Assessment of a rapid method of Pneumococcal antigen detection in routine sputum bacteriology

THESIS

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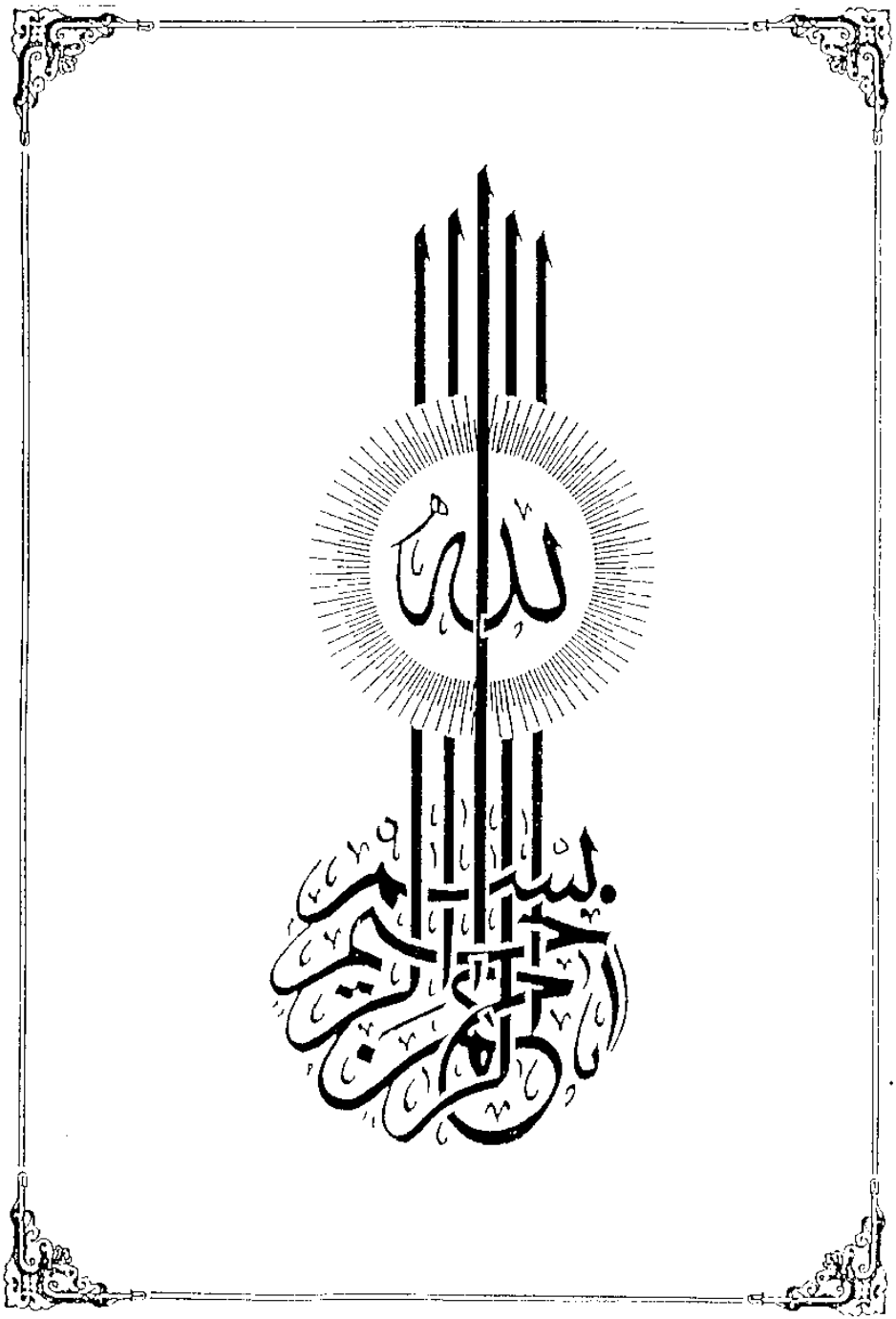

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INTRODUCTION

It is widely accepted that Streptococcus pneumoniae still accounts for most cases of pneumonia especially for fatal community acquired diseases (Boeresma et al., 1992) . It is therefore important to be able to confirm or exclude a pneumococcal aetiology .

Beside accuracy of identification it is of interest to know how quickly a diagnosis could be arrived at .

Further as antimicrobial treatment has often been started before admission or before acceptable sputum specimens can be obtained therefore it is of interest to find out how therapy influences the usefulness of different methods of detection (Kalin and Lindberg , 1985) .It was reported by Whitby et al., (1985) that rapid diagnosis of pneumococcal chest infection by examination of expectorated sputum was valuable for early institution of appropriate treatment .

AIM OF THE WORK

The aim of this work is to compare the merits of sputum culture with the direct sputum gram stained film and the antigen detection by latex agglutination test as a new aid for rapid management .

III. REVIEW of LITERATURE

A-Streptococcus Pneumoniae

1- *MORPHOLOGY*

Pneumococci are nonmotile , nonsporulating , capsulated,gram positive ovoid bacteria ,that are 0.5 micrometer to 1.0 micrometer by 1.2 micrometer to 1.8 micrometer in size . In clinical specimens , a lance like asymmetry of individual cocci may be apparent in mirror image pairs , the classic diploids, with blunted contours juxtaposed .The two cells developed in a smoothly continuous capsule .

Short chains also occur and are seen most commonly with serotype 3. Capsulation is generally demonstrable with clinical materials because only the smooth (capsulated) form is virulent (Hoeprich,1983).

2- *NUTRITIONAL REQUIREMENTS*

The nutritional requirements of Streptococcus pneumoniae are complex and not precisely known.Glucose is a primary substrate for energy production . Not only must this nutrient be available for optimal growth , but also the culture medium must be well buffered to accommodate the organic acids generated by fermentation (Hoeprich , 1983).

Pneumococci are aerobic and facultatively anaerobic, growth

is favoured by the presence of 5% to 10% CO_2 .

Optimum temperature for growth is 37C and ranges from 25-40C. Grows on ordinary media,but better on media with 5 - 10 % serum, blood or heated blood,which supply nutrients(Dugid and Ross, 1989).

Blood increases survival of pneumococci by protecting them from theH_2O_2 formed during growth (Holt , 1962).

3- CULTURAL CHARACTERS

Colonies on blood agar are tiny ,smooth transparent and low convex ,they become flattened or depressed centrally , showing the "draughtsman form " as they grow to a diameter of about 1 mm. Some strains , e.g. of type 3 , which form very large capsules tend to form larger , mucoid colonies , which may remain convex Some culture media e.g. Columbia agar , induce other strains to form such large atypical colonies .

A partial clearing of blood and a greenish discolouration (alpha - haemolysis) is produced underneath and in a narrow zone around the colonies . This resembles the alpha haemolysis formed by colonies of Sreptococcus viridans , from which the pneumococci must be distinguished .

In primary cultures on blood agar , the flattened or the draughtsman form of pneumococcal colonies with their narrow zone

of alpha haemolysis helps to identify the pneumococci .

Growth may be better anaerobically than aerobically and the heamolysis may then resemble the beta - haemolysis (Duguid and Ross,1989). An oxygen labile haemolysin (pneumolysin) is formed by some strains . All the pneumococci whether capsulated or not cause greening on blood agar, that is to the practiced eye characteristically more intense than the similar change caused by Streptococcus viridans (Hoeprich , 1983).

Pneumococci tend to die fairly quickly in cultures , in the course of a day or two , particularly in aerobic cultures in media without blood . The dead organisms tend to undergo autolysis . Thus an aerobic culture in shallow nutrient broth may be uniformly turbid after 6 - 12 hr. and become clear by autolysis within 24 hr.

On repeated subculture the capsulated , smooth colony forming pneumococci (S form) may give rise to a few non capsulated, rough colonied mutant (R form) organisms . The R-forms give granular instead of smooth growth in broth and are non virulent (Duguid and Ross, 1989) .

In fluid media ,the diplococcal form gives rise to turbidity, but non capsulate variants that form long chains produce a cotton wool like deposit (Smith and Easman , 1990).

4- SELECTIVE CULTURE

A major problem with the use of plain sheep blood agar for isolation of pneumococci from sputum is the usual presence of heavy growth of mouth flora , which makes selection of typical pneumococcal colonies among the other bacterial colonies tedious and difficult.

The addition of 5 ug of gentamicin per ml.to sheep blood agar suppresses growth of many of these interfering pharyngeal bacteria, but not pneumococci (Barber, 1966.)Therefore growth on the gentamicin plate is lighter , making selection of pneumococcal colonies easier and more accurate , since fewer colonies are competing for space and attention of the examiner John et al. (1975) had demonstrated the superior sensitivity of the gentamicin plate over the plain sheep blood agar in isolation of pneumococci from clinical specimens.

5- SENSITIVITY TO CHEMICHAL AND PHYSICAL AGENTS

Bile solubility and autolysis All capsulated pneumococci , but not all non capsulate variants are lysed by bile (Lund ,1959). The bile or other detergents , activates N - acetylmuramyl -L - alanine amidase which is responsible for the lysis of cultures of pneumococci by attaking peptidoglycan .

The other major component of the cell wall of the pneumococci is a choline containing teichoic acid which is necessary for the

action of the autolytic enzyme in vivo but not in vitro(Garcia et al.,1987) .The bile salts used are sodium deoxycholate and sodium taurocholate , the test is done either in a test tube or by adding the bile salt solution directly to the colony(Koneman et al.,1983).

Optochin susceptibility

Ethylhydrocupreine hydrochloride,a quinine derivative , has a detergent like action and causes selective lysis of pneumococci (Koneman et al.,1983).The susceptibility of S.pneumoniae to optochin is so uniform , so marked that is in contrast to viridans group and other streptococci (Morgenroth and Levy ,1911).

The optochin disc testing has become routine for confirmation of identity in most clinical laboratories (Hoeprich , 1983).

Optochin impregnated paper like discs , either 6mm or 10mm in diameter are used to perform the test. Characteristically , strains of pneumococci have zones of growth inhibition of 14 mm (6 mm disc) or 16 mm (10 mm disc) or greater (Duguid and Ross,1989). An organism showing lesser zone diameters should be tested for bile solubility to confirm that it is S.pneumoniae(Koneman et al.,1983).

However rare optochin resistant pneumococci have been described by Kontiainen (1987).

Susceptibility to antibiotics

Most strains are highly sensitive to benzyl penicillin , other penicillins , cephalosporins , erythromycin , tetracyclines clindamycin and cotrimoxazole (Duguid and Ross, 1989) .

Although these many antimicorbics are active against S. pneumoniae, the aminocyclitols are notable exceptions, for example , gentamicin , 5ug / ml final concentration in blood agar aids in isolation of pneumococci from contaminated specimens (Hoeprich , 1983). Reports of pneumococci with multiple resistance to antibiotics continue to appear . Until 1967 , pneumococci were considered to be universally susceptible to penicillin,with a minimum inhibitory concentration (MIC) of 0.01 mg / L or less . Since then there have been numerous reports of strains of pneumococci with MIC of 0.1 mg/L or more .

Howes and Mitchell (1976) had described pneumococcal strains resistant to penicillin and other antibiotics .
A major outbreak of disease caused by pneumococci with multiple resistance was reported from South Africa (Jacobs ,1978) . Penicillin resistant isolates have been described in South Africa Spain and France but have rarely been recovered in United States (Joseph and Lynch,1993).

Pneumococci can be divided into 3 categories according to their penicillin sensitivity : strains are considered to be sen-

sitive if their MIC is below 0.01 mg / L, relatively resistant if the MIC is between 0.01 and 0.9mg/L and resistant if the MIC is above 1.0 mg /L (Parker and Duerden ,1990).

Penicillin resistant pneumococci do not form penicillinase The available evidence suggests that the resistance is chromosomally dtermined (Buu-Hoi and Horodniceanu, 1980).

Alterations in the susceptibility of pneumococci to penicillin may be correlated with changes in the penicillin binding proteins present in the cell membrane (Tomasz , 1987).

Sensitivity to physical agents

Pneumococci are quite susceptible to physical agents such as heat at 56 C for 20 min. which is lethal (Hoeprich , 1983). Pneumococci die fairely quickly in laboratory cultures , which should be freezedried rather than subcultured for maintenance of them (Duguid and Ross,1989).

6- ANTIGENIC STRUCTURE OF PNEUMOCOCCI

84 different serotypes of pneumococci have been identified (Henricksen, 1979).

According to Eugene (1991) the different serotypes are based on biochemical difference in the polysaccharide capsule of the different strains of the organism and in turn on the ability of the