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Is *Streptococcus pseudopneumoniae* a neglected respiratory pathogen?

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Abstract:

Streptococcus pseudopneumoniae **is a member of Viridans streptococci, associated with chronic obstructive lung disease and lower respiratory infection. It is non-capsulated, bile insoluble and optochin susceptible in ambient air but intermediate or resistant in 5%CO_2. But, its role as a pathogen is yet to be established. The objective of the study was to detect the prevalence and to determine the clinical significance of *S.pseudopneumoniae* in sputum samples. The study period was 5 months. Good quality sputum samples (>25 neutrophils and <10 epithelial cells/LPF) of patients suspected of LRTIs, were inoculated onto chocolate agar. Alpha haemolytic colonies were identified by testing for capsule by India ink preparation, bile solubility and optochin susceptibility in ambient air and 5%CO_2. Antibiotic sensitivity testing was done by Kirby-Bauer's disc diffusion method. Out of 66 alpha haemolytic colonies, 4 were *S.pseudopneumoniae*, 17 were *S.pneumoniae* and 45 were other Viridans streptococci. Antibiotic sensitivity patterns of *S.pseudopneumoniae* and *S.pneumoniae* were analyzed. *S.pseudopneumoniae* is misinterpreted as *S.pneumoniae* unless specifically looked for. As there is scant information available, studies have to be encouraged for better determination of clinical importance of *S.pseudopneumoniae*. This will help in right diagnosis and successful treatment.**

Key words: ***Streptococcus pseudopneumoniae,*** **Lower respiratory tract infection, Respiratory pathogen, Antibiotic resistance**

Introduction:

Streptococcus pseudopneumoniae is a recently designated species, included in the *Streptococcus oralis – Streptococcus mitis* group (members of the Viridans streptococci) and is associated with chronic – obstructive lung disease and lower respiratory tract infections.[1,2] It is usually missed out in routine diagnostic practices, as it closely resembles *Streptococcus pneumoniae,* but it is different from the latter, in being non-capsulated, bile insoluble & optochin sensitive in ambient air & intermediate or resistant in 5%CO_2. Perhaps, its clinical relevance & role as a respiratory pathogen is yet to be established. Even studies regarding antimicrobial susceptibility of *S.pseudopneumoniae* are scarce. So, this study was conducted as an attempt to isolate & identify the clinical significance of the organism.

Objectives:

- To detect the prevalence & antibiogram of *S.pseudopneumoniae* in sputum samples.
- To determine the clinical significance of *S.pseudopneumoniae*

Materials & Methods:

The study period was 5 months from March 2013 to July 2013, conducted in A. J. Institute of Medical Sciences and Research Centre, Mangalore.

Samples: 302 sputum samples of patients, clinically suspected of having LRTI, were sent for routine culture and sensitivity testing.

Microscopy: Gram staining was performed and 261 good quality sputum samples (>/=25neutrophils & <10 epithelial cells/low power field) were included in the study & processed further for culture. Rest of the sputum samples (n=41) with >10 epithelial cells/low power field were excluded.[3,4]

Culture and identification: The samples were inoculated onto chocolate agar & incubated at 37^0C for 18 – 24 hrs. Gram staining of the alpha – haemolytic colonies was done and those showing gram positive cocci in pairs & chains were further identified by testing for capsule by India ink preparation (under 40X objective), bile solubility & optochin susceptibility in 5% CO_2 & ambient air.[1-4]

Bile solubility test: It was performed by taking two test tubes, each with 0.5ml suspension of the isolate prepared in phosphate – buffered saline (PBS). One tube was control and the other was the test. To the test, 0.5ml of 10% deoxycholate was added and 0.5ml of normal saline to the control tube. The tubes were incubated at 37°C for 2 hrs. A visible clearing of the suspension in the test was considered to be positive.[5]

Optochin sensitivity test: It was done by placing 5µg optochin disk at the centre of a lawn culture from overnight growth on two chocolate agar plates. They were, then, incubated for 18 to 24 hrs at 35 – 37°C in both 5%CO_2 and ambient air environments. Optochin susceptibility was defined as a zone of inhibition of >/=14mm.[6-9] *S.pseudopneumoniae* was confirmed if the isolate was sensitive to optochin in ambient air but intermediate or resistant under 5%CO_2.

Antibiotic sensitivity test: It was tested by Kirby – Bauer's disc diffusion method as per CLSI guidelines.[6,9] *S.pneumoniae* ATCC 49619 procured from HiMedia laboratories (P) Ltd, Mumbai was used as control.[10]

Results and Discussion:

Amongst 261 samples processed, 66 samples showed pure growth of alpha–haemolytic colonies (**Figure I**). Out of which 4 were *S.pseudopneumoniae*, 17 were *S.pneumoniae* and 45 were other Viridans streptococci (**Figure II**). This is similar to a study by Wessels E et al who detected 2 *S.pseudopneumoniae* isolates amongst 54 alpha – haemolytic isolates.[5]

In this study, *S.pseudopneumoniae* was isolated in 1.53% of the good quality sputum samples. The studies conducted by Mohammedi et al and Keith et al, showed its presence in 4% of the cases.[1,2] Low incidence of *S.pseudopneumoniae* was noted even in a study by Harf-Monteil et al.[3]

Analysis of antibiotic sensitivity patterns showed that the isolates were resistant to most of antibiotics which is in-accordance with a study conducted by Laurens et al.[7] 75% of the *S.pseudopneumoniae* isolates in this study, were resistant to fluoroquinolones, 50% were resistant to penicillin, cotrimoxazole, clindamycin, chloramphenicol, erythromycin and gentamicin, 25% to tetracycline and zero resistance was observed for vancomycin and linezolid (**Table I**).

Figure I: Total number of samples with alpha haemolytic isolates in the study group (out of 261)

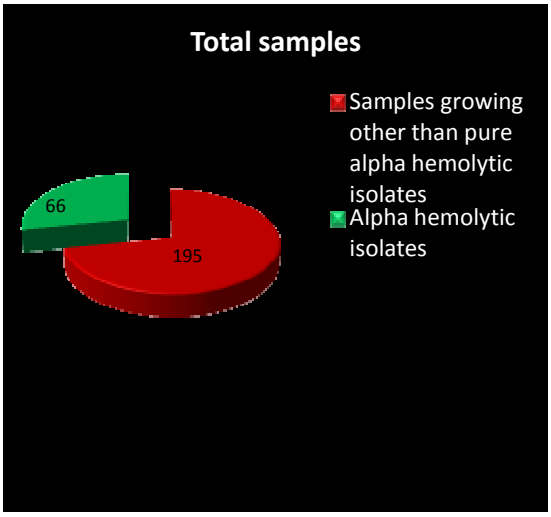

Figure II: Prevalence of *Streptococcus pseudopneumoniae* and *Streptococcus pneumoniae* among alpha–haemolytic isolates (out of 66)

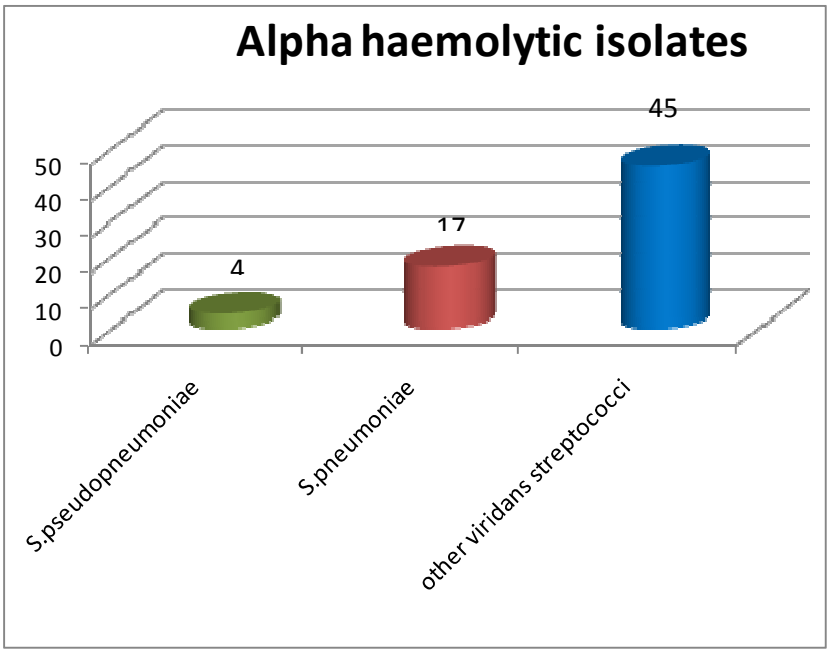

Table I: Percentage antibiotic susceptibility pattern of the isolates

Antibiotic (potency)	*S.pseudopneumoniae*	*S.pneumoniae*
Penicillin G (10units)	50	41.18
Cotrimoxazole (25 µg)	50	30.77
Clindamycin (2 µg)	50	82.3
Chloramphenicol (30 µg)	50	88.23
Erythromycin (15µg)	50	64.7
Gentamicin (10 µg)	50	72.73
Levofloxacin (5 µg)	25	53.33
Linezolid (30 µg)	100	100
Ofloxacin (5 µg)	25	35.3
Tetracycline (30 µg)	75	76.47
Vancomycin (30 µg)	100	100

In this study, it was observed that *S.pseudopneumoniae* was the only organism isolated in 4 good quality sputum samples from patients suffering from LRTIs, 2 of them had a history of COPD. This is similar to the studies by Mohammadi et al[1] and Harf-Monteil et al[4]. It was also noted that the clinical condition of those patients improved after switching over to the antibiotics which *S.pseudopneumoniae,* isolated from their samples, were sensitive to, thus suggesting that it may be the probable pathogen. Though the pathogenic role of *S.pseudopneumoniae* in LRTI can be suspected from of this study, a more definite and precise conclusion can be derived, only when the same will be done on a larger scale.

Conclusions:

In the laboratory, *S.pseudopneumoniae* is often misinterpreted as *S.pneumoniae* unless specifically looked for. This study shows that *S.pseudopneumoniae* is the only organism grown from good quality sputum samples and thus might be suspected of pathogenicity. It is also observed that this organism exhibits high percentage of resistance to most of the commonly used antibiotics which may lead to therapeutic failure. Since there is scant information available regarding this organism in India, studies like this need to be done on a larger scale and also characterization of this organism to a genetic level is necessary; for better determination of its prevalence, drug resistance and clinical importance. These will in – turn help in appropriate diagnosis and successful treatment

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