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RESEARCH ARTICLE

URINE AGAR MEDIUM: DEVELOPMENT OF A NEW HUMAN URINE-BASED BACTERIAL CULTURE MEDIUM

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ABSTRACT

Objective: The primary goal was to create a novel medium from human urine and compare its Colony morphology and nutritional value with that of nutrient agar medium. **Materials and methods:** This study was conducted in the Department of MLT, Government Medical College Thiruvananthapuram, over a period of 3 months. Prior to media preparation, human urine was collected and sterilized. Compare the colony morphology of different bacteria on urine agar medium with nutrient agar and also evaluate the nutritional value of urine agar medium. **Result:** The novel urine agar medium supports the growth of both gram-positive and gram-negative pathogens. Colonies are well differentiated. This medium enhances the pigment production of *Pseudomonas* and *Staphylococcus*. Medium is comparable to a 1/4th concentration of nutrients when compared to nutrient agar.

INTRODUCTION

There are several reasons for cultivating bacteria on artificial culture media in the laboratory. The fact that it may be used to diagnose infectious diseases is among the primary reasons. Bacterial involvement in the illness process can be determined by isolating it from body fluids that are typically thought to be sterile. Separating bacteria from mixtures can also be accomplished conveniently by culturing them on solid media. It is crucial to offer the same environmental and nutritional conditions that the bacteria would find in their natural home when growing them. Therefore, every nutritional element that a bacterium receives in its natural habitat must be supplied by an artificial culture medium.

A culture medium typically includes water, trace elements, some growth stimulants, and a source of carbon and energy as well as nitrogen[1]. In addition to these, ideal pH, oxygen tension, and osmolarity must also be taken into account. Nutrient agar is a baseline medium that provides all the conditions needed for bacterial growth. Here, urine was used as a growth medium. Urine is both a biofluid and a waste product. Urine has significant usefulness despite being primarily thought of as a waste product because it includes a wide array of metabolites[2]. The macro- and micromolecules, vitamins, amino acids, inorganic salts, sources of carbon and nitrogen, and other elements required for bacterial growth are all included in these metabolites. Therefore, human urine is an excellent culture medium.

MATERIALS AND METHODS

Over the course of three months, this investigation was carried out in the microbiology lab of the Department of MLT, Government Medical College Thiruvananthapuram. Urine agar medium, nutrient agar, and different biochemical mediums were prepared as part of this study. Standard strains of *E. coli* (ATCC 25923), *Staphylococcus* (ATCC 25922), and *Pseudomonas* (ATCC 27853) were taken, and the strains were confirmed by gram staining and biochemical reactions.

Collect the investigator's urine sample and place it in a sterile beaker. (The person who collects the urine sample should not have any medication, infection, or menstruation prior to 5 days.) Adjust the pH to 7.4 by using the Lovibond comparator. Sterilize the urine sample by membrane filtration. For this, seitz filter with an asbestos filter pad is attached to a side-armed conical flask through a tight rubber cork with a single channel. The side arm of the conical flask is connected to the suction apparatus by a siliconized rubber tube. (All these equipment were pre-sterilised.). Pour the urine sample into the Seitz filter vessel and switch on the suction apparatus. Filtration is carried out at 240 mmHg. Leaving sterile filtrate in the container. The container is plugged with non-absorbent cotton and then placed in a water bath at 37 °C; raise the temperature to 50 °C. Simultaneously, agar and glucose are

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dissolved in water by boiling and sterilized by autoclaving at 121 °C for 15 minutes at 15 lbs. Cool the agar to 50 °C and add aseptically to the sterile warm urine solution. Mix well by rotating. Pour aseptically 15 ml into the sterile petridishes, allow to set, and store at 4 °C. Check the sterility by placing a fresh plate in an incubator for 48 hours. Each 100 ml of urine agar medium contains 2 g of agar, 0.5 g of glucose, 95 ml of urine, and 5 ml of water.

Nutrient agar is routinely used for the isolation of non-fastidious microorganisms that are not exacting in their nutrition. In addition to preparing the nutrient agar plate, make two more sets of the medium by reducing the concentration to half and a quarter. The strength of each ingredient is listed in Table 1. Dissolve peptone, meat extract, and NaCl in water and adjust the pH to 7.4. Filter and add agar; dissolve by heating. Sterilize by autoclaving at 121 °C for 15 minutes at 15 lbs. Pour aseptically into sterile petridishes[3].

Table 1 Composition of Nutrient agar medium

Ingredients	NA	½ NA	¼ NA
Peptone	1gm	0.5gm	0.25gm
Meat extracts	1gm	0.5gm	0.25gm
NaCl	0.5gm	0.25gm	0.125gm
Agar	2gm	2gm	2gm
Distilled water	100ml	100ml	100ml
Ph	7.4	7.4	7.4

Bacterial suspension is made from the verified strains of *E. coli*, *Staphylococcus*, and *Pseudomonas* by adding a single colony to the peptone water. Mix, then let the suspension sit at 37°C for 20 minutes. Check the turbidity and, if necessary, correct it with sterile normal saline to meet the 0.5 McFarland criteria. Inoculate the bacterial suspension into the culture plates. Incubate at 37^0c for 24 hours along with control plates. Observe and compare the colony morphology of organisms on UAM and NA. Nutritional level concentration of urine agar is evaluated by comparing the colony morphology with ½ and 1/4th strength of nutrient agar.

RESULT

After 24 hours of incubation, colonies appear on both urine agar and nutrient agar. The morphology of each organism in urine agar medium is studied and compared with that on nutrient agar medium. On nutrient agar, *E. coli* forms circular, smooth colonies with a diameter of 3 mm, but on UAM, the colonies grow to a diameter of 1 mm (Fig. 1). The colony size on urine agar corresponds to 1/4th of the nutrient agar strength. Colony features are listed in Table 2.

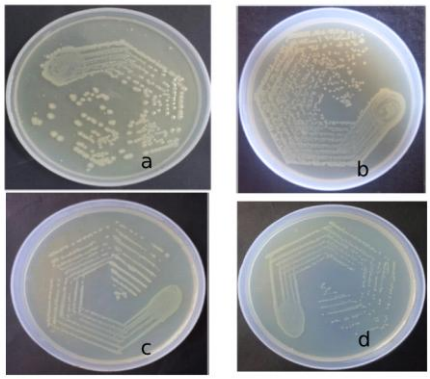

Fig 1: Colonies of *Escherichia coli* on a) Nutrient agar b) Urine agar medium, c) Half strength Nutrient agar, d) Quarter strength Nutrient agar

Table 2 Colony characteristics of *E.coli*

Colony characteristics	NA	UAM	½ NA	¼ NA
Size	1 – 3mm	0.5 – 1mm	1 – 2mm	0.5 – 1mm
Shape	Circular	Circular	Circular	Circular
Edge	Entire	Entire	Entire	Entire
Elevation	Convex	Low convex	Convex	Convex
Surface	Smooth	Smooth	Smooth	Smooth
Consistency	Moist	Moist	Moist	Moist
Opacity	Opaque	Opaque	Opaque	Opaque
Odour	Nil	Nil	Nil	Nil
Pigment	Nil	Nil	Nil	Nil
Change in medium	Nil	Nil	Nil	Nil

Staphylococcus colonies on both media have a butyrous, opaque consistency with a golden yellow pigment. Bacterium produce 2 mm-diameter colonies on nutrient agar, 1 mm-diameter colonies on urine agar, and 1/4th strength nutrient agar medium (Fig. 2). Compared to nutrient agar, colonies are more brightly pigmented, and colony sizes are comparable with the 1/4th strength of nutrient agar medium (Table 3).

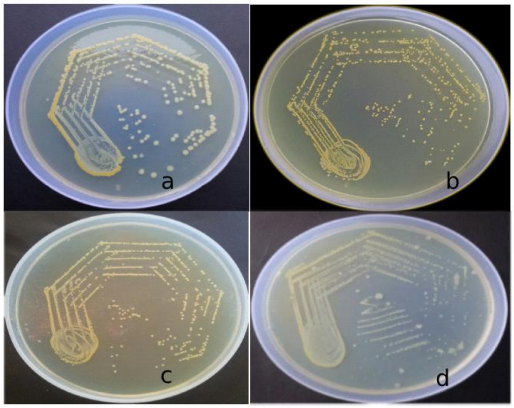

Fig 2: Colonies of *Staphylococcus aureus* on a) Nutrient agar b) Urine agar medium, c) Half strength Nutrient agar, d) Quarter strength Nutrient agar

Table 3 Colony characteristics of *Staphylococcus aureus*

Colony characteristics	NA	UAM	½ NA	¼ NA
Size	2mm	1mm	1-1.5mm	0.5 – 1mm
Shape	Circular	Circular	Circular	Circular
Edge	Entire	Entire	Entire	Entire
Elevation	Convex	Low convex	Convex	Convex
Surface	Smooth	Smooth	Smooth	Smooth
Consistency	Butyrous	Butyrous	Butyrous	Butyrous
Opacity	Opaque	Opaque	Opaque	Opaque
Odour	Nil	Nil	Nil	Nil
Pigment	Golden yellow	Golden yellow	Golden yellow	Golden yellow
Change in medium	Nil	Nil	Nil	Nil

Pseudomonas produces translucent, 3 mm-diameter mucoid colonies with serrated edges and a greenish-yellow pigment.

On urine agar medium with 2 mm-diameter dry-serrated flat colonies, the pigments are more vividly yellow-green in color. It produces 2 mm-serrated mucoid colonies on 1/4 nutrient agar (Fig. 3). It emits a distinct earthy odor across all media. Features of colonies are enumerated in Table 4.

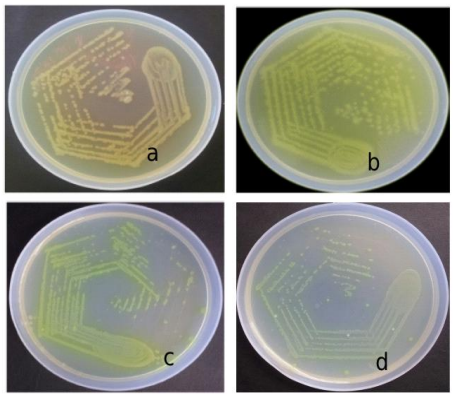

Fig 3: Colonies of *Pseudomonas aeruginosa* on a) Nutrient agar b) Urine agar medium, c) Half strength Nutrient agar, d) Quarter strength Nutrient agar

medium (AUM) is also available[4]. This medium contains artificial urine and agar at a pH of 7.2–7.4. Urine is artificially synthesized to avoid person-to-person sample variation. In urine medium, these problems were overcome by taking a pooled human urine sample.

A combination of human urine and Digitaria exilis extract is used as a broth for the bacterial isolation[5]. Modifications of urine medium are widely used for the industrial production of *Spirulina plantensis*[6]. Human urine is a major ingredient in the media for the demonstration of *Leishmania braziliensis*[7] and as a fertilizer for Brassica oleracea[8]. Urine agar medium will support the growth of *E. coli*, *Pseudomonas*, and *Staphylococcus* with similar characteristic colony morphologies. It enhances the pigmentation of *Pseudomonas* and *Staphylococcus*. The medium is more economical than the nutrient agar medium. So we can replace urine agar medium with nutrient agar for the primary isolation of *E. coli*, *Staphylococcus*, and *Pseudomonas*. The medium has a slight aromatic odor, which is one of the major disadvantages.

Table 4 Colony characteristics of *Pseudomonas aeruginosa*

Colony characteristics	NA	UAM	½ NA	¼ NA
Size	1 – 3mm	1 – 2mm	1.5-2.5mm	1 – 2mm
Shape	Irregular	Irregular	Irregular	Irregular
Edge	Serrated	Serrated	Serrated	Serrated
Elevation	Low convex	Flat	Low convex	Low convex
Surface	Smooth	Rough	Smooth	Smooth
Consistency	Mucoid	Dry	Mucoid	Mucoid
Opacity	Translucent	Translucent	Translucent	Translucent
Odour	Earthy odour	Earthy odour	Earthy odour	Earthy odour
Pigment	Greenish yellow	Greenish yellow	Greenish yellow	Greenish yellow
Change in medium	Nil	Nil	Nil	Nil

DISCUSSION

All body fluids are good culture mediums for bacterial isolation. However, it is impractical to gather them and use them as a medium for culture. Because urine is readily available, we have chosen it for the medium preparation in this instance. This study was also undertaken to compare the colony characteristics of E. *coli*, *Staphylococcus aureus*, and *Pseudomonas* aeruginosa on nutrient agar and urine agar mediums. After 24 hours of incubation, colonies appeared on both culture mediums. When compared with nutrient agar, the size of the colony is relatively small in urea agar. Nutrient agar: *E. coli* (2–3mm), *Staphylococcus* (2 mm), and *Pseudomonas* (1–3mm). Urine agar: E. coli (0.5–1 mm), *Staphylococcus* (1 mm), and *Pseudomonas* (1-2 mm). But all the other characters remain the same. On nutrient agar, *Staphylococcus* produces golden-yellow pigmented colonies, and Pseudomonas produces greenish-yellow flat colonies. Similarly, on urine agar, the organism produces the above-mentioned pigmented colonies with intense color. This study will help to find out the amount of nutrients present in urine agar. For this, different concentrations of nutrient agar are prepared and incorporated for the inoculation of test organisms. Nutritionally, the urine agar is similar to the 1/4th concentration of the nutrient agar. A simple artificial urine

CONCLUSION

When compared to nutrient agar medium, this study demonstrates that there is little variation in the colony morphology of *E. coli*, *Pseudomonas*, and S*taphylococcus* on urine agar medium. The difference in colony size and pigment production is the most obvious shift. They produced rather tiny colonies on urine agar media. In urine agar media, bacteria like *Pseudomonas* and *Staphylococcus* form more vividly colored colonies than in nutrient agar. Both gram-positive and gram-negative pathogens can grow in the medium. This work also contributes to the discovery that the nutritional medium is comparable to the 1/4th concentration of nutrient agar.

CONFLICTS OF INTEREST

Nil

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