather, all pathogenic, commensal as well as environmental bacteria, mobile genetic elements and bacteriophages, from which pathogenic bacteria can acquire resistance [4].

Traditional methods of the microorganism's identification require the detection of differences in morphology, growth, form, biochemical activity and metabolism to recognise the genera and species of bacteria. Colony morphology of different germs is usually described by an exact observation of the characteristic features of a colony, size, color, shape.

The current project has many parallels to what scientists have been doing for a long period time. From detection microorganisms by physical and functional characteristics to the adaptation of more modern techniques and equipments, microbiologists are continually developing a variety of tools to discover the identities of previously unknown microscopic life. Different microorganisms often have their own different characteristics of identification. For example, the identification of microorganisms with different morphological characters, such as fungi, often bases on their morphological characteristics as the main indicators; the detection of some bacteria or yeasts, related to the morphological, physiological and biochemical characteristics; the identification of most bacteria with the same morphological features, often uses more physiological, biochemical, and other parameters.

We propose in this study various identification methods of microorganisms, including culture and selective media as well as biochemical identification techniques.

Moreover, bacterial or fungal colonies of a single species, when grown on specific media under controlled conditions are described by their colony morphology, characteristic size, growth rate, shape, colour, consistency, metabolic reaction, and sometimes pigmentation. When growth conditions are carefully controlled, colonial morphology is important for preliminary identification and for differentiation of organisms. Biochemical tests are among the most important methods for microbial identification. Most of biochemical methodology related to the detection of different microorganisms, are characterized on the selective detection of enzymes activity. The reason is to differentiate pathogens, concentrate on the specific problems concerning the desired organism present at certain level.

Thus, most of the bacteria are classified firstly on the main characteristics of their morphology and physiology. Biochemical and physiological tests can then be used to distinguish among genera within a group, and between species once you have identified the correct genus.

MATERIALS AND METHODS

The study was carried out at the Institute of Biochemistry after H. Buniatyan NAS RA, EcoSense Laboratory, Eurasia international university and Yerevan State Medical University after Mkhitar Heratsi. Colony morphology observations form a major identifying criterion for fungi/yeasts and bacteria. All the media supported growth as per the requirement hence they were used in this study. Detection and isolation of pure cultures was carried out based on morphological characteristics where colony shape, pigmentation and size were the distinguishing factors for both bacteria and fungi. The vials with different clinical materials such as wounds, respiratory system, biomaterials (urine, sperm and urogenital smears) were taken to identify microorganisms as bacteria and fungi/yeasts.

Identification and detection of bacteria and fungi using morphological characteristics

Based on morphological differences [5], colonies were isolated from their cultures. The characteristics observed included (different forms, such as circular, irregular, etc.), elevation (flat or raised), pigmentation (red, yellow, white, pink and etc), size (small, medium, large) and texture. Colony morphology observations formed a major identifying criterion for bacteria and in addition fungal isolates were identified using cultural characters, morphology and by examining spore arrangements after observing under a microscope (XS-208C, Biobase, China, Leica 3000, Germany).

Identification of specific microbes using differential, selective media and biochemical features

Staphylococcus aureus: The culture from the vials was sub-cultured on the plates with Columbia CNA Agar with 5% Sheep Blood (Becton Dickinson GmbH, Heidelberg, Germany) as soon as possible after it is received. It was then incubated at a temperature of 35°C for 42 to 48 hours preferably in an aerobic atmosphere enriched with carbon dioxide, and read after 18 to 24 hours and after 42 to 48 hours. Growth of white colonies surrounded by yellow zones indicated presence of *Staphylococcus aureus* and then confirmed by coagulation tests (Staphylase test. DR0595A, UK). Place a drop of reagent and control on the filter paper. With wooden stick emulsified a portion of the isolated colony each of the drop to make two thick suspensions. *S. aureus* is coagulase positive, i.e. is possible to identify flakes, because this reagent consists of sheep erythrocytes with fibrinogen (if the strain of staphylococci consists of that factor attach to that reagent and elicite the flakes). That's mean the coagulase test is used to differentiate *Staphylococcus aureus* (positive) which produce the enzyme coagulase, from *S. epidermis* and *S. saprophyticus* (negative) which do not produce coagulase.

Streptococcus pyogenes: The culture from the vials was sub-cultured on the plates with Columbia Agar with 5% Sheep blood (Becton Dickinson GmbH, Heidelberg, Germany) as soon as possible after it is received. It was then incubated at a temperature of 35°C for 42 to 48 hours, and read after 18 to 24, which identified the typical appearance of *S. pyogenes* colonies with dome-shaped with a smooth or moist surface and clear margins. They display a white-greyish color and are surrounded by a zone of β-hemolysis that is often two to four times as large as the colony diameter, and then confirmed by microscopically, *S. pyogenes* appears as Gram-positive cocci, arranged in chains.

Escherichia coli: The culture from the vials was sub-cultured on the plates with Columbia agar with 5% Sheep Blood as soon as possible after it is received. It was then incubated at a temperature of 35°C for 42 to 48 hours, and read after 18 to 24, which identified the typical appearance of *E.coli* colonies with straight, rod shape bacillus.

Candida albicans-yeast: The biomaterials were sub-cultured on the plates with Columbia Agar with 5% Sheep Blood (Becton Dickinson GmbH, Heidelberg, Germany). It was then incubated at a temperature of 35°C for 42 to 48 hours, and read after 18 to 24, which identified the typical appearance of *Candida albicans* with small, oval, measuring 2-4 µm in diameter.

The biomaterials were sub-cultured on the plates with Chocolate Agar with Bacitracin (Becton Dickinson GmbH, Heidelberg, Germany).

It was then incubated at a temperature of 35°C for 48 hours. Grow of small, oval microorganisms is identified the typical appearance of *Candida albicans*.

The biomaterials were sub-cultured on the plates with Sabouraud Agar with gentamicin and chloramphenicol (Becton Dickinson GmbH, Heidelberg, Germany). It was then incubated at a temperature of 30°C-35°C for 42 to 48 hours. Grow of small, oval microorganisms is identified the typical appearance of *S. pyogenes* colonies with dome-shaped with a smooth or moist surface and clear margins. They display a white-greyish color and are surrounded by a zone of β-hemolysis that is often two to four times as large as the colony diameter, and then confirmed by microscopically, *S. pyogenes* appears as Gram-positive cocci, arranged in chains.

RESULTS AND DISCUSSION

Isolation and identification of microorganisms in biosafety laboratories

Under the investigation methods the bacterial and fungal isolates were obtained and identified from different laboratory places and different biomaterials. The bacterial contaminants were Escherichia coli, *Streptococcus pneumonia*. The laboratories walls, classrooms, floors and tables contained most of the contaminating microorganisms as bacteria and fungi.

Identification of bacteria and fungi by nutrition and selective media and microscopic characteristics

Microscopic examination of the different biomaterials is an excellent opportunity to assess general health status and diagnose diseases. For this purpose, different samples were observed under the microscope. Training and optimization of different deep learning methods for instance segmentation are used to detect different microorganisms.

Isolation of specific organisms was carried out based on morphological characteristics, where shape, size and colouration were the distinguishing factors for both bacteria and fungi.

The accuracy of the results is assessed by quantitative and qualitative evaluation standards

The test for microbes carried using selective LB Agar-Miller, a nutritionally rich medium designed for growth of cultures, showed the growth of single, pairs and irregular clusters of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, etc. (Table 1).

Table 1: Microscopic characteristics of identified bacteria.

Bacterial species	Shape	Arrangements	Motility
Staphylococcus aureus	Cocci	Singles, pairs and irregular clusters	Non-motile
Escherichia coli	Straight rods, cocobacilliary	Singles/pairs	Non-motile
Streptococcus pyogenes	Round-to-ovoid coccus	Pairs	Non-motile
Candida albicans	Hyphae, pseudohyphae, yeasts	Singles/groups	Non-motile

The antifungal and antibacterial assays using differential and selective media

The culture from vials carried using selective media (Columbia CNA Agar with 5% Sheep Blood) showed the growth of *Staphylococcus aureus* (Figure 1). *Staphylococcus aureus* usually grow as opaque, 1-3 mm in diameter within 24-36 hours in air at 34°C -37°C. Colonies are smooth, yellowish (gold-coloured) pigment on blood agar (Figure 1).

Figure 1: The selective media test: Columbia CNA Agar with 5% Sheep blood. *Staphylococcus aureus* isolated from the vials.

The second method for the confirmation of *S. aureus* is the coagulase testing, which is the accepted basis of *S. aureus* identification. *S. aureus* is unique as bacterium in its ability to coagulate blood, and it also is able to produce fibrinogen-binding proteins that alleviate clumping. Clumps of cells are able to avoid detection by the host's immune system due to a fibrinogen coat.

S. aureus is coagulase positive, i.e. is possible to identify flakes, because this reagent consists of sheep erythrocytes with fibrinogen (if staphylococci specific strain consists of this factor, is able to attach to that reagent and elicit the flakes) (Figure 2).

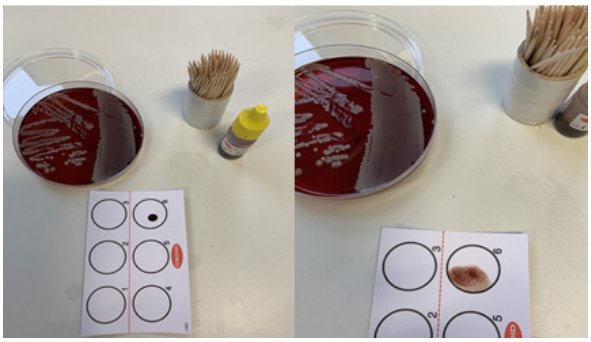

Figure 2: The coagulase testing, *Staphylococcus aureus* identification *via* appearance of flakes.

The culture from the vials carried using selective media (Columbia CNA Agar with 5% Sheep blood) showed the growth of *Streptococcus pyogenes* (Figure 3). Colonies are surrounded by a zone of beta-hemolysis (the complete disruption of erythrocytes and the release of hemoglobin) (Figure 3).

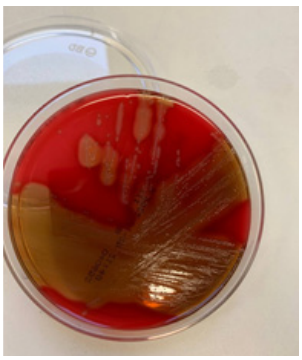

Figure 3: The selective media test: Columbia CNA Agar with 5% Sheep blood, *Streptococcus pyogenes* isolated from the vials.

The biomaterials were sub-cultured on the plates with Columbia Agar with 5% Sheep Blood, Sabouraud Agar with gentamicin and chloramphenicol and Chocolate agar with bacitracin showed the growth of small, oval microorganisms, which are identified the typical appearance of yeasts *Candida albicans* (Figure 4).

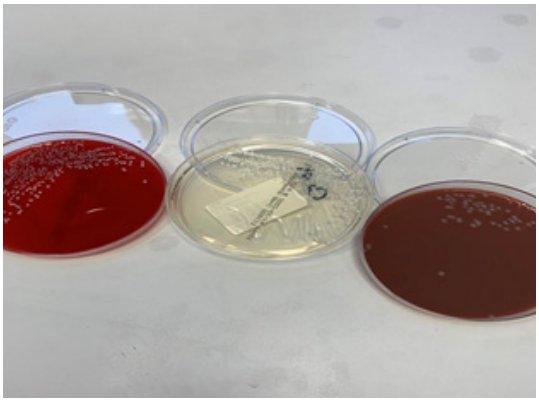

Figure 4: The selective media test: Columbia Agar with 5% Sheep Blood. *Candida albicans* isolated from biomaterials, The selective media test: Sabouraud Agar with gentamicin and chloramphenicol. *Candida albicans* isolated from biomaterials, The selective media test: Chocolate Agar with Bacitracin, *Candida albicans* isolated from biomaterials.

The culture from the vials carried using Columbia CNA agar with 5% Sheep Blood selective media showed the growth of *Escherichia coli*. These colonies with straight, rod shape bacteria on blood agar (Figures 5 and 6) [6-10].

Figure 5: The selective media test: Columbia CNA Agar with 5% Sheep blood, *Escherichia coli* isolated from the biomaterials.

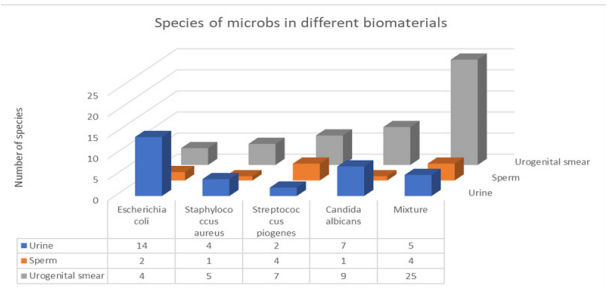

Figure 6: The frequency of pathogenic microbes present in biomaterials. **Note:** (■): Urogenital smear, (■): Sperm, (■): Urine.

CONCLUSIONS

Isolation and identification of microorganisms in biosafety laboratories

In segregation of microbial contaminants in biosafety laboratories objective, the extent of microbial contamination in biosafety laboratories was high. In current studies the rate of occurrence of bacteria isolates were much higher compared to the fungal isolates. *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* bacteria and *Candida albicans* fungi were isolated. These type biological contaminants can be transmitted by infected people and animals.

Through microscopic observations different species of bacteria and fungi were identified from same laboratory sites. These include *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* bacteria and *Candida albicans* fungi.

The antifungal and antibacterial assays using differential and selective media

We observed from this experiment that different microorganisms (Bacteria-*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli*, fungi-*Candida albicans*) were cultured and successfully identified using specific selective media and in addition biochemical methods. Despite the abandonment of culture by a large number of microbiologists, culture media remain a fundamental tool for bacteriologists for the isolation of commensal but also pathogenic bacteria. The use of additional biochemical methods as evidence could also allow identifying specific species for which selective agar or media were suitable for their growth. Hence, these types of selective or culture media for the selection of certain bacteria with an important medical role for prevention and treatment infectious diseases.

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