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Evaluation of different plating media for growth of *Helicobacter Pylori* isolated from duodenal ulcer patients.

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ABSTRACT

Objective: Helicobacter pylori infection is the most common cause of duodenal ulcers, which are sores in the lining of the first part of the small intestine. The bacterium damages the protective lining, allowing stomach acid to cause an ulcer. *H. pylori* is regarded as a highly fastidious organism, typically requiring 3-4 days of growth on complex media containing blood or serum in a low-oxygen atmosphere. The growth of the H. pylori has been achieved only by inoculating the clinical specimen and storage in blood agar only. The objective of this research was to compare the growth of *H. pylori* in different blood agar medium to elucidate whether the medium provided considerable growth *of H. pylori in vitro*.

Methods: *H. pylori* was inoculated in human, goat, sheep and chicken blood agar plates and incubated in anaerobic under microaerophilic (5% O_2, 10% CO_2 and 85% N_2) conditions at 37°C for 4 days. After successful incubation, significant differences in growth were observed and further confirmed based on colony morphology, Gram staining and biochemical tests.

Results: In human blood agar plates, it was observed that creditable colony size and growth of *H. pylori*. In goat blood also showed more or less equal results same like human blood; whereas minimal growth was observed in chicken, and no growth was observed in sheep blood agar plates. Even though the *H. pylori* colonies were observed on chicken blood agar plates, the urease test of the colonies are very slow reactive than human and goat blood agar.

Conclusion: The study is suggesting the usage of goat blood as the alternative for the human blood agar for isolation and subculturing of *H. pylori* from clinical samples.

Keywords: Duodenal ulcer, Complex media, Blood agar, Skirrow supplement, *H. pylori*.

INTRODUCTION

H. pylori strains are variable in their growth response to different culture conditions, but no systematic studies on the different atmospheres and growth systems have been published. *H. pylori* can grow on different solid media containing blood or blood products (blood or lysed blood agar plates). For the successful cultivation and maintenance of microbial pathogens in the laboratory, the need of pre-tested culture media is essential. The major factors that are necessary for the isolation of clinical pathogens are providing all essential nutrients, ions and moisture, enrich with supplements, maintenance of pH and osmotic pressure, and able to neutralize the toxic materials produced [1]. From the inception of the Clinical Microbiology laboratory settings, the usage of blood agar supports the growth of fastidious clinical isolates significantly.

Helicobacter pylori are the gram-negative spiral-shaped bacterium which is isolated from gastric ulcers in 1982 [2] that has subsequently been classified into a new genus, *H. pylori* [3]. The major cause of chronic gastritis and peptic ulcer disease are by *H. pylori* and is an important risk factor for the development of gastric malignancies [4,5]. Although various methods have been developed for detecting *H. pylori* infection, the usage of bacterial culture remains extremely important to isolate *H. pylori* and susceptibility testing [6]. For the cultivation and other microbiological studies of *H. pylori,* the usage of blood agar is mandatory added with a battery of prophylactic supplement (antibiotics) to prevent contamination. The concentration of 5% blood is not only useful as a growth inducer also considered for the diagnosis by its hemolytic nature.

In most laboratories, defibrinated sheep blood is the best supplement for bacteriological culture due to its inhibitory nature of the growth of non-pathogenic commensals. The information of usage of other blood sources apart from human and sheep blood are scanty [7]. Also, some study suggested that human blood may certain antibiotics and antimicrobial agents which may also inhibit growth and cause false hemolysis. Thus, need for alternative blood sources for the development of novel blood agar to avoid the above said laboratory issues. Some studies suggested the usage of expired human blood from blood banks is often the most accessible source, but there is no exploration of wide usage of other blood sources and its effect on microbial growth and other biochemical and morphological characteristics [8].

In order to avoid the occupation risk to bloodborne pathogens become more real and cannot ignore; it has become mandatory to identify other blood types to find out suitability, availability, feasibility and easy handling to prepare blood agar plates [7,8]. Various factors, including transport conditions, bacterial density, culture medium, and microaerophilic condition, directly influence the yield of culture also play a very important role in the *H. pylori* bacteria culturing [9]. This study therefore describes and evaluates other animal blood types for the ability to support *H. pylori* growth compared with human blood. It also helps to identify variations in colony morphology and other growth characteristics including biochemical tests, compare the growth rate of *H. pylori*. Thus, this study tries to determine the limitations of the usage of different blood types for *H. pylori* growth.

MATERIALS AND METHODS

Blood collection

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Human blood was obtained from the blood bank of MNMC Hospital, blood samples of goat, Sheep, and Chicken were obtained from animal house of MNMC. Health related questions were enquired and antibiotics used in the animals in the preceding month. 20 ml blood was collected by cardiac puncture in chicken, jugular veins of the goat and sheep. Fresh human blood was collected aseptically in EDTA bottles and stored at a low temperature until use.

Preparation of Skirrow supplement

The antibiotic supplement is recommended for the selective isolation of *H. pylori* which is composed of polymyxin B (1.250IU), vancomycin (5 mg) and trimethoprim (2.5mg). In this study, commercially available Skirrow supplement (HIMEDIA FD003) was used; further rehydrated the contents of one vial aseptically with 2 ml of sterile distilled water which is sufficient to 500ml of blood agar medium.

Preparation of different blood agar medium

Human, goat, sheep, and chicken blood were aseptically collected from respective sources. For 100ml of blood agar base, 5% of blood and 0.2% of Skirrow supplement were aseptically added and mixed thoroughly. Further, the medium was poured into sterile Petri dishes.

Sources of the *H. pylori* isolates

Duodenal ulcer biopsy samples obtained from the Department of Gastroenterology, MNMC was processed as per the procedure of isolation and identification of *H. pylori* (Guetarni and Bensoltane, 2013). The grinded biopsy materials were inoculated in the blood agar medium enriched with Skirrow supplements and incubated for 3 to 4 days at microaerophilic (5% O_2, 10% CO_2, and 85% N_2) conditions at 37°C. The colonies were identified as small, round, bulgy and glossy (translucent), intense urease, catalase and oxidase production for *H. pylori* [10].

Cultivation in various blood agar plates

The microbiologically confirmed *H. pylori* were inoculated each in the respective blood agar plates and incubated for 3 to 4 days at microaerophilic (5% O_2, 10% CO_2, and 85% N_2) conditions at 37°C. Further, the colonies were observed for *H. pylori* growth and confirmed by rapid biochemical tests including urease, catalase and oxidase [10].

RESULTS

After successful incubation under the microaerophilic condition, the growth of *H. pylori* was observed and recorded. No previous report observed in the determination of *H. pylori* growth in different blood culture medium. The positive results of colony morphology, gram staining and rapid biochemical test including urease, catalase, and oxidase supported the growth of *H. pylori.* The detailed description of the results was depicted in Table 1.

Table 1: Description of *H. pylori* on various blood agar medium.

Blood agar	Growth range	Colony	Gram's staining	Biochemical Tests		
				Urease	Catalase	Oxidase
Human	Significant growth	Small, round, bulgy & glossy (translucent)	Gram-negative rods (spiral)	Positive	Positive	Positive
Goat	Significant growth	Small, round, bulgy & glossy (translucent)	Gram-negative rods (spiral)	Positive	Positive	Positive
Chicken	Sparse growth	Small, round & glossy (translucent)	Gram-negative rods (spiral)	Slow reactive	Slow reactive	Slow reactive
Sheep	Scanty growth	Small, round & glossy (translucent)	Gram-negative rods (spiral)	Slow reactive	Slow reactive	Slow reactive

For comparing the all the plates the growth of H. pylori in human blood agar medium showed significant growth followed by goat and chicken blood agar medium plates. Very less (scanty) growth was observed on sheep blood agar medium.

The growth of H. pylori in different blood agar medium varies each other; thereby Human and goat blood agar provided the same type of colonies whereas chicken and sheep blood showed and sparse and scanty growth respectively. The bulginess and the translucency vary in different blood agar medium, whereas human and goat blood showed better that chicken and sheep. The rapid biochemical test results of H. pylori also varied. Human and goat blood are rapid in reactivity of urease, catalase and oxidase tests but slow reactive was observed in chicken and sheep blood.

DISCUSSION

The results presented in this study confirm that although *H. pylori* colonies are found in all blood agar plates relatively the colony size and other features varied. All the blood agars prepared from animals and human supported the growth of *H. pylori* and there was no significant difference in the morphology and cultural characteristics on the various blood agar types. This was further confirmed by comparing with descriptions from Bergey's Manual [11]. However, substantially smaller colony sizes and slower in rapid biochemical tests were noted among blood agar. Literature suggested that there are no variations in the colony morphology among different blood agar in other infectious bacteria [1].

CONCLUSION:

The culturing of H. pylori from gastric antral biopsy specimens is a reference technique in bacteriology and is essential for identifying virulence factors antimicrobial susceptibility testing Although it is usually considered as a tedious, time-consuming and expensive procedure, culturing on solid medium is the standard method for the isolation of H. pylori from gastric biopsy specimens The gold standard method for the isolation of infectious materials is the successful culturing of microorganism. In recent years, the culturing of *H. pylori* from gastric antral biopsy specimens is a reference technique in bacteriology and is essential for identifying virulence factors antimicrobial susceptibility testing [12]. Although it is usually considered as a tedious, time-consuming and expensive procedure, culturing on solid medium is the standard method for the isolation of *H. pylori* from gastric biopsy specimens [13,14].

These findings have profound implications of using alternative animal blood for the preparation of blood agar, wherein most laboratories expired human blood is commonly supplemented. Thus this study will give some ideas to the laboratory personals and policy makers to alternate the human blood to other animal blood for the cultivation of various pathogenic bacteria including *H. pylori*. To our knowledge, this is the first time that different blood sources have been shown to be suboptimal for the growth of *H. pylori.* The comparative analysis of the growth of other bacteria in different blood agar was established [7,8,15,16,17,18]. Even some study suggested that citrated sheep blood agar is the best alternative for *Streptococcus pneumoniae*, *S. pyogenes,* and *Staphylococcus aureus* [15], but in this study, the sheep blood is not much successful for the culture and maintenance of *H. pylori*.

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