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Review Article

Brain Heart Infusion Agar : A Surrogate of Agar Blood

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Abstract
Blood agar is needed for culture of various organisms. As sheep blood is needed for it's preparation, many small laboratories specially those of rural areas can not prepare it due to difficulty in collection of sheep blood. So, either they do not do the culture or do it without blood agar which cause missing of some important organisms. In this study Brain heart infusion agar was used together with Blood agar to assess it's efficacy as an alternative of Blood agar. In total 1256 various samples were cultured, on Blood agar , Brain heart Infusion agar and MacConkey agar. Out of 1256 samples 404 samples showed growth of various organisms. It was noted that all bacteria including *Enterococcus sp.* grew equally in blood agar and BHIA. Brain heart infusion agar can be used as a surrogate of blood agar.

Key words: Brain heart infusion agar, Blood agar, bacteria

Introduction:
Blood agar is an essential media for culture of different samples. But, for it's preparation, sheep blood is needed. Sheep blood is not easily available for small laboratories. So, small laboratories either do not do the culture of samples or do it using Nutrient agar instead of Blood agar which cause missing of some important and common bacteria causing clinical infection. Brain heart infusion agar(BHIA) is a highly nutritious media and is effective for cultivation of a wide variety of fastidious and non-fastidious microorganisms including Neisseria and Streptococci.[1,2]

This media can be used as a surrogate of blood agar media. A trial was given to justify the efficacy of this media in place of blood agar.

Material and methods
The study was done in the Department of Microbiology, Bangladesh University of Health Sciences, Mirpur, Dhaka-1216 Bangladesh from 01 January 2018 to 31 December 2018.

In total 1256 samples including Urine(n=919), Blood (n=130) (for sub culture), Pus /Wound Swab(n=155), Sputum (n=40)andThroat swab(n=12) was cultured in blood agar, BHIA and MacConkey agar.[3,4,5] Identification of common organisms was done by standard procedure.[3,4,6]

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Identification of Gram positive cocci using BHIA.
For identification of gram negative bacteria,haemolysis is not important and can be identified by usual biochemical tests. But for identification of gram positive cocci, haemolysis in blood agar media is an important factor which is absent in BHIA. This was overcome by using biochemical tests as follows:

Step I. Catalase test was done with gram positive cocci. Catalase positive ones were staphylococci which was further identified by Coagulase test and novobiocin sensitivity.

Step II. Coagulase negatives were streptococci which were further tested for all of 'Bacitracin sensitivity', 'hippurate test', 'Optochin sensitivity', 'Growth in 6.5% Sodium chloride in TSB (trypticase soy broth)' and 'BES(bile esculin agar)' with interpretation as follows:

Bacitracin sensitive: *S. pyogenes;*
Hippurate test positive: *S. agalactae;*
Black colony in BES and growth in 6.5% NaCl in TSB : Group D, *Enterococcus sp*;

Black colony in BES and No growth in 6. 5% NaCl in TSB : Group D, *Non-Enterococcus sp*; Optochin sensitive : *S pneumonia;*
All negative :Viridans Streptococci.[3,6]
Further tests may be done for full identification of organisms.[7,8,9,10]

Result:

Out of 1256 samples, 404 showed growth of bacteria(Table-I). It was noted that all bacteria,grew equally in blood agar and BHIA (Fig.I, Fig.II and Fig.III) except that haemolysis was present in Blood agar which was absent in BHIA. Growth of *Enterococcus* species in BHIA was excellent and was similar to or more than blood agar.

Discussion:

As sheep blood for blood agar media is not easy available, it's substitute is necessary for culture of samples. In this study, Brain Heart Infusion Agar (BHIA) was compared with blood agar for culture of various samples and organisms. Performance of BHIA was equal to Blood agar for isolation of common organisms. *Enterococcus* sp. needs enriched media, such as blood agar, for their growth. It was evident from this study that *Enterococcus* sp. grew equally in BHIA and blood agar.

So, Brain Heart infusion agar can be used as surrogate media of Blood agar for culture of all organisms.

Table-I: Name of bacteria grown and their proportion

Name of bacteria	Number	Percentage
Escherichia coli	193	47.8
Klebsiella sp.	79	19.6
Pseudomonas sp.	31	7.7
S.aureus	25	6.2
Acinetobacter sp.	24	5.9
Enterococcus sp.	12	3.0
Enterobacter	09	2.2
Proteus	09	2.2
S.typhi	08	2.0
S. saprophyticus	07	1.7
Citrobacter	06	1.5
S.paratyphi	01	0.2
Total	**404**	**100%**

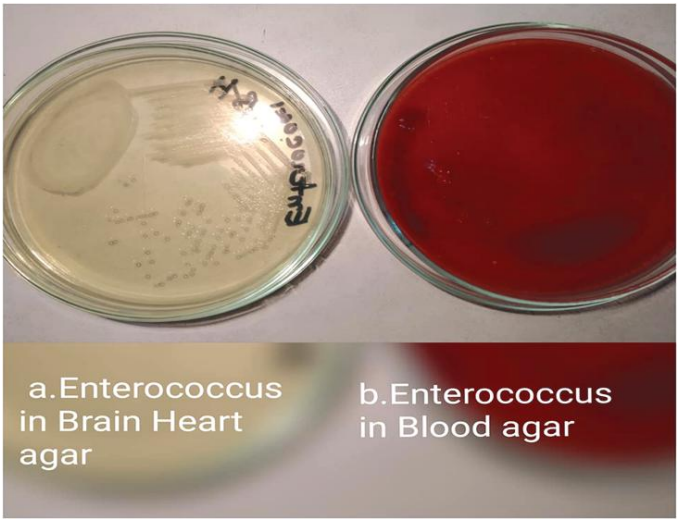

Fig. I

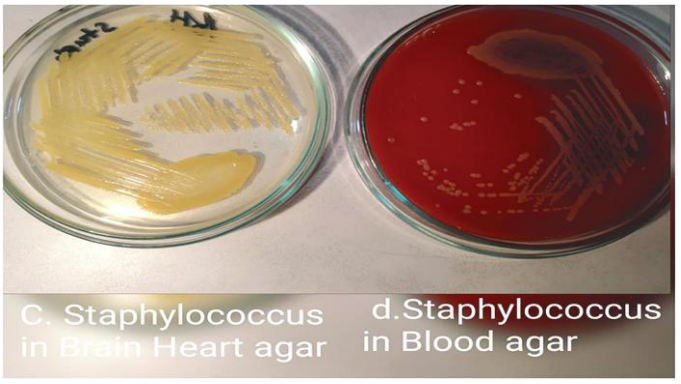

Fig. II

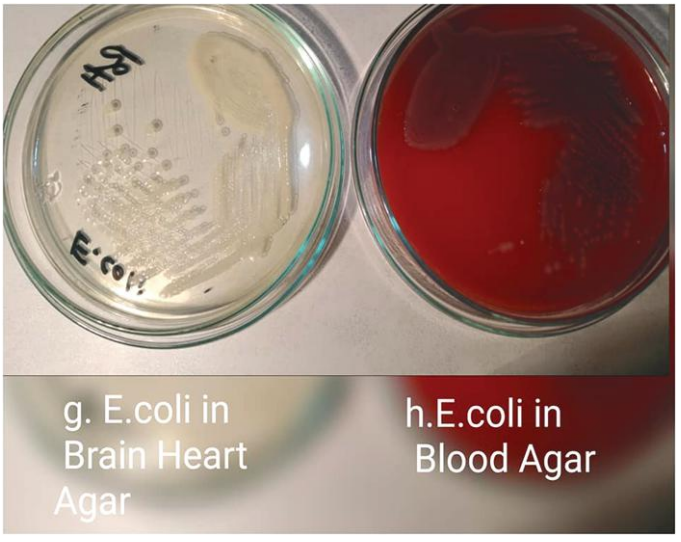

Fig. III

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