NEOMYCIN ROBERTSON'S COOKED MEAT MEDIUM (N.RCM) FOR THE SELECTIVE ISOLATION OF ANAEROBES

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ABSTRACT: As Robertson's cooked meat medium (RCM) promotes the simultaneous multiplication of facultative anaerobes along with anaerobes, it may be interfered with the isolation rate of anaerobes. In the present study RCM was made selective to overcome this difficulty by the incorporation of Neomycin. The efficacy of modified RCM was evaluated by growing stock cultures of various anaerobes and aerobes well as by inoculating clinical specimen directly and comparing the results with routine RCM. Among the 160 stock cultures of aerobes 8 of the 28 neomycin resistant aerobes grew in N.RCM but none of the 132 neomycin sensitive aerobes. Out of the 150 clinical samples tested in the study,77 anaerobes and 97 aerobes were isolated from routine RCM whereas 89 anaerobes and 8 Neomycin resistant aerobes from NRCM (i.e. NRCM inhibited 91.7% of aerobes and gave an increased yield of 13.5% anaerobes).These findings suggest that NRCM can be employed to suppress the aerobes and at the same time to enhance the isolation of anaerobes.
Key Words: RCM, Anaerobes, N.RCM

INTRODUCTION

Robertson's cooked meat medium (RCM) is suitable for the culture of all non sporing as well as spore forming anaerobes. It is used by many workers as an enrichment medium to resuscitate organisms present in small numbers on the original swab but lost to direct culture in the course of transportation. This medium is useful in detecting the production of toxins and for the demonstration of saccharolytic & proteolytic property of Clostridia. RCM is also utilized for the preservation of anaerobes (Willis, 1977) Cooked meat medium with glucose and vitamin K is recommended as a suitable medium for anaerobic isolates to be examined by Gas Liquid Chromatography.(Willis, 1977).

Successful isolation may be attained by inoculating RCM at the bedside or in the operation theatre rather than some hours later in the laboratory. The use of RCM inoculated directly will not allow quantitation , but early qualitative results of a possible significant pathogen can alert the clinician.

However due to the simultaneous multiplication of aerobes along with the anaerobes in this medium, isolation rate of anaerobes may be overlooked. This difficulty was overcome by addition of Neomycin to the medium in the present study. The efficacy of N.RCM was evaluated by growing stock cultures of aerobes & anaerobes in the medium as well as by inoculating clinical specimens and comparing the results with routine RCM.

MATERIALS AND METHODS

Neomycin RCM employed in the present study was prepared by incorporating 100µg/ml neomycin in nutrient broth. Conventional RCM was prepared as described by Willis,1997.For the convenience of preparation of RCM,meat particles were minced, cooked dried and stored in an airtight container. This study was conducted in the Department of Microbiology in our institute for duration of 1 year.

Testing stock cultures

In the first phase of study, stock cultures 160 aerobes and 75 anaerobes were grown in NRCM and RCM. Stock cultures of anaerobes had been stored in RCM at 4°C and aerobes on nutrient agar slopes at 4°C.Stock cultures of aerobes included in the study were as follows:- Escherichia coli (48) Klebsiella pneumoniae (27)Proteus mirabilis (19)Citrobacter fruendii (14)Staphylococcus aureus (25) β-hemolytic Streptococci (6)Miscellaneous bacteria(21) making a total of 160.Stock culture of 75 anaerobes tested in the first phase of study were B.fragalis group (29) Prevotella intermedia(5) Porphyromonas gingivalis (2) Fusobacterium nucleatum (7) Peptostreptococci (19) Clostridia species(8) and Miscellaneous anaerobes(5).

For each anaerobic stock culture, a loop full of coked meat broth culture was streaked on one half of a freshly prepared Supplemented Brain Heart Infusion blood agar(BHI.BA) containing yeast extract, cysteine, Vitamin K and hemin with a metronidazole disc (5µg)) to verify the purity of culture. The plates were incubated for 48 hours at 35°C in a Gas pak anaerobic jar (Hi Anaerobic –Mark II). A single colony from the supplemented BHI BA, which was sensitive to metronidazole was cultured in 5 ml thiglycollate medium, incubated overnight and at 0.01 ml quantities were inoculated to RCM and N.RCM.

Stock cultures of each aerobe were streaked on to one half of the blood agar plate, which was then incubated aerobically for 24 hours at 37oc to verify the purity. Single colony was transferred to 5 ml of nutrient broth, incubated over night and inoculated 0.01 ml quantities into RCM and NRCM. The growth of the organism was noticed visually as well as by sub culturing in the recovery medium, supplemented BHI BA for anaerobes and BA for aerobes.

Neomycin resistance was checked in the first phase of the study by disc diffusion technique employing disc containing with 30µg of neomycin by Kirby Bauer disc diffusion technique.

CLINICAL STUDY

In the second phase of the study evaluation of N.RCM was performed by inoculating clinical specimen and comparing the results with conventional RCM The samples were inoculated in both RCM and N.RCM for 24 hours and then processed aerobically and anaerobically according to the standard procedures. (Willis.A.T. 1977, Wadsworth, 1985, Koneman,1997)

RESULTS

Stock cultures of all the 75 anaerobes and 28 out of 160 aerobes were resistant to neomycin by Kirby Bauer's disc diffusion Technique. Among the 160 aerobes, all grew in routine RCM, but only 8 of them in N.RCM. All the 75 stock cultures of anaerobes grew in RCM as well as in N.RCM.

To evaluate the efficacy of N.RCM, 150 clinical samples were processed, which included female genital tract infections (28), wound infections (19), chronic suppurative otitis media (17), Respiratory tract infections (13), intra abdominal infections (20), Urinary tract infections (12), Diabetic foot ulcer (16), and periodontal infections (25). Details of the aerobic & anaerobic isolates are given in the Table 2.

TABLE 1;Details of Stock cultures used in the study.

Bacteria	Number tested	Neomycin disc diffusion		Growth in	
		Sensitive	Resistant	RCM	NRCM
Escherichia coli	48	43	5	48	1
Klebsiella pneumoniae	27	23	4	27	2
Proteus mirabilis	19	16	3	19	1
Citrobacter fruendii	14	9	5	14	1
Staphylococcus aureus	25	23	2	25	3
β-hemolytic Streptococci	6	6	0	6	
Miscellaneous bacteria	21	12	9	21	
Total	**160**	**132**	**28**	**160**	**8**
B.fragalis group	29		29	29	29
Prevotella intermedia	5		5	5	5
Porphyromonas gingivalis	2		2	2	2
Fusobacterium nucleatum	7		7	7	7
Peptostreptococci	19		19	19	19
Clostridia species	8		8	8	8
Miscellaneous anaerobes	5		5	5	5

TABLE 2: Details of Bacterial Isolates from 150 Clinical Samples:

Aerobes	Number	Anaerobes	Number
Escherichia coli	25	Bacteroides fragilis	31
Klebsiella pneumoniae	13	Prevotella	12
Proteus mirabalis	5	Porphyromonas	15
Citrobacter fruendii	3	Fusobacterium	9
Pseudomonas aeruginosa	15	Peptostreptococci	26
Staphylococus aureus	22	Clostridia	3
β-hemolytic Streptococci	8	Miscellanous anerobes	3
Miscellaneous bacteria	6		
Total	97		89

Table 3. Comparison of growth of bacteria in RCM & N.RCM

	Bacteria	Growth in	
		RCM	NRCM
Stock cultures	Aerobes (160)	160 (100%)	8
	Anaerobes (75)	75	75
Clinical samples	Aerobes (97)	97 (100%)	8 (8.3%)
	Anaerobes (89)	77 (86.5%)	89 (100%)

When RCM & N.RCM were employed in parallel for the processing of 150 clinical samples, a total of 97 aerobes and 89 anaerobes were isolated. Details of the bacterial isolates from these clinical samples are given in the Table 3. All 97 aerobes isolated from clinical samples grew in RCM, whereas only 8 of the neomycin resistant aerobes in N.RCM (8.3%). i.e.N.RCM inhibited 91.7% of aerobes.
In NRCM there was an increased isolation rate of anaerobes compared to the routine RCM (89 anaerobes from NRCM and 77 (86.5% from routine RCM))ie .an increase of 13.5% anaerobes from NRCM .Comparison of growth of bacteria in RCM and NRCM of stock cultures as well as clinical samples is described in the table 3.

DISCUSSION:

The animalcules of Leeuwenhoek were not only the first described microorganisms, they were also the first described anaerobes. However these observations were overlooked or forgotten and anaerobiosis was rediscovered 200 years later by Louis Pasteur (Willis,1977). Technical shortcomings in obtaining appropriate specimens, inadequate transport, prolonged culture procedure and morphological resemblance of facultative anaerobes are some of the limiting factors likely to reduce the frequency of isolating anaerobes. To enhance the isolation rate of anaerobes, various techniques and media were employed by different researchers.
Incorporation of animal tissues to media for the cultivation of anaerobic bacteria was attempted by workers such as Theobald smith in1890 Tarezzi in 1905.Von Hiibtler in 1899 employed an brain emulsion water and demonstrated the colour changes resulting from the growth of saccharolytic and proteolytic anaerobes. In 1916 Miss Robertson substituted bullock heart for the brain emulsion and later Holman modified it using beef muscle for studying anaerobes of war wounds (Holman 1918, Miles et.al 1985).
Antibiotics were used by number of workers for the selective isolation of anaerobes. Review of literature has showed that neomycin was considered as the selective agent of choice for anaerobes. Neomycin sulphate was introduced by Lowbury and Lilly in 1955 for the selective isolation of Clostridium perfringens type A(Lilly et.al 1955). It was later used for the isolation of most common Clostridia by Willis and Hobbs (Willis et.al,1959). Finegold et.al used a mixture of Neomycin (200µg/ml) and vancomycin (7.5 µg/ml) in fresh blood agar for the isolation of Bacteroides species. Neomycin blood agar containing 100 µg/ml neomycin had been used by Willis as a selective medium for Clostridia and anaerobic cocci.The same concentration has been employed in the present study also.
Neomycin is an amino glycoside isolated from Streptomyces species. The most important mechanism of resistance against this antimicrobial agent is the production of deactivation enzymes aminoglycoside phosphoryl transferase and acetyl transferase (Stelling.J,2000) Neomycin has the advantage that it is heat stable and may be added to the agar base medium before sterilisation. Neomycin at a concentration of 100 µg/ml as used in the present study makes the RCM medium more selective for anaerobes by inhibiting most of the aerobe (91.7%) Isolation rate of anaerobes from clinical samples was also increased by 13.5% when N.RCM was included, as the over growth of aerobes is inhibited.

CONCLUSION:

Incorporation of Neomycin at a concentration of 100µg/ml makes the RCM medium more selective for anaerobes, by inhibiting most of the aerobes. Isolation rate of anaerobes from clinical samples was increased by 13.5% in the present study by the inclusion of N-RCM, as the overgrowth of aerobes is inhibited. In short, N RCM can be employed as an excellent selective as well as transport medium for the samples processed specifically for anaerobes.

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