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Full Length Research Paper

Comparison of a modified peptone water transport medium with two commercially available transport media for the recovery of aerobes from swab specimens

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A laboratory modified peptone water medium was evaluated alongside Stuart and Amies media for their relative suitability as transport media for aerobic bacteria isolated from wound specimens obtained from Central Hospital, Benin City, Nigeria. The survival rates of isolates from the three media were assessed quantitatively for a period of seven successive days, using the pour-plate method for making viable counts. The percentage of isolation was in the following decreasing order, *Pseudomonas aeruginosa* (36.4%), *Klebsiella aerogenes* (27.3%), *Escherichia coli* (22.7%), *Staphylococcus aureus* (18.2%) and *Proteus sp* (13.6%). The survival rate of aerobic bacterial isolates was enhanced in the modified peptone water transported medium (77.3%) compared with that obtained in Stuart medium (22.7%) and Amies medium 54.5%). Statistical analysis using the students t-test at 90% confidence limit showed significant difference ($p < 0.10$) when modified peptone water was compared with both Stuart and Amies transport media.

Key words: Modified peptone water, Stuart, Amies, transport medium, comparison, swab specimens.

INTRODUCTION

Microbiological culture media are used to isolate, identify and study the characteristics of microorganisms. Thus, they are indispensable tools in routine diagnostic microbiology. For a culture medium to be effective, it must contain all the nutrients the microorganisms require for growth. The most important requirement of a culture medium is its ability to allow detectable growth from a minute inoculum within the shortest period of incubation (Baker et al., 2001).

Many clinical specimens are conveniently collected on applicator swabs and then transported to the laboratory often with a delay of several hours. Unfortunately certain bacterial pathogens are notoriously incapable of surviving prolonged exposure on swabs; thus a number of transport media, such as Cary-Blair, Stuart and Amies transport media, have been devised (Barry et al., 1972). Microorganisms, being living things, grow rapidly, reproduce and die. Transport media are not only formulated to slow down these three processes, they also disallow the overgrowth of commensals which may be present in the specimen. It is imperative that clinical specimens collected on applicator swabs reach the laboratory in a transport medium if they cannot be inoculated directly into appropriate nutrient media. Even in a transport medium, swabs cannot be held for excessively long periods but must be processed as soon as possible.

Hindiyel et al. (2001) carried out a comparative study of three transport systems (the new Starplex Star Swab 11, the new Pan-vi-pak Amies agar gel collection and transport swabs, and the BBL rt A-Cul) for the maintenance of anaerobic and fastidious aerobic organisms. The new Pan-vi-pak systems performed better than the other

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Table 1. Stuart transport medium (OXOID CM 11) – (STM).

Composition	g/liter distilled water
Sodium glycerophosphate	10.0
Sodium thioglycollate	0.5
Cysteine hydrochloride	0.5
Calcium chloride	0.1
Methylene blue	0.0001
Agar	5.0
pH 7.4 ± 0.2	

Table 2. Amies transport medium (OXOID CM 425) – (ATM).

Composition	g/liter distilled water
Charcoal pharmaceutical	10.0
Sodium chloride	3.0
Sodium hydrogen phosphate	1.05
Potassium chloride	0.2
Sodium thioglycolate	1.0
Calcium chloride	0.1
Magnesium chloride	0.1
Agar	4.0
pH 2 ± 0.2	

swabs evaluated by maintaining the viabilities of both anaerobic and fastidious aerobic organisms for 24 h for majority of the organisms evaluated. This time period should be sufficient for transport of specimens to the clinical microbiology laboratory without compromising recovery. Egwari and Rotimi (1991) carried out a comparative study in Nigeria, to evaluate the survival of *Bacteroides* spp. in Amies transport medium stored at different temperatures. In the quantitative assessment, the *Bacteroides* spp. were recovered from Amies transport medium stored at 20°C for six weeks. At 10°C no *Bacteroides* spp. were recovered after two weeks. At both storage temperatures, *Bacteroides fragilis* showed the highest survival rate. In the quantitative assessment, the test strains were recovered after four months of storage at 20°C in Amies medium.

The present study reports the results of a comparative evaluation of Stuart and Amies media, and a laboratory modified medium for the recovery of aerobes from swab specimens.

MATERIALS AND METHODS

Culture media

Stuart transport medium (OXOID CM 11) – (STM) (Table 1.), Amies transport medium (OXOID CM 425) – (ATM) (Table 2.), Modified peptone water medium – (MPWM) (Table 3), MacConkey agar (OXOID CM 7) and Nutrient agar (OXOID CM 3) were used for the study.

Table 3. Modified peptone water medium – (MPWM).

Composition	g/liter distilled water
Peptone	10.0
Sodium chloride	5.0
Calcium chloride	0.1
Magnesium chloride	0.1
Distilled water	1 L

Table 4. Distribution of bacterial isolates from patients' wound specimens.

Bacterial isolate	No of isolates	Percentage of sample isolates
P. aeruginosa	8	36.4
K. aerogenes	6	27.3
E. coli	5	22.7
S. aureus	4	18.2
Proteus sp	3	13.6

Sample size = 25; sample with growth = 22.

Collection and processing of specimens

The clinical specimens were obtained from the general and out-patient department of the Central Hospital, Benin City, Nigeria. Patients already on antibiotics were excluded from the study. The exudates from twenty five abscesses were collected from consenting patients, using sterile swab sticks moistened with sterile physiological saline in order to avoid absorption of specimens by the cotton fabrics of the swab sticks.

Specimens were aseptically collected in quadruplicates. Each of the three transport media was immediately inoculated with a swab stick containing the clinical specimen, while the fourth swab specimen was inoculated and streaked out on blood and MacConkey agar plates and finally smeared onto a clean grease free glass slide and gram stained. The plates and the transport broth media were incubated at 37°C for 18 - 24 h. Bacterial colonies growing on culture plates after incubation were identified using conventional biochemical tests (Chessbrough, 2003). Bacterial colony counts for each transport medium were made daily for seven days using the pour-plate method (Nester et al., 2004).

RESULTS AND DISCUSSION

A total of 25 swab specimens were obtained from patients with various wounds which included gluteal abscess, trauma and wound sutures, amputation, burns and diabetic ulcers. Of the total specimens, 22 (88%) showed bacterial growth while 3 (12%) yielded growth after 48 h incubation at 37°C. Table 4 shows the general distribution of bacterial isolates from wound swabs. *Pseudomonas aeruginosa* was the most prevalent organism, with a

Table 5. Summaries of mean, standard deviation (SD) standard error of mean (SEM) and survival rates of bacterial colonies.

Media	1 day	2 days	3 days	4 days	5 days	6 days	7 days
S TM mean	1.1×10^5	4.6×10^4	439×10^3	310×10^2	28×10^1	19×10^1	7.7×10^1
ATM mean	1.4×10^5	4.6×10^4	4000×10^3	380×10^2	30×10^1	22×10^1	14×10^1
MPWM mean	1.5×10^5	6.2×10^4	4500×10^3	407×10^2	31×10^1	23×10^1	32×10^1
STM SD	11329	5239	874	193	25	14	17
ATM SD	32557	13110	1472	151	17	13	19
MPWM SD	19518	17429	1463	86	8	29	25
STM SEM	2400	1117	186	41	5.3	30	3.6
ATM SEM	6900	2800	314	32	3.5	2.5	4.1
MPWM SEM	4200	3700	312	18	1.7	6.2	5.3
STM sum	2.43×10^6	1.22×10^6	9.7×10^4	6850	610	426	170
ATM sum	2.0×10^5	1.01×10^5	8.71×10^4	8420	660	470	317
MPWM sum	3.3×10^6	1.35×10^6	9.9×10^4	8950	682	498	695

Table 6. T-values for the transport media (confidence limit; 90%).

Media/Days	1	2	3	4	5	6	7
MPWM (T. cal) (against)	8.31	4.12	0.30	2.16	0.55	0.58	3.62
STM (T. val)	1.303	1.303	1.303	1.303.	1.303	1.303	1.303
MPWM (T. cal) (against)	1.23	3.44	1.13	0.73	0.53	0.59	2.68
ATM (T. val)	1.303	1.303	1.303	1.303	1.303	1.303	1.303

T. val = Table of value for T; T. cal = calculated T value.

Table 7. Percentage survival rates of bacterial isolates.

Transport medium	% Survival rate
STM	22.7
ATM	54.5
MPWM	77.3

prevalence rate of 36.4% while the least isolated organism was *Proteus* sp. Table 5 shows the summaries of mean standard deviation, standard error of mean and sum of survival recovered from STM, ATM and MPWM over a period of 7 days. Table 6 shows the t -values for the compared transport media MPWM vs. STM and MPWM vs. ATM. The percentage survival rates of bacterial colonies in Stuart, Amies and modified peptone water media were 22.7, 54.5 and 77.3% respectively (Table 7).

The isolation of pathogenic bacteria involved in clinical infections is dependent on proper method of collection, transportation and culture of clinical specimens sent for bacteriological analysis. This study compared the ability of two commercially available transport media (STM and ATM) and laboratory modified peptone water (MPWM) to maintain the viability of bacteria in clinical wound specimens.

The three media supported growth of aerobes at 24 h. There was a gradual decline in the number of bacterial colonies counted (Table 5). The laboratory modified peptone water medium showed a more superior survival rate of viable bacteria. On the seventh day of incubation, the percentage survival rates of bacteria observed with the transport media were in the following decreasing order: MPWM, 77.3%; ATM, 54. 5% and; STM, 22.7%.

This enhanced survival rate in the MPWM may have been due to the fact that peptone is a hydrolyzed product of protein which consists of a rich mixture of proteins, polypeptides and amino acids, whose break down provides the carbon requirement for the organism and therefore aid their survival (Bakers et al., 2001). The incorporated salts, that is, calcium and magnesium chlorides are known to help maintain the permeability of bacterial cells to nutrients, thus contributing to their enhanced survival (OXOID Manual, 1998). Permeability of cell to nutrient for growth is the most crucial factor for bacterial metabolism.

The sodium chloride was to provide the right tonicity for bacterial survival. It may be agreed that the absence of nutrient in a transport medium (such as in Stuart's medium) retards the growth of commensal organisms within the sample which can multiply and overgrow the less hardy pathogens. However, the absence of nutrients in Stuart's and Amies media can be detrimental to the viability of less hardy pathogens. Formulations of bacteriological transport media do not necessarily have to be complex. Cost effectiveness is also worth considering. It must be

stressed that for a good diagnostic process, apart from using a suitable transport medium, the proper collection and processing of clinical samples is an important aspect in the successful identification of specific causative agent of disease. Gram stain result for the swab specimens analyzed remained consistent as the days of incubation progressed beyond the first 24 h period. This may have been due to the fact that aerobes are known to survive relatively well on culture.

Statistical analysis of results, using the student's t-test at 90% confidence limit, is shown in Table 6. Results indicate a significant difference ($p < 0.10$), showing that the modified peptone water medium performed better than STM and ATM in supporting bacterial growth.

In this study, recovery of bacteria was studied for up to 7 days. Most routine medical laboratories obviously do not encounter such prolonged delays before processing specimens. Reference laboratories often process a large number of mailed specimens, making delays inevitable. Our modified peptone water medium could sustain bacterial growth for as long as 7 days as shown in this study. This medium is less costly than either Stuart or Amies media.

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