Research article

Human, cattle and goat blood as substitutes for sheep blood in blood-supplemented culture media

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Abstract

Introduction and Objective: Sheep blood is the recommended type of blood for supplementation of agar media. In Sri Lanka, due to lack of availability of sheep blood, expired citrated human blood is used which gives poor haemolysis and causes difficulties in identification of some organisms. In addition, human blood contains antibodies and other antibacterial factors including antibiotics which may inhibit bacterial growth. Human blood may also contain blood borne pathogens which could be a risk for laboratory staff. The objective of this study was to explore available alternatives in the Sri Lankan setting.

Methods: Clinical isolates and standard strains of *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus pneumoniae* and *Haemophilus influenzae* were tested for growth, identification characteristics and antibiotic susceptibility on human, cattle, goat and sheep blood agars. The performances were compared. Identification of *Listeria monocytogenes* as well as *Streptococcus agalactiae* was carried out using the CAMP test.

Results: All tested organisms gave similar isolation rates at the tested dilutions in the four tested agar plates. Human blood gave noticeably smaller colonies. *S. pyogenes* and *S. agalactiae* gave equally large zones of beta-haemolysis and *S. pneumoniae* gave obvious alpha-haemolysis on all animal blood agar plates. Both types of haemolysis were faint on human blood.

Typical arrow head shape haemolysis for *S. agalactiae* and match-head shape haemolysis for *Listeria* were seen in the CAMP test on the three animal blood agar plates whereas human blood gave negative results. All blood agar plates gave comparable positive results in the satellitism test for *H. influenzae*. There was no difference in bacitracin and optochin sensitivity tests for identification of *S. pyogenes* and *S. pneumoniae* respectively.

Inhibitory zones were unreadable when antibiotic susceptibility was done for *H. influenzae* on goat and cattle chocolate agar. Sheep and human chocolate agar were inferior to Haemophilus test medium. ABST results were equivalent but goat blood gave hazy, irregular margins for other organisms.

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Conclusions: Cattle and goat blood show similar performance to sheep blood in growth and identification tests for common fastidious pathogens. Cattle blood is equivalent to sheep blood for ABST, but goat blood is inferior.

Keywords: Sheep blood agar, Human blood agar, Blood supplemented media

Introduction

Artificial culture media play a very important role in microbiological laboratories. Robert Koch (1843-1910) is considered as the father of artificial culture media.[1] Agar media supplemented with 5% blood are widely used in laboratories for routine isolation, identification and antibiotic susceptibility testing (ABST) of bacterial pathogens.[1,2] Some fastidious bacteria such as *Neisseria* and *Haemophilus* species require "X" factor (Haemin) and "V" factor (Nicotinamide adenine dinucleotide -NAD) which are found in blood cells. Hemin (factor X) is available from non-haemolysed as well as haemolysed blood cells. The lysis of RBC during the heating process releases intracellular coenzyme NAD (V Factor) into the agar. The heating process also inactivates the growth inhibitors in blood.[3]

Blood supplementation allows the visualization of haemolysis which assists identification of some organisms. *Streptococcus* species give different types of haemolysis (β-haemolysis and α-haemolysis).[4] Blood supplementation minimizes misidentification of colonies, thereby reducing specimen processing cost and turnaround time, and helps accuracy of reports.[4] Defibrinated sheep blood is largely preferred because it prevents growth of some nonpathogenic commensals (eg: *Haemophilus haemolyticus)* due to growth inhibitors and also gives the best haemolytic patterns.[1] Horse blood has also been recommended and is used in some countries.[4] However, in many developing countries, including Sri Lanka, sheep and horse blood are difficult to obtain due to unavailability of these animal sources.[5,6] Buying these blood products from commercial sources is not feasible due to the high cost. Citrated human blood is therefore used in many resource poor settings, despite its many shortcomings.[2,6] Human blood is not generally recommended for enrichment of agar media because of poor bacterial isolation rates and inability to demonstrate proper organism characteristics.[1,2,6] Presence of antibodies, antibiotics and various inhibitory components in human blood can account for this.[2] The haemoglobin level of animals such as sheep and cattle are around 8-16 mg/dl[7] in comparison with the haemoglobin level of human red cell concentrate (20 mg/dl). Human blood agar therefore gives a darker colour compared to other animal blood agars, which makes observation of colony characteristics a difficult task.

The CAMP test is commonly used for identification of *Streptococcus agalactiae* and it is useful identification test for *Listeria monocytogenes*. The CAMP reaction depends on the synergistic haemolytic activity of two factors; CAMP factor and sphingomyelinase. The CAMP factor is secreted by group B streptococci (GBS) and is a protein with exotoxin and pore-forming properties which are suggested to be important for GBS pathogenesis. Sphingomyelinase is secreted by *Staphylococcus aureus* strains.[2,8] As the sphingomyelin content of human and rabbit blood cells is low, they do not support the CAMP reaction well. The sphingomyelin content of sheep, cattle and goat blood is high, and these blood types support the CAMP reaction well.[2,9]

The use of human blood is associated with safety risk to laboratory personnel, especially due to transmission of blood-borne viral infections such as hepatitis B, C and HIV and is therefore considered unsuitable for use in clinical diagnostic laboratories**.**[2,9]

Due to these drawbacks of human blood supplemented media, a suitable alternative is needed in countries where use of sheep blood is not feasible. In Sri Lanka expired citrated human blood obtained from blood banks is commonly used for preparation of media. Taking into consideration the importance of proper blood enriched media in laboratory practice, it is a timely need to look for a suitable substitute. This study was carried out to look for a better source of blood which is affordable and freely available to be used for bacteriology in Sri Lanka.

The objective of the study was to compare the performance of citrated human, cattle, goat and sheep blood as enrichment substance in blood supplemented culture media. These two animal species are commonly available in Sri Lanka, making them a credible option to be considered for this purpose.

Methods

This is a laboratory based descriptive cross-sectional study, carried out in the Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura. All procedures were performed according the laboratory manual of the Sri Lanka College of Microbiologists, 2011.[10]

Citrated sheep, cattle and goat blood were collected from healthy animals (following examination by a veterinary surgeon) who had no recorded history of blood borne disease and had not received an antibiotic within the previous three months. Blood collection was performed under strict aseptic conditions by experienced professionals and transported in a cold box. Citrated human blood was obtained from the National Blood Transfusion Service.

5% blood and chocolate agar, blood Mueller-Hinton agar and Haemophilus Test Medium (HTM) were prepared following standard microbiological methods.[10]

3% of prepared culture plates were incubated overnight at 35˚C for sterility. Standard stock cultures of *Streptococcus pyogenes* (ATCC 12384), *Streptococcus agalactiae* (ATCC 12386), *Streptococcus pneumoniae* (ATCC 49619), *Haemophilus influenzae* (ATCC 49247) and two clinical isolates each of these four organisms (confirmed identification using standard biochemical tests and/or API kits) were tested and compared for growth, identification characteristics and antibiotic susceptibility on the four different types of blood agar. Three clinical isolates of *Listeria monocytogenes* (confirmed identification using standard biochemical tests and motility test) were used for the CAMP test.

Colony count, colony size and morphology on each type of blood agar were checked.

Colony count

Each of the above bacterial strains from an overnight culture was inoculated in Brain Heart Infusion Broth (BHI) to make a suspension (turbidity~0.5 McFarland standard) and serially diluted in BHI. The neat suspension (~0.5 McFarland standard) and1/10 and 1/100 dilutions of the neat (approximately 10^8, 10^7 and 10^6 CFU/ml) were used for testing. Agar plates were inoculated using a calibrated 1 µl bacteriological loop and incubated overnight at 35 ˚C in 5-10% CO_2. All tests were done in duplicate. The colony count was given as the average of the two readings in units of CFU/ml.

Colony morphology

Sizes of two separated colonies were measured in millimeters and the average taken. Colony morphology was checked by the naked eye. Haemolytic patterns (α, β) for *Streptococcus* sp. with diameters of the haemolytic zones (mm) were recorded.

Identification tests

CAMP (Christie-Atkins-Munch-Peterson) test for *Streptococcus agalactiae* and *Listeria monocytogenes* and satellitism test for *Haemophilus influenzae* were performed.[10]

0.04U bacitracin and 5µg optochin sensitivity tests were done for *Streptococcus pyogenes* and *Streptococcus pneumoniae* respectively.

Antibiotic susceptibility testing (ABST)

ABST was done according to the guidelines by Clinical Laboratory Standard Institute (CLSI), 2012.[11] For *Haemophilus influenzae*, ABST was performed on Haemophilus Test Medium (HTM) in addition to the four test chocolate agar plates and zones of inhibitions compared.

Results

Colony count

Table 1: Colony count (CFU/ml) of tested organisms on all four blood agars at different dilutions

	Sheep	**Goat**	**Cattle**	**Human**
Streptococcus pyogenes				
Clinical 1 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 2 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
ATCC 12384 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 1 (1/10)	10^4-10^5	10^4-10^5	10^4-10^5	10^4-10^5
Clinical 2 (1/10)	NG	NG	NG	NG
ATCC 12384 (1/10)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 1 (1/100)	10^3-10^4	10^3-10^4	10^3-10^4	10^3-10^4
Clinical 2 (1/100)	NG	NG	NG	NG
ATCC 12384 (1/100)	NG	NG	NG	NG
Streptococcus agalactiae				
Clinical 1 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 2 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
ATCC 12386 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 1 (1/10)	10^4-10^5	10^4-10^5	10^4-10^5	10^4-10^5
Clinical 2 (1/10)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
ATCC 12386 (1/10)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 1 (1/100)	10^4-10^5	10^4-10^5	10^4-10^5	10^4-10^5
Clinical 2 (1/100)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
ATCC 12386 (1/100)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Streptococcus pneumoniae				
Clinical 1 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 2 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
ATCC 49619 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 1 (1/10)	10^3-10^4	10^3-10^4	10^3-10^4	10^3-10^4
Clinical 2 (1/10)	10^3-10^4	10^3-10^4	10^3-10^4	10^3-10^4
ATCC 49619 (1/10)	NG	NG	NG	NG
Clinical 1 (1/100)	NG	NG	NG	NG
Clinical 2 (1/100)	NG	NG	NG	NG
ATCC 49619 (1/100)	NG	NG	NG	NG
Haemophilus influenzae				
Clinical 1 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 2 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
ATCC 49247 (0.5M)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 1 (1/10)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 2 (1/10)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
ATCC 49247 (1/10)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 1 (1/100)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
Clinical 2 (1/100)	$>10^5$	$>10^5$	$>10^5$	$>10^5$
ATCC 49247 (1/100)	10^4-10^5 (DTC)	10^4-10^5 (DTC)	10^4-10^5 (DTC)	10^4-10^5 (DTC)

NG – No growth DTC – Difficult to count

Morphological characteristics

Table 2: Colony morphology of organisms on different types of agar

	Sheep	Goat	Cattle	Human
Streptococcus pyogenes ATCC 12384				
Colony size	1-2 mm	1-2 mm	1 mm	<1 mm
Haemolytic zone	Obvious	Obvious	Obvious	Faint
Diameter	>2 mm	>2 mm	>2 mm	1.5-2 mm
Streptococcus pyogenes Clinical isolate 1				
Colony size	<1 mm	<1 mm	<1 mm	<1 mm
Haemolytic zone	Obvious	Obvious	Obvious	Faint
Diameter	1.5-2 mm	1.5-2 mm	1.5-2 mm	1.5-2 mm
Streptococcus pyogenes Clinical isolate 2				
Colony size	<1 mm	<1 mm	<1 mm	<1 mm
Haemolytic zone	Obvious	Obvious	Obvious	Faint
Diameter	1.5-2 mm	1.5-2 mm	1.5-2 mm	1.5-2 mm
Streptococcus agalactiae ATCC 12386				
Colony size	<1 mm	<1 mm	<1 mm	<1 mm
Haemolytic zone diameter	1-1.5 mm	1-1.5 mm	1-1.5 mm	1-1.5 mm
Streptococcus agalactiae Clinical isolate 1				
Colony size	1-1.5 mm	1-1.5 mm	1-1.5 mm	<1 mm
Haemolytic zone diameter	1.5-2 mm	1.5-2 mm	1.5-2 mm	<1 mm
Streptococcus agalactiae Clinical isolate 2				
Colony size	1-1.5 mm	1-1.5 mm	1-1.5 mm	<1 mm
Haemolytic zone diameter	1.5-2 mm	1.5-2 mm	1.5-2 mm	<1 mm
Streptococcus pneumoniae ATCC 49619				
Colony size	<1 mm	<1 mm	1 mm	<1 mm
Haemolytic zone diameter	2-3 mm	2-3 mm	2-3 mm	<1 mm
Streptococcus pneumoniae Clinical isolate 1				
Colony size	<1 mm	<1 mm	1 mm	<1 mm
Haemolytic zone diameter	1-2 mm	1-2 mm	1-2 mm	<1 mm
Streptococcus pneumoniae Clinical isolate 2				
Colony size	<1 mm	<1 mm	1 mm	<1 mm
Haemolytic zone diameter	1-2 mm	1-2 mm	1-2 mm	<1 mm
Haemophilus influenzae ATCC 49247				
Colony size	1 mm	Powderly	Powderly	1 mm
Haemophilus influenzae Clinical isolate 1				
Colony size	1 mm	Powderly	Powderly	1 mm
Haemophilus influenzae Clinical isolate 2				
Colony size	1 mm	Powderly	Powderly	1 mm

The colony sizes of all clinical isolates of *Streptococcus pyogenes* were the same on all blood types. The ATCC strain (12384) however gave very small colonies (<1 mm) on human blood agar (HBA) with minimal haemolysis.

The ATCC strain (12386) of S*treptococcus agalactiae* gave <1 mm colonies in all four blood agar plates while the clinical isolates grew poorly (<1 mm) only on HBA. Clinical isolates and the ATCC strain gave good β haemolysis on the 3 animal blood agar plates. Although the ATCC strain also gave good β haemolysis on HBA, the clinical isolates performed poorly (<1 mm).

Colony sizes of both clinical isolates and ATCC strains (49619) of *Streptococcus pneumoniae* were larger (1 mm) on cattle blood agar (CBA) than on the other 3 blood agar plates (<1 mm). The ATCC strain (49619) gave larger α haemolytic zones (2-3 mm) on the animal blood agar

plates in comparison with the clinical isolates (1-2 mm). α haemolysis was negligible (<1 mm) on HBA for all isolates.

Beta haemolysis and alpha haemolysis were obvious on SBA, GBA and CBA but faint on HBA as shown in Figures 1 and 2.

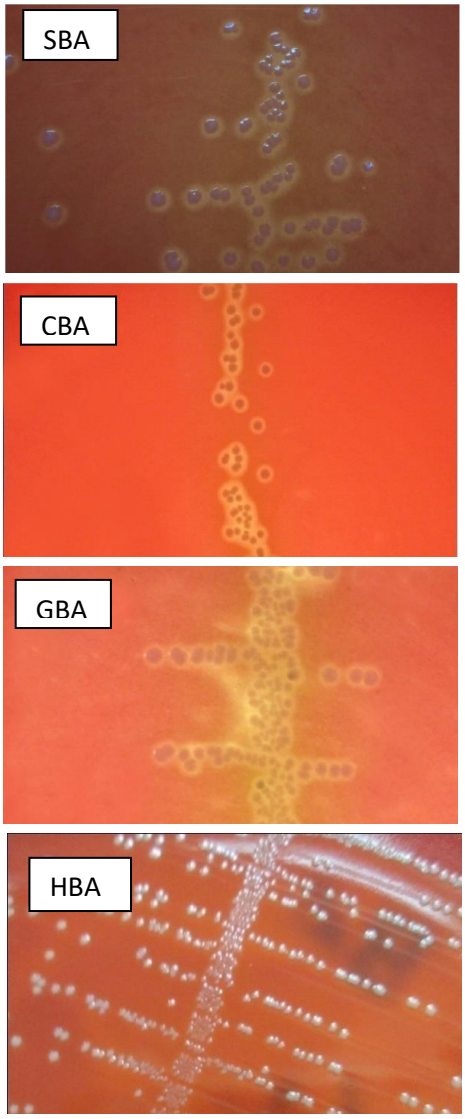


Figure 1: β-haemolysis of *Streptococcus agalactiae*

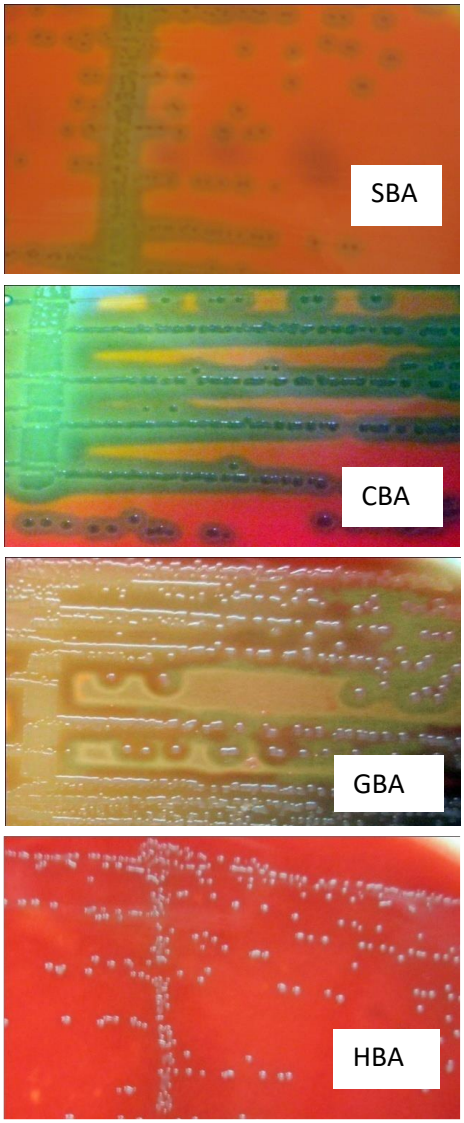


Figure 2: α-haemolysis of *Streptococcus pneumoniae*

Haemophilus influenzae

Clinical isolates and ATCC strains gave 1 mm colonies in Sheep Chocolate Agar (SCHA) and Human Chocolate Agar (HCHA) whereas on Cattle Chocolate Agar (CCHA) and Goat Chocolate Agar (GCHA) they were powdery colonies (Figure 3).

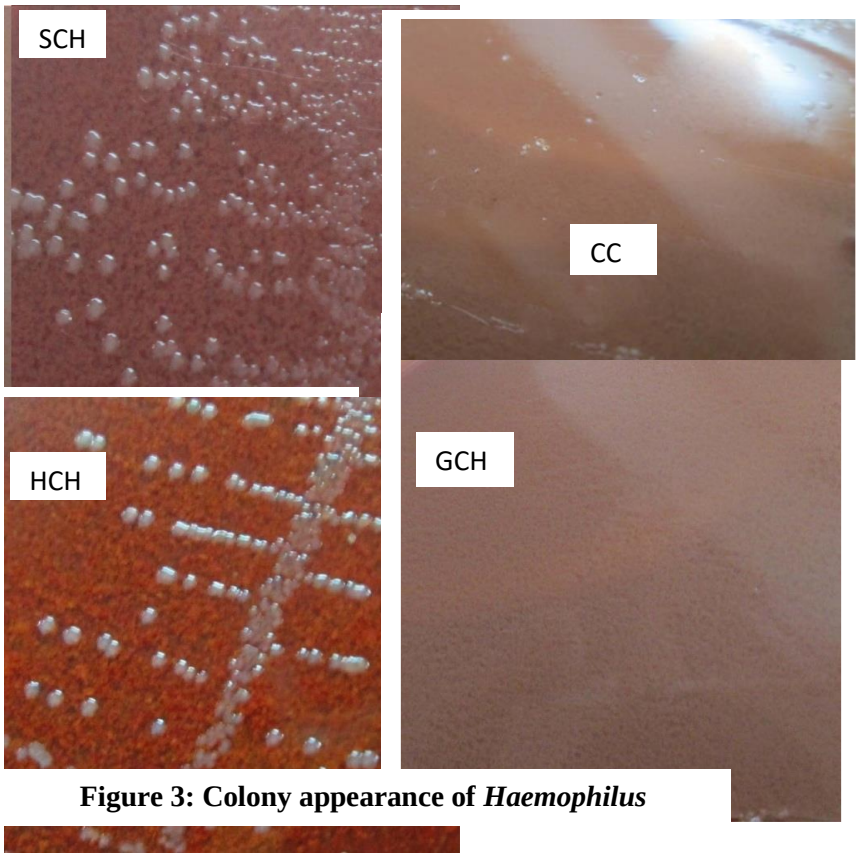
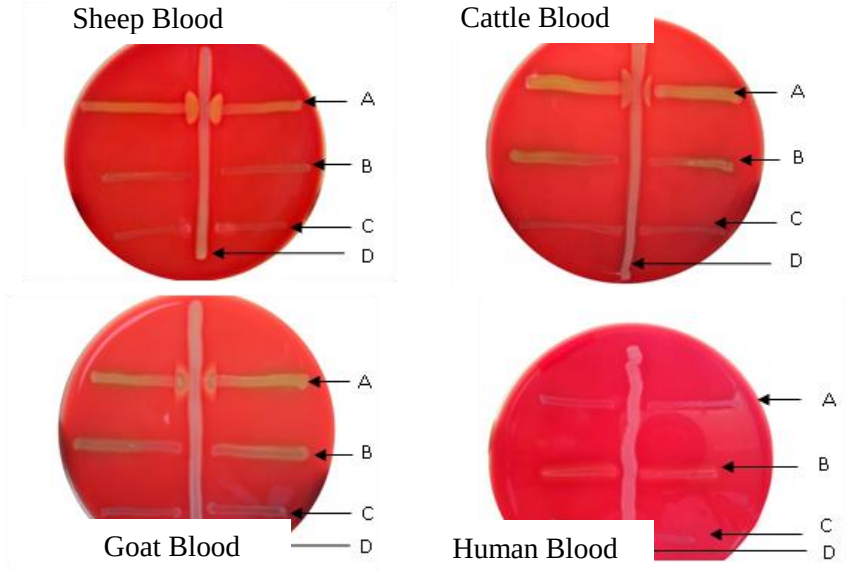


Figure 3: Colony appearance of *Haemophilus*

Identification tests

All clinical and standard strains of *S. pyogenes* were sensitive to 0.04U bacitracin and *S. pneumoniae* were sensitive to 5 µg optochin in all four blood types. *S. agalactiae* gave obvious arrow head shaped haemolysis on SBA, CBA, and GBA in CAMP test but this was absent in HBA. *Listeria* gave match head shaped haemolysis on all types of animal agar and was absent in HBA (Figure 4).



A – *S. agalactiae* B – *Enterococcus faecalis*

Figure 4: CAMP test on different types of blood agar

For *Haemophilus influenzae*, all four blood types gave satellitism. However, performance of GBA and HBA were poor.

Antibiotic Sensitivity Testing (ABST)

Inhibition zone sizes of standard strains of *Streptococcus* sp. were within the recommended range and clinical isolates of all these organisms gave similar results on all 4 types of blood Mueller-Hinton agar (BMHA) for all tested antibiotics (Figure 5).

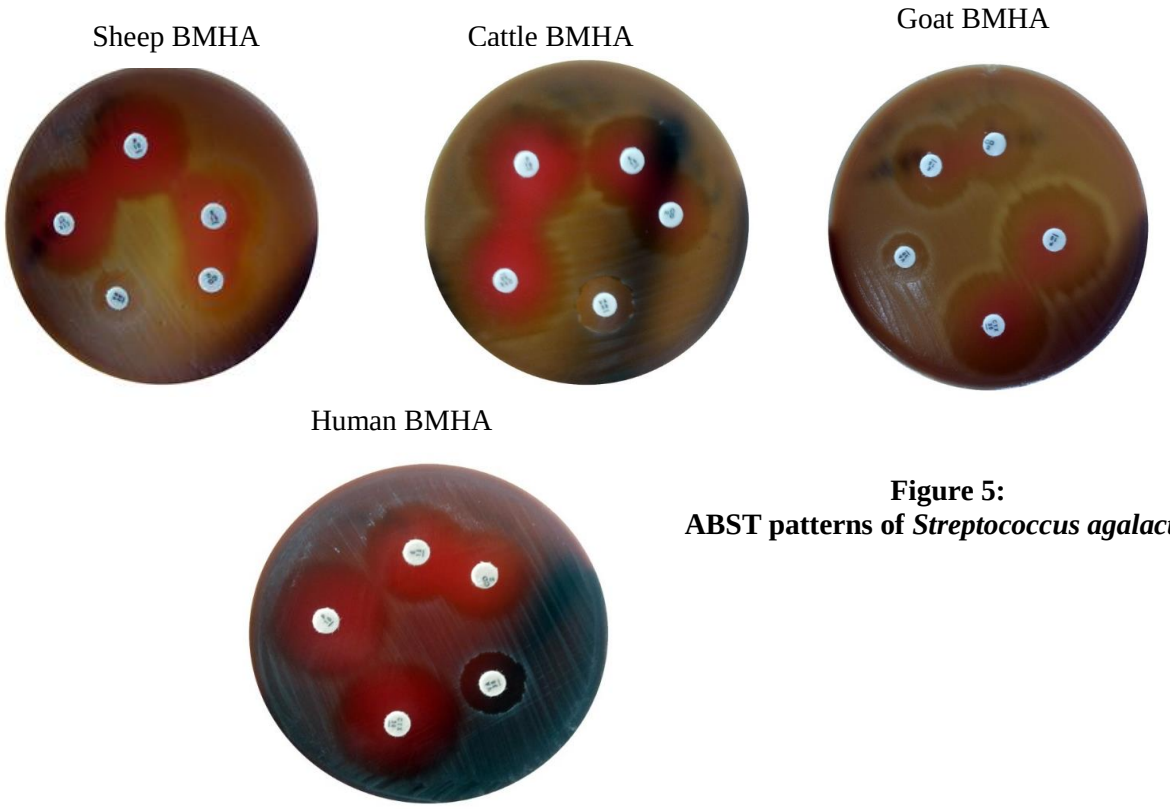


Figure 5:
ABST patterns of *Streptococcus agalactiae*

Table 3: ABST results of *Streptococcus pyogenes*

Streptococcus pyogenes		Clindamycin 2 µg		Erythromycin 15 µg		Vancomycin 30 µg		Cefotaxime 30 µg		Penicillin 10 U	
		1	2	1	2	1	2	1	2	1	2
		mm		mm		mm		mm		mm	
Clinical -1	Sheep	26	27	24	24	18	18	26	31	31	32
	Goat	25	24	30	31	16	16	29	29	31	33
	Cattle	26	26	25	26	17	18	32	30	32	35
	Human	20	23	24	23	17	16	32	32	32	31
Clinical -2	Sheep	25	26	17	16	18	18	45	45	35	35
	Goat	18	20	13	14	17	17	38	37	35	33
	Cattle	20	20	14	15	20	20	40	39	36	36
	Human	21	19	16	16	18	17	39	39	39	37
ATCC	Sheep	40	42	30	30	17	18	33	32	32	35
	Goat	36	35	30	31	16	16	32	32	31	31
	Cattle	40	42	29	30	17	17	33	35	32	33
	Human	36	35	28	28	15	15	31	28	30	28

Table 4: ABST results of *Streptococcus agalactiae*

Streptococcus agalactiae		Clindamycin 2 µg		Erythromycin 15 µg		Vancomycin 30 µg		Cefotaxime 30 µg		Penicillin 10 U	
		1	2	1	2	1	2	1	2	1	2
		mm		mm				mm			
Clinical -1	Sheep	23	25	23	24	16	18	32	31	32	32
	Goat	22	24	24	23	17	16	32	29	29	33
	Cattle	20	19	22	22	15	18	33	30	30	29
	Human	21	23	21	23	14	16	32	32	33	31
Clinical -2	Sheep	17	20	24	25	14	16	29	31	30	28
	Goat	19	20	23	24	15	17	28	30	29	27
	Cattle	21	20	24	25	16	15	32	29	34	36
	Human	21	19	23	21	14	14	31	28	33	35
ATCC	Sheep	27	28	28	30	19	18	33	32	32	30
	Goat	26	24	27	27	16	16	32	32	31	31
	Cattle	22	21	29	30	18	17	33	35	32	33
	Human	23	22	28	28	16	15	31	28	30	28

Table 5: ABST results of *Streptococcus pneumoniae*

Streptococcus pneumoniae		Chloramphenicol 30 µg		Erythromycin 15 µg		Vancomycin 30 µg		Cefotaxime 30 µg		Oxacillin 1 µg	
		1	2	1	2	1	2	1	2	1	2
		mm		mm		mm		mm		mm	
Clinical -1	Sheep	18	20	R	R	25	25	38	37	27	25
	Goat	17	16	R	R	29	29	41	38	28	30
	Cattle	20	19	R	R	30	30	50	43	28	29
	Human	16	15	R	R	27	27	38	35	25	24
Clinical -2	Sheep	37	35	11	09	24	24	31	34	26	28
	Goat	34	33	10	11	24	23	39	36	21	25
	Cattle	36	34	R	R	21	20	33	31	22	22
	Human	31	29	R	R	21	24	32	33	21	20
ATCC	Sheep	36	37	38	35	24	23	44	42	25	28
	Goat	30	28	36	34	23	22	39	38	24	25
	Cattle	36	30	37	38	22	21	44	45	22	21
	Human	34	32	36	33	20	18	42	40	22	25

R - Organisms grow up to the disk.

Table 6: ABST results of *Haemophilus influenzae*

Haemophilus influenzae		Co-amoxiclav 10 µg		Cefuroxime 30 µg		Ciprofloxacin 5 µg		Cefotaxime 30 µg		Ampicillin 10µg	
		1	2	1	2	1	2	1	2	1	2
		mm		mm		mm		mm		mm	
Clinical -1	Sheep	22	23	26	28	24	22	29	27	23	22
	Goat	-	-	-		-	-	-	-	-	-
	Cattle	-	-	-		-	-	-	-	-	-
	Human	17	16	25	24	22	24	28	27	22	24
	HTM	25	26	29	30	26	28	32	30	28	29
Clinical -2	Sheep	21	22	25	25	25	24	30	29	17	16
	Goat	-	-	-	-	-	-	-	-	-	-
	Cattle	-	-	-	-	-	-	-	-	-	-
	Human	16	18	24	25	22	25	27	28	15	15
	HTM	24	26	30	31	29	28	31	32	27	28
ATCC 49247	Sheep	17	18	-	-	35	34	29	29	15	14
	Goat	-	-	-	-	-		-	-	-	-
	Cattle	-	-	-	-	-		-	-	-	-
	Human	18	16	-	-	30	32	27	27	10	11
	HTM	20	22	-	-	40	38	37	35	19	21

As the *H. influenzae* strains did not grow properly on CCHA and GCHA, the test was repeated, with similar results.

The inhibition zone sizes of the standard strain of *H. influenzae* were within the recommended range on HTM for all antibiotics. However, they were not within the recommended range for cefotaxime on SCHA and for ciprofloxacin and cefotaxime on HCHA.

There were no zones of inhibition (ZOI) of clinical isolates I and II with co-amoxiclav on HCHA. However, these isolates were sensitive on HTM and SCHA. Although the other ABST results were same, zone diameters on HCHA and SCHA were smaller than zone sizes of HTM.

Hazy margins of the zones of inhibition were obtained with GBMHA (figure 5) which made reading of the zone diameter difficult.

Figure 6: Hazy margins on GBMHA in antibiotic sensitivity testing

Discussion

Group A and B streptococci and pneumococci grew well on the four types of blood agar giving colony counts of $>10^5$ CFU/ml at 0.5 MF turbidity. A few strains failed to grow in dilutions which could be due to non-maintenance of standard stock cultures or simply the insufficiency of the number of organisms to give a visible growth.

Colony size was smaller on HBA than on other blood agar for all tested organisms which suggests inferiority of human compared to animal blood as shown previously.[1,12]

All four types of blood agar gave β-haemolysis for group A and B streptococci and α-haemolysis for pneumococci. However, β and α-haemolysis were minimal and difficult to observe on HBA. Haemolytic zones on HBA were also smaller compared to the other 3 types of blood agar. The RBC concentrate (Hb 20 g/dl) is used to prepare HBA which is higher than the concentration in other animal bloods (8-16 g/dl). Prepared HBA plates were darker in colour than others due to the high content of haemoglobin, which may be the reason for the difficulties in detecting haemolysis. β haemolysis of *S. agalactiae* was observed on HBA on removal of the colonies. These findings are similar to the findings of Egwuatu et al (2014)[1] and Russell et al (2006).[9]

Colony counts of *H. influenzae* were the same on all four types of chocolate agar. Colony size on GCHA and CCHA were smaller than on SCHA and HCHA. Colonies of *H. influenzae* in the present study were smaller than previously reported.[3] Sooriyar et al (2015)[12] found HCHA superior to SCHA for *H. influenzae* whereas in the present study, the colony sizes on HCHA and SCHA were similar.

Haemophilus sp. require NAD or NADP (Nicotinamide Adenine Dinucleotide Phosphate). Animal blood naturally contains thermolabile growth inhibitors for such compounds and can be removed by heating.[4,5] The temperature and time required to remove these inhibitors can vary for different animal bloods. For SCHA and HCHA, 80 °C for 15 minutes is adequate.[5] GCHA and CCHA were also prepared by heating at 80°C for 15 minutes in the current study. The powdery colonies on these two media may have been due to inadequate heating for removal of inhibitors. Increasing the temperature above 80 °C in the preparation of chocolate agar using cattle/goat blood may give better results. Chandar Anand et al[3] have commented that pig blood required a higher temperature of 100 °C for removal of inhibitors. Gratten et al[4] have noticed good growth of *Haemophilus* on GCHA after gentle heating and adding NAD supplements. It would be useful to investigate the usefulness of these two methods to improve isolation of *H. influenzae*.

All four types of blood agar gave positive results for satellitism. Satellitism was seen better on CBA and SBA plates compared with GBA and HBA plates.

Obtaining anti-sera for serotyping of streptococci is difficult in Sri Lanka, due to high cost. The CAMP test is often used as a presumptive identification test for group B streptococci and *Listeria monocytogenes*. The CAMP test gave satisfactory results on SBA, GBA and CBA whereas HBA gave negative results. The CAMP factor secreted by Group B streptococci and sphingomyelinase secreted by *S. aureus* are the essential factors for this reaction. Sphingomyelin content of sheep, cow and goat red blood cells are high, whereas in human red blood cells it is low.[2,12] which may explain the failure of HBA. HBA, the most commonly available routine medium in Sri Lankan laboratories is therefore unsuitable for this test.

Optochin and bacitracin sensitivity tests as well as ABST results for group A and B streptococci and pneumococci were similar in all four types of blood Mueller-Hinton agar (BMHA). All standard isolates gave zone diameters within the recommended range and clinical isolates were similar in their zone size interpretation results.

The drawback of GCHA and CCHA is that they cannot be used for the ABST of *H. influenzae* due to poor growth, resulting in unreadable inhibitory zones. HTM gave the best results for ABST of *Haemophilus influenzae* in this study. In addition, accurate reading of the ZOI was difficult with goat blood supplemented MHA (Figures 5 and 6).

When comparing goat and cattle blood agar, cattle blood agar is better than goat blood agar for the CAMP test which is useful in identification of Group B streptococcus and *Listeria* because GBA gave arrow head shaped synergistic haemolysis with hazy margins in CAMP test. CBA gave typical arrow head shape with clear-cut margins.

Limitations of the study

Pig blood is another possible source which was considered for this study. However, phlebotomy of pigs was difficult due to their thick fat layer. Only about 10ml of blood could be obtained from one pig at a time which would make it difficult to obtain sufficient supplies for routine laboratory services. High volumes of blood could be obtained from slaughter houses. However, with current methods of slaughter, ensuring a sterile blood supply would not be possible. This source was therefore removed from the study.

This is only a preliminary study which was done using a small number of organisms. To confirm the results, further studies are needed with larger numbers of different organisms. Further, the different types of blood agar require testing for isolation of organisms from different clinical specimens as isolation might vary according to the site of the specimen.

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Conflicts of Interest

There are no conflicts of interest.

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