ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of a New Rapid Paper-Based Sugar Fermentation Technique for Identification of Medically Important Bacteria.

Bhattacharyya S*, Kumari C, Sarfraz A, Jaiswal NK, Kumar R, Roy H, Ahmed S, Prasad R, Mehdi MA, and Singh A.

Department of Microbiology, AIIMS, Patna-801505, India.

ABSTRACT

Identification of medically important bacteria by biochemical tests often takes long time, and delay in identification can lead to delay in initiation of empirical antibiotic administration. We here report a new, in-house sugar fermentation technique developed by us which is successful and gives comparable results of with that of sugar fermentation in broth. This can correctly identify medically important bacteria from pure culture and help in timely commencement of antibiotic therapy.

Keywords: sugar, fermentation, bacteria, antibiotic

Corresponding author

ISSN: 0975-8585

INTRODUCTION

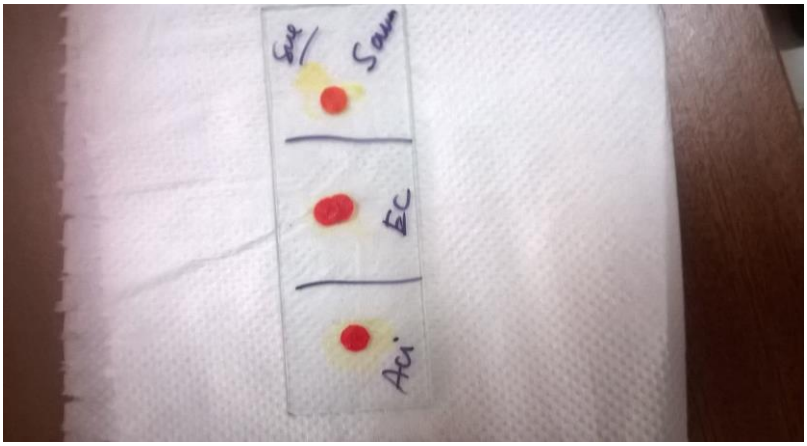
Identification of medically significant bacteria like *Staphylococcus aureus* and *Escherichia coli* requires putting up of biochemical tests following isolation of colonies from samples[1].These biochemical tests are interpreted after 18-20 hours, or overnight incubation at 37^{o}C [1]. Sugar fermentation is one of the major tests needed for bacterial identification [2]. For example, maltose fermentation differentiates *Proteus vulgaris* [positive] from *Proteus mirabilis* [negative][3]. However, overnight incubation at 37^{o}C is mandatory for broth-based frementation tests to come positive [2]. This can delay start of antibiotic therapy and lead to increased patient mortality and mortality [4].

MATERIALS AND METHODS

This was a laboratory-based observational study, carried out in Department of Microbiology, AIIMS Patna as a departmental project from May 2015 to July, 2015. Ten clinical isolates each of *S. aureus*, *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were selected for the study. They were identified by staining, oxidase strip test and standard biochemicals and conventional sugar fermentation using Peptone water with 1% [w/v] Phenol red. In the new method, 10 µl of peptone water containing 1% sugar and Phenol Red was put on sterile 6 mm. Filter paper disks made from Whatman no. 1 filter paper. The paper disks were dried for 5 minutes, and with straight wire or loop, 2-3 colonies of the bacteria to be tested were smeared on the disk after placing the disk on a sterile glass slide, and kept at room temperature. Disks were observed for orange to yellow colour development, which indicated positive sugar fermentation. For each test, Known *E. Coli* and *P. aeruginosa* were kept as positive and negative controls, respectively. Each test was carried out three times for each isolate. Test was read for 15 minutes. After 15 miniutes, any colour development was disregarded. Simultaneously the isolates were tested for sugar fermentation by conventional method also, using the same medium in test tubes. Lactose, maltose, glucose and sucrose were the sugars used in this study.

RESULTS

Results showed 100% concordance between the new paper based method and broth-based conventional method. Thus the new paper-based method was found to very suitable and useful alternative to conventional broth-based fermentation test.



DISCUSSION

Sugar fermentation for checking breakdown of saccharides like sucrose and lactose is a very useful test to differentiate between bacterial groups [5]. Scientists have made efforts to reduce the time needed for results of sugar fermentation tests to come, by using rapide broth-based methods [6]. However, these methods stiil use broth and need a lot of expertise, besides requirements like buffer solution containing sugars and indicators, and still need about 30 minutes to come positive from pure culture [6]. Other methods have relied on colour change using small amounts of liquid media with chemical indicators, and incubating for about 4 hours [7]. Our method was a new one, and we have named it APM method [after name of the institute]. In

this new APM method, however, liquid media have to be added in the said amount to sterile disks and used freshly. On storage at both rommom temperature and 4 ^{o}C, spontaneous colour change was found. However, it gave excellent and reproducible results when it was freshly poured ojn the paper disks and used. As far as we know, this is the first such method used, where freshly made cloured sugar-containing disks have been used. This can dramatically reduce the time for bacteria identification, more so when combined with Gram staining, Catalase and Oxidase and other tests, like spot indole test. This consequently will be beneficial for starting of timely empiric antibiotic therapy. Further studies, are however warranted in this context.

ACKNOWLEDGEMENTS

The authors acknowledge the help of Mr. Mahesh Kumar, lab attendant, Department of Microbiology of the institute.

REFERENCES

[1] Clarke PH, Cowan ST. Biochemical Methods for Bacteriology. J Gen Microbiol 1952;6: 187-97.
[2] Carbohydrate Fermentation Protocol. ASM Microbe Library.
[3] MacFaddin, J. F. 2000. Biochemical tests for identification of medical bacteria, 3rded. Lippincott Williams & Wilkins, Philadelphia, PA.
[4] Senga P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JF, Raoult D. Clin Infect Dis 2009;49 [4]: 543-551.
[5] http://www.srmuniv.ac.in/downloads/ISOLATION_AND_IDENTIFICATION.pdf.
[6] Young H, Paterson IC, MacDonald DR. Br J Vener Dis 1976 ; 52[3]: 172–175.
[7] Schreckenberger PC, Blazevic DJ. J Clin Microbiol 1976;3:313-17.