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Cultural, biochemical and microscopic evaluation of some common microorganisms isolated from different environmental sources

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Abstract
Colony morphology is a crucial bacterial adaptation process to deal with environmental stressors, and it may be a sign of phenotypic variation. There are a number of conventional techniques for identifying bacteria that rely on the observation of either the morphology of individual cells or colony features. However, the present study clearly and systematically demonstrated the cultural, biochemical and microscopic characteristics of common microorganisms including Fungi, *E. coli*, *Klebsealia* spp., *Staphylococcus aureus*, *Staphylococcus epidermidies*, *Pseudomonas putita*, *Pseudomonas fluorescence*, *Pseudomonas aeruginosa*, *Vibrio* spp., *Salmonella* spp., *Shigella* spp., *Bacillus* spp. This study attempted to portray the size, margin, pigmentation, form, elevation of different species on agar plates and the degree of growth, nature of turbidity and nature of surface growth in liquid media. Furthermore, the microscopic evaluation of all the strain was also carried out through staining procedure.

Keywords: Microorganisms, microscopic characteristics, cultural characteristics, biochemical characteristics

1. Introduction
The isolation of samples using bacteriological methods like culture in particular or distinct media is a widespread practice, particularly in clinical laboratories. On agar surfaces, microorganisms form colonies that can be used by scientists and medical professionals to distinguish between genera or even species. Due to significant technical improvements in bacterial identification process, a formidable array of methods for detecting, identifying, and differentiating bacteria are now accessible. Molecular techniques such as ELISA, PCR, and MALDI-TOF MS have considerably improved bacterial identification to speed up analysis and reduce handling (Singhal *et al*., 2015) [1]. However, culture-based approaches are still necessary to determine the bacterial count before and after antibiotic administration to ascertain the microbiological effect of antibiotic courses and whether phenotypic selection is occurring. Treatments with antibiotics can induce changes in bacterial behavior that may not be identifiable by molecular techniques. Clinical identification can be challenging due to the frequent occurrence of unique biochemical and metabolic characteristics in atypical colony morphologies. For instance, small colony variations (SCV) of Staphylococcus aureus, which exhibit altered metabolic activity, can interfere with coagulase tests and produce false negative results (Hilmi *et al*., 2013) [2]. Therefore, by identifying intra-strain diversity, defining colony morphology frequently augments traditional microbial identification (Sousa *et al*., 2013) [3-6].

Intra-population diversity resulting from bacterial phenotypic and genetic adaptability provides advantages and increases the likelihood of bacterial survival in evolving and changing environments (Boles *et al*., 2004; Goerke *et al*., 2007) [4, 5]. Changes in colony morphology serve as a macroscopic representation of the diverse biological strategies employed by microbes to cope with various stressful conditions, including starvation, oxygen deprivation, antibiotics, and host defenses (Sousa *et al*., 2013) [3-6]. Moreover, variations in colony appearance can reflect differences in virulence (Martin *et al*., 1993) [7], antimicrobial resistance (Lewis, 2005; Massey *et al*., 2001) [8, 9], and persistence (Lechner *et al*., 2012) [10]. Despite being considered outdated by some authors, colony morphology characterization remains valuable for understanding the diversity of individual microbes, arising from genetic alterations and reversible changes (Braga *et al*., 2013; Weile and Knabbe, 2009) [11, 12].

In chronic infections such as cystic fibrosis (CF), the presence of multiple colony morphology variants is commonly observed. A notable clinical feature in CF is the conversion of Pseudomonas aeruginosa from a non-mucoid form to a mucoid form, which is more difficult to eradicate (Hogardt and Heesemann, 2010; Lyczak *et al*., 2002) [13, 14]. Mucoid variations can show multidrug resistance and greater resistance to medicines such gentamicin, aminoglycosides, ciprofloxacin, and imipenem (Agarwal *et al*., 2005; Manno *et al*., 2005) [15, 16]. Various other morphotypes have been identified in bacteria associated with chronic and acute infections. Some of the most common and well-studied examples include small colony variations (SCV) (Haussler *et al*., 2004; Haussler *et al*., 1999, 2003; Hoffman *et al*., 2006; Massey *et al*., 2001; Wellinghausen *et al*., 2009) [17, 18, 19, 20, 21], rough (small) colonies (Drenkard and Ausubel, 2002) [22], and hyperpiliated colonies (Haussler *et al*., 2003) [19].

When bacteria from biofilms are cultivated on agar media, different morphological patterns appear in the colonies, which provide evidence that bacteria can undergo phenotypic modifications to support the production of biofilms (Sauer *et al*., 2002) [23].

This study aimed to systematically investigate the influence of experimental conditions on various colony morphological features, such as form, margin, texture, size, and color.

2. Materials and methods

2.1 Study area and microbiological profiling

This study involved the isolation of 11 different types of microorganisms from environmental samples, including soil and water. The microorganisms identified were Fungi, *E. coli*, *Klebsiella spp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas putida*, *Pseudomonas fluorescence*, *Pseudomonas aeruginosa*, *Vibrio spp., Salmonella spp., Shigella spp., and Bacillus spp*. The isolation process was conducted between February 2017 and April 2017, following established protocols (APHA, 1998; Sousa *et al*., 2013) [24, 3-6].

To identify and quantify pathogenic bacteria and fungi, a 1 ml of each sample was diluted up to a factor of 10^{-5}. This dilution was performed according to the standardized guidelines. (Capuchino and Sharman 2014) [25].

2.2 Standard protocol for the detection of microorganisms

Enumeration of microorganisms in this study was performed using several techniques, including the spread plate, streak plate, and membrane filter methods. For soil samples, 10g of soil was homogenized with 90 ml of distilled water and subsequently diluted up to a factor of 10^{-7}. From the 10^{-7} dilution, 0.1 ml of the sample was plated onto Nutrient agar and Sabouraud Dextrose agar to isolate total viable bacteria and fungi, respectively. Additionally, selective media such as MacConkey agar, Mannitol Salt agar, Cetrimide agar, TCBS agar, Starch agar, and *Salmonella-Shigella* agar were utilized for the detection of specific microorganisms (Chowdhury *et al*., 2016; Acharjee *et al*., 2013) [26, 27]. Similarly, using the same dilution, one loop full of the inoculum was streaked using the four-quadrant streaking method on the same media. For water samples, 100 ml of water was passed through a membrane filter with a pore size of 0.45 µm. The filter paper containing captured microorganisms was then placed onto different selective media. Incubation of all inoculated plates was carried out at 37 °C for 24 hours, except for Sabouraud Dextrose agar plates, which were incubated at 25°C for 48 hours.

2.3 Colony morphology on agar media and liquid media

After incubation, the agar plates were examined to observe the cultural or colony characteristics of the isolates, including their size, pigment, form, margin, and elevation (Capuchino and Sharman, 2014) [25]. Inocula of the isolates were then transferred from the agar plates into liquid media to assess their growth turbidity (Capuchino and Sharman, 2014) [25].

2.4 Microscopic observation

To detect the shape, arrangement, and different groups of the isolates, bacterial cultures from the agar plates were prepared for gram staining. Additionally, cultures from the Sabouraud Dextrose agar (SDA) plate were used for simple staining (Capuchino and Sharman, 2014) [25].

2.5 Biochemical tests for the confirmative identification

Finally, standard biochemical tests were conducted to identify all the pathogenic isolates found in the samples. These tests included the Triple Sugar Iron test, citrate test, IMVIC (Indole, Methyl Red, Voges-Proskauer, Citrate) test, MIU (Motility, Indole, Urease) test, catalase test, Nitrate test, Gelatine test, Starch test, and oxidase test. The previously described methods (Table 2) were followed for performing these tests (Alfrad and Bensons, 2007; Cappuccino and Sherman, 1996) [28, 29].

3. Results and Discussion

Several microorganisms have been identified and researchers are working on the habitat, multiplication rate, environmental benefits, virulence factors, growth strategy, physiological properties, genetic variation, metabolic activity and clinical significance of different microorganisms (Acahrjee *et al*. 2013, Choudhury *et al*., 2016) [27, 26]. Some of our study findings reported from the microbiology laboratory of Stamford University Bangladesh on the antibiogram properties of different pathogen as well as antimicrobial properties of some medicinal plants against different pathogenic laboratory strain. For conducting the basic research of microbiology, it is very essential to know the cultural, microscopic and biochemical characteristics of the isolates those are commonly uses in our basic research. For this reason present study tried to archive the different characteristics of some common microorganisms such as Fungi, *E. coli*, *Klebsealia* spp., *Staphylococcus aureus*, *Staphylococcus epidermidies*, *Pseudomonas putita*, *Pseudomonas fluorescence*, *Pseudomonas aeruginosa*, *Vibrio* spp., *Salmonella* spp., *Shigella* spp., *Bacillus* spp. (table 1)

Table 1: Isolation of different microorganisms from environmental samples

Media	Isolates	Positive/Nagative
Nutrient agar	Total Viable Bacteria	+
Saborout Detrose agar	Fungi	+
MacConkey agar	*E. coli*/*Klebsealia*	+
Manitol Salt agar	*Staphylococcus* spp.	+
Pseudomonas agar	*Pseudomonas* spp.	+
TCBS agar	*Vibrio* spp.	+
Salmonella-Shigella agar	*Salmonella*/*Shigella* spp.	+
Starch agar	*Bacillus* spp.	+
Clostridium agar	*Clostridium* spp.	-

3.1 Biochemistry of the isolates

All the isolates were introduced for the identification of their biochemical properties against different parameters like their ability of different carbohydrate utilization, ability of the production of H_2S gas, motility, citrate, oxidase, catalase, starch, nitrate and gelatin utilization ability (table 2). *E. coli* utilized lactose, sucrose and dextrose in TSI, showed the positive result MR, motility, nitrate and manitol. *Klebseilla* spp. showed positive result for citrate, catalas, nitrate, urease, manitol *Klebseilla* spp. also utilized lactose, sucrose and dextrose. *Shigella* spp. showed positive result for MR, motility, catalas and nitrate. *Salmonella* spp. showed positive result for MR, motility, citrate, catalase, and nitrate. *Salmonella* only produced H_2S. *Vibrio* spp. showed positive result for indole, MR, citrate, motility and oxidase. *Staphylococcus* spp. showed positive result for MR, motility, catalase, nitrate, manitol and gelatin. The special characteristic of *Bacillus* spp. was to hydrolyze gelatin and starch. *Pseudomonas* spp. showed positive result for citrate, motility, oxidase catalase and nitrate (table 2).

Table 2: Biochemical identification of the microorganisms

Microorganisms	TSI			H_2S reaction	Indole	MR	VP	Citrate	Motility	Oxidase	Catalase	Nitrate	Unease	Starch hydrolysis	Manitol	Gelatin utilization
	Slant	Butt	Gas													
E. coli	Y	Y	+	-	-	+	-	-	+	-	+	+	-	-	+	-
Klebsiella spp.	Y	Y	+	-	-	-	-	+	-	-	+	+	+	-	+	-
Shigella spp.	R	Y	-	-	-	+	-	-	+	-	+	+	-	-	-	-
Salmonella spp.	R	Y	-	+	-	+	-	+	+	-	+	+	-	-	-	-
Vibrio spp.	Y	Y	-	-	+	+	-	+	+	+	-	-	-	-	-	-
Staphylococcus spp.	Y	R	+	+	-	+	-	-	+	-	+	+	-	-	+	+
Bacillus spp.	Y	R	-	-	+	+	-	-	+/-	-	+	+	-	+	-	+ (rapid)
Pseudomonas spp.	R	R	-	-	-	-	-	+	+	+	+	+	-	-	+	+ (rapid)

TSI: Triple Sugar Iron Test; Y: Yellow (Acid); R: Red (Alkaline); MR: Methyl red; VP: Voges-Proskauer

3.2 Cultural and microscopic characteristics of isolates.

On agar plates to describe the colony of different bacteria researchers can use different terms like colony morphology, colony types, colony variants and morphotypes. Presence of colonies on the agar media indicated that a group of bacteria grown from the single colony on agar surface those were different by their character on different media. All the strain on different agar media showed distinct size, shape, arrangement, margin, elevcation, pigmentation and also gram reaction through microscope (table 3).

Table 3: Morphological and microscopic characteristics of isolated microorganisms on nutrient agar plate.

Isolates	Cultural characteristics					Microscopic characteristics		
	Size	Pigment	Form	Margin	Elevation	Shape	Arrangement	Staining
Fungi	Large	Blackish	Rhizoid	Filamentous	Convex	Bird's nest & flask fungi	Chain	-
E.coli	Small	Dry pink	Circular	Entire	Convex	Rod	Single	Gram negative
Klebsealia spp.	Small	Gummy pink	Circular	Entire	Flat	Rod	Single	Gram negative
Staphylococcus aureus.	Large	Yellow	Irregular	Endulate	Raised	Cocci	Cluster	Gram positive
Staphylococcus epidermidies	large	Pink	Irregular	Endulate	Raised	Cocci	Cluster	Gram positive
Pseudomonas putita.	Pin point	Whitish	Circular	Entire	Raised	Rod	Single	Gram negative
Pseudomonas fluorescence.	Large	Greenish	Irregular	Lobate	Raised	Rod	Single	Gram negative
Pseudomonas aeruginosa	Small	Non fluorescent	Irregular	Lobate	Raised	Rod	Single/Pairs/short chain	Gram negative
Vibrio spp.	Large	Yellow	Irregular	Undulate	Raised	Curved/Comma	Single polar flagellum	Gram negative
Salmonella spp.	Small	Black centre	Circular	Entire	Convex	Rod	Cluster	Gram negative
Shigella spp.	Small	Pink	Circular	Entire	Convex	Rod	Short chain	Gram negative
Bacillus spp.	Moderate	Violet	Irregular	Lobate	Raised	Rod	Chain	Gram positive

3.3 Cultural characteristics of isolates on liquid media

Suspension on liquid broth is essential for conducting different experiment such as Antibiogram, anti-microbiual activity, MIC, MBC, response of bacterial growth against.

Table 4: Morphological characteristics of isolated microorganisms on liquid medium

Isolates	Cultural characteristics		
	Degree of growth	Nature of turbidity	Nature of surface growth
Fungi	High	Cloudy	Wrinkled
E. coli	High	Cloudy	Smooth
Klebsiella spp.	High	Cloudy	Smooth
Staphylococcus aureus.	High	Cloudy	Smooth
Staphylococcus epidermidies	High	Murky	Smooth
Pseudomonas putita.	High	Murky	Rough
Pseudomonas flourescence.	Moderate	Murky	Rough
Pseudomonas aeruginosa	Moderate	Murky	Rough
Vibrio spp.	Low	Murky	Rough
Salmonella spp.	Moderate	Murky	Rough
Shigella spp.	Moderate	Cloudy	Rough
Bacillus spp.	Moderate	Murky	Rough

Different temperature, pH and salinity (Acharjee *et al*. 2013) [27]. In this study, we found the growth parameters of different bacteria on liquid media based on the three important categories like degree of growth, nature of turbidity and nature of surface growth (table 4). The degree of growth for the fungi, *E. coli Klebsiella* spp. *Staphylococcus* spp, and *Staphylococcus epidermidies* was very high while fungi, *E. coli Klebsiella* spp. *Staphylococcus* spp showed cloudy growth. The surface area of the colony was smooth for *E. coli Klebsiella* spp. *Staphylococcus* spp, and *Staphylococcus epidermidies* while the rough area was observed for *Pseudomonas putita*, *Pseudomonas fluorescence*, *Pseudomonas aeruginosa*, *Vibrio* spp., *Salmonella* spp., *Shigella* spp. and *Bacillus* spp. The cell turbidity appearance was murky for *Pseudomonas putita*, *Pseudomonas fluorescence*, *Pseudomonas aeruginosa*, *Vibrio* spp., *Salmonella* spp., and *Bacillus* spp. and except *Shigella* spp (Table 4). Meanwhile, the degree of growth was moderate for *Pseudomonas fluorescence*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp. and *Bacillus* spp while the *Vibrio* spp. showed the lower growth.

The degree of growth Several previous studies reported that the identification of colony morphology of different bacteria on different media is highly depend on the environmental condition but the variability is not remarkable (Hay *et al*., 2009; Rakhimova *et al*., 2008; Starkey *et al*., 2009, Sousa *et al*. 2013) [30, 31, 32, 3-6].

4. Conclusions

The recognition of typical colony morphologies is the fundamental aspect among others or even individual for clinical diagnosis. The colony morphology manual of bacteria on agar media is commonly used in scientific and clinical laboratories as an additional tool to identify various bacterial species, which makes this type of study highly common. The ultimate objective is to predict antibiotic resistance, expression of virulence factors, and persistence capacity based on morphological features, useful for assisting clinical diagnosis on bacterial profiling, present study will propose a guideline for many authors, clinicians, and technicians to better understand bacterial adaptation and evolution, purposes of fundamental science.

5. Conflict of Interest

Not available

6. Financial Support

Not available

7. References

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