An Evaluation of Media Suitable for the Transportation by Air of *Streptococcus pneumoniae*.

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Introduction

•Documentation of the changing global epidemiology of *Streptococcus pneumoniae* has become increasingly important with the expansion of multiply antibiotic resistant clones in certain regions and the widespread introduction of pneumococcal vaccines in the same and other areas.
•For many developing countries the expertise and facilities to perform molecular typing is not available which necessitates the international transportation of isolates to suitably equipped laboratories.
•Transportation by air to optimise the viability of the fastidious pneumococcus has become complex because of stringent International Civil Aviation Organisation (ICAO) regulations for the transportation of infectious substances (1).
•We decided to compare various transport media to assess which would be most suitable to use for delivering large numbers of *S. pneumoniae* internationally by air in order to facilitate studies of the molecular epidemiology of *S. pneumoniae* carriage in resource-poor regions of the world.

Methods

•Seventeen isolates of *S. pneumoniae* were chosen which included two laboratory strains, three PMEN multi-resistant clones and twelve clinical isolates of various serotypes and antibiotic sensitivities (Table 1).
•Single colonies taken from blood agar plates were incubated for 24 hours in a CO_2 enriched atmosphere at 37°C were inoculated into 10ml Brain Heart Infusion (BHI) broth (Oxoid, UK) and grown at 37°C in a water bath until the midlog growth phase was reached (optical density 0.6).
•1200µl of sterile glycerol was added to each culture and 100µl of these cultures were inoculated to each of the following media: chocolate blood agar slopes (Oxoid, UK), Robertson's cooked meat broth (2ml) (Oxoid, UK), Transwab ® with Charcoal Medium for Aerobes and Anaerobes (Medical Wire & Equipment Co. Ltd., UK) and Dorset Egg Media (MAST, UK).
•100µl of BHI/glycerol cultures for each isolate were cultured on 5% horse blood agar as positive controls.
•All 17 isolates on each different media plus the controls were cultured in air at room temperature for 32 days or until no further growth was detected or the culture of *S. pneumoniae* was swamped by contaminating micro-organisms.

Results

•Table 2 illustrates the contamination rates and some physical properties of the four media tested. Contamination occurred most frequently with Robertson's Cooked Meat Broth and least often over the 32 days of culture with Transwabs®.
•Figure 1 shows the percentage viability calculated for the 17 isolates of *S. pneumoniae* on different media. Contaminated cultures were considered as non-viable as well as cultures for which there was no further growth.

Discussion

•Various media are used as transport or storage media for *S. pneumoniae*, including chocolate blood agar, Dorset egg agar[2], Skim milk-tryptone-glucose-glycerine (STGG) and Skim milk-glucose-glycerine (SGG)[3,4,5].
•STGG and SGG function best as storage media for frozen isolates while at room temperature and in liquid form maintain viability for only a short number of days[5]. Unfortunately neither liquid nor frozen media are practical for air transportation[1].
•Dorset egg agar has previously been found to be a very useful media for the cultivation of *S. pneumoniae* at room temperature with viability of 93% of isolates at 30 days incubation in a previous study[2]. It is also cheap to manufacture and is solid. Unfortunately, in many countries it is not commercially available and although relatively simple to produce in-house, it cannot currently be imported into many countries because of concerns regarding egg products and the spread of avian influenza from endemic countries. When commercially produced it is often bulky, manufactured as slopes in glass Bijou bottles which can be heavy, fragile and therefore impractical to transport in large quantities by air.
•Transwabs® have in this study shown themselves to be good at maintaining the viability of *S. pneumoniae* isolates with low contamination rates and are less fragile and weigh less. Additionally they were found to allow more efficient packaging of cultures. They are also an economically viable method, can be stored at room temperature, are individually packaged in sterile conditions, quality controlled to meet National Committee for Clinical and Laboratory Standards (NCCLS) criteria by manufacturers[6] and bypass the need for in-house media production or the need for an autoclave (production of SGG and STGG requires an autoclave for in-house media production). They also comply with WHO guidance for the transport of Infectious Substances[7].

Figure 1: Viability of *Streptococcus pneumoniae* on different media at room temperature

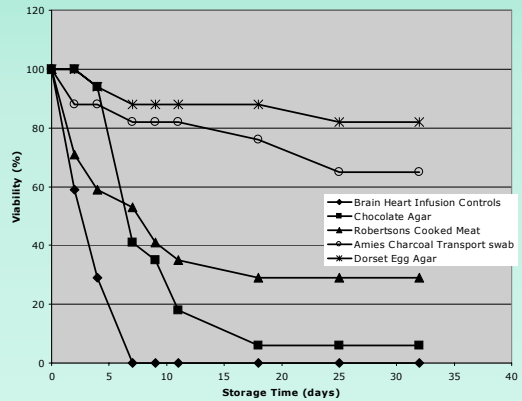

Table 1 : Characterisation of the 17 Streptococcus pneumoniae strains used. Antibiotic sensitivity was performed using Clinical and Laboratory Standards Institute criteria for disc sensitivity testing.

Isolate	Serotype	Multi Locus Sequence Type	Oxacillin sensitivity	Erythromycin sensitivity
1	6A	376	resistant	resistant
2	14	9	sensitive	resistant
3	23F	9	sensitive	sensitive
4	19A	9	sensitive	resistant
5	14	9	resistant	resistant
6	18	9	sensitive	resistant
7	14	67	sensitive	resistant
8	14	9	resistant	resistant
9	8	9	sensitive	sensitive
10	19A	75	sensitive	resistant
11	6B	156	resistant	sensitive
12	9V	156	resistant	resistant
13	14	156	sensitive	sensitive
14	14	9	sensitive	sensitive
15	4	205	sensitive	sensitive
16	1	227	sensitive	sensitive
17	19F	9	sensitive	sensitive

Table 2 : Comparison of physical properties and contamination rates of different media

	Media				
	Chocolate agar slope	Robertson's Cooked meat broth	Transwab	Dorest Egg Agar	Brain Heart Infusion Control
Contamination Rate	18%	53%	12%	23%	6%
Mean weight (standard deviation)	22.4g (0.8g)	25.7g (0.5g)	12.5g (0.2g)	36.3g (0.4g)	Not tested
Fragility	Fragile	Fragile	Non Fragile	Fragile	Non Fragile
State at Room Temperature	Solid	Liquid	Solid	Solid	Liquid

References

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