

**Prevalence of Streptococcus pneumonia in patients diagnosed with pneumonia by culture and PCR**

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**Abstract:** Pneumonia is an acute infection of the lower respiratory tissues accompanied by signs and symptoms and evidence of chest infiltration on x-ray or altered breath sounds on auscultation. Depending on their co-morbidities, residents may develop community-acquired, end-of-life or aspiration pneumonia. *Streptococcus pneumonia* (pneumococcus) is responsible for approximately two-thirds of bacterial community-acquired pneumonia cases as well as the most deaths from pneumonia, particularly in the elderly. The risk is highest in individuals with diminished immune competence, smokers and those with chronic conditions including cardiovascular or pulmonary disease, and diabetes. The aim of this study was to determine the bacterial etiology in adult patients with pneumonia infection by implementing polymerase chain reaction. A total of 126 cases (45 were male and 81 were female; age range: 1-80 years, mean age: 44) who were admitted to our hospital and clinically diagnosed as pneumonia between November 2011 - June 2012, were included in the study. Respiratory samples (sputum and blood) obtained from patients were searched by PCR method (Sinagen companies) in terms of the presence of *Streptococcus pneumonia*. The samples were simultaneously inoculated onto 5% sheep blood agar. The bacterial etiology was identified in 63 (50%) of 126 patients with pneumonia and a total of 73 pathogens were detected. The leading organism was *S. pneumonia*. It was concluded that PCR/RLBH method supplemented the determination of bacterial etiology in our study cases by *S. pneumonia*. **Materials & Methods:** After completing the questionnaire, patient tracking numbers of 126 sputum samples collected and then Gram stain, culture, and PCR were performed. **Results:** Of the 126 samples, 35.7% were male and 64.3% female mean age was 44 years. A number of 45 patients were positive whereas the results for PCR were showed positive for 63 patients. **Discussion:** The result of this study has shown the importance of streptococcus pneumonia in the society and because PCR is a fast method for detection of bacteria and can have good results in treating pneumonia before wasting time.

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### Introduction

Pneumonia is primarily due to infections caused by bacteria or viruses and less commonly by fungi and parasites. Although there are more than 100 strains of infectious agents identified, only a few are responsible for the majority of the cases. Coinfections with both viruses and bacteria may occur in up to 45% of infections in children and 15% of infections in adults<sup>[1]</sup>. A causative agent may not be isolated in approximately half of cases despite careful testing<sup>[2]</sup>. Among the agents bacteria are the most common cause of community-acquired pneumonia (CAP), with *Streptococcus pneumoniae* isolated in nearly 50% of cases<sup>[3][4]</sup>. *Streptococcus pneumoniae* is a major pathogen, causing otitis media, sinusitis, pneumonia, bacteremia, and meningitis in children worldwide. It also offers some

threat of mortality, mostly in developing countries<sup>[5][6]</sup>. Other commonly isolated bacteria include: *Haemophilus influenzae* in 20%, *Chlamydia pneumoniae* in 13%, and *Mycoplasma pneumoniae* in 3% of cases;<sup>[3]</sup> *Staphylococcus aureus*; *Moraxella catarrhalis*; *Legionella pneumophila* and Gram-negative bacilli.<sup>[2]</sup> In addition to above bacteria many viruses such as rhinoviruses, coronaviruses, influenza virus, respiratory syncytial virus (RSV), adenovirus, and *parainfluenza*<sup>[1][7]</sup> account for approximately a third<sup>[6]</sup> In adults, and in children for about 15% of pneumonia cases<sup>[8]</sup>. People following organ transplantation or those who are otherwise immunocompromised present high rates of *cytomegalovirus pneumonia*.<sup>[8][9]</sup> Those with viral infections may be secondarily infected with the bacteria *Streptococcus pneumoniae*, *Staphylococcus aureus*, or *Haemophilus influenzae*, particularly when

other health problems are present<sup>[10][8]</sup>. A number of drug-resistant versions of the above infections are becoming more common, including drug-resistant *Streptococcus pneumoniae* (DRSP) and methicillin-resistant *Staphylococcus aureus* (MRSA)<sup>[10]</sup>. The spreading of organisms is facilitated when risk factors are present<sup>[2]</sup>. Alcoholism is associated with *Streptococcus pneumoniae*, anaerobic organisms and *Mycobacterium tuberculosis*; smoking facilitates the effects of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Legionella pneumophila*. In addition, exposure to birds is associated with *Chlamydia psittaci*; farm animals with *Coxiella burnetii*; aspiration of stomach contents with anaerobic organisms; and cystic fibrosis with *Pseudomonas aeruginosa* and *S. aureus*<sup>[2]</sup>. *S. pneumoniae* is more common in the winter<sup>[2]</sup> and should be suspected in persons who aspirate a large amount anaerobic organisms<sup>[10]</sup>. A number of parasites can affect the lungs, including: *Toxoplasma gondii*, *Strongyloides stercoralis*, *Ascaris lumbricoides*, and *Plasmodium malariae*, which typically enter the body through direct contact with the skin, ingestion, or via an insect vector. However, some parasites such as *Ascaris* and *Strongyloides* genera, stimulate a strong eosinophilic reaction, which may result in eosinophilic pneumonia<sup>[11]</sup>. Among fungi, fungal pneumonia is uncommon, but occurs more commonly in individuals with weakened immune systems due to AIDS, immunosuppressive drugs, or other medical problems<sup>[2][12]</sup>. *Histoplasma capsulatum*, *blastomyces*, *Cryptococcus neoformans*, *Pneumocystis jiroveci*, and *Coccidioides immitis* are the most often causes<sup>[2]</sup>. It is worth noting that the number of cases has been increasing in the latter half of the 20th century due to increasing travel and rates of immune suppression in the population<sup>[12]</sup>.

## Materials & Methods

A total of 126 sputum were collected from patients (age 1-80 years) hospitalized with lower respiratory infections at Shahid Beheshti Hospital in Yasouj, Iran, from November 2011 to June 2012. The sputum's obtained were divided into two samples: one was used for bacterial culture and the other for PCR. Informed consent was obtained from the patients prior to the collection of samples.

### Bacterial culture

A portion of each of the sputum specimens was cultured on blood agar plates for 48 hours at 37 °C in an atmosphere enriched with 5% CO<sub>2</sub>. Isolates were identified as *S. pneumoniae* by typical colony morphology, Gram-positive staining, optochin disk sensitivity.

### PCR

Nucleic acid from each sputum specimen was extracted using a (sinagen company), according to the manufacturer's protocol. The extracted DNA suspension was kept frozen at -70 °C until further use. The PCR products were analyzed by gel electrophoresis on a 1.4% agarose gel at 120 volts for 45 minutes, stained with ethidium bromide, and visualized with ultraviolet Trans illumination. Tables 1 & 2. After various examining modes of molecular size, material composition and dual temperature stage the molecular tests was achieved. Polymerase chain reaction using the PCR primers listed has been done and the products were electrophoresed using agarose gel 1/5% (Figure1).

Table 1. Primer sequences used and their properties.

Primer	Sequences	Target	Organism	Product (bp)
S.p.F	5AAGGGCA CTTGATC	Position 106 to 127	S.pneumonia	484
S.p.R	ACTAC-C-3' 5CTACGCA TTTACCGC TCA-C3'			

Table 2: *Streptococcus pneumoniae* determination by PCR regime

Stage	Temperature	time
pre-incubation	95	'3
Denaturation	94	"30
Annealing	66	"45
Extension	72	'1
Final Extension	72	'5

## Results

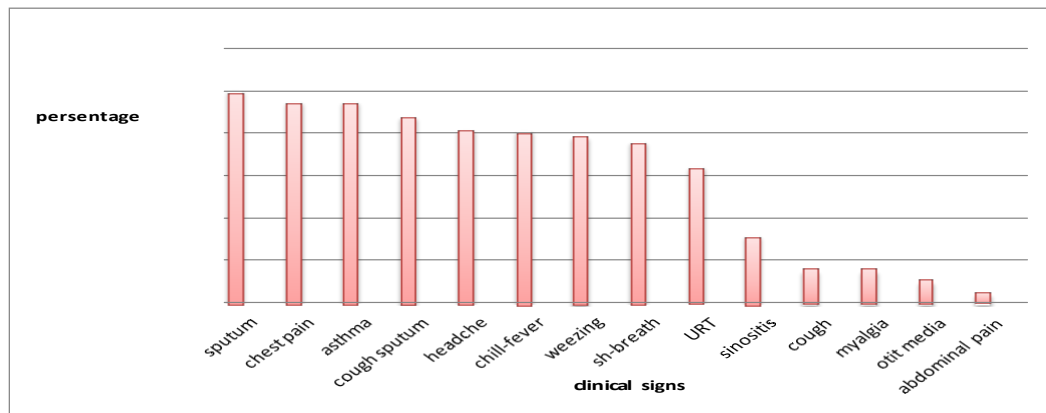
Of the 126 samples, 81 (62.3) Female 45 (37.7) were male. Their average age was 44 years. Among the 126 total cases, all of them underwent simultaneous bacterial culture and PCR analysis for pneumococcal isolation, while 49 were only analyzed by PCR. Bacterial culture yielded positive results in 35.7% (45/126) cases and PCR in 50% (63/126). Both methods revealed identical results in 79% (108/126) of tests. Among 2.5% (18/126) that showed different results, 33.8 (22/65) showed negative results with culture and positive results with PCR, and 0 (0/63) showed positive results with culture and negative results with PCR. The PCR result has shown that 63 patients (50%) had a disease with *S. pneumoniae*. Some clinical signs were evaluated in the following chart with the percentage of positive samples for *S. pneumoniae*.

Base on the chi-square test at the 5% level there is no correlation between clinical symptoms and *S. pneumoniae* infection ( $P > 0.05$ ). Regarding to the chi-square test there is not

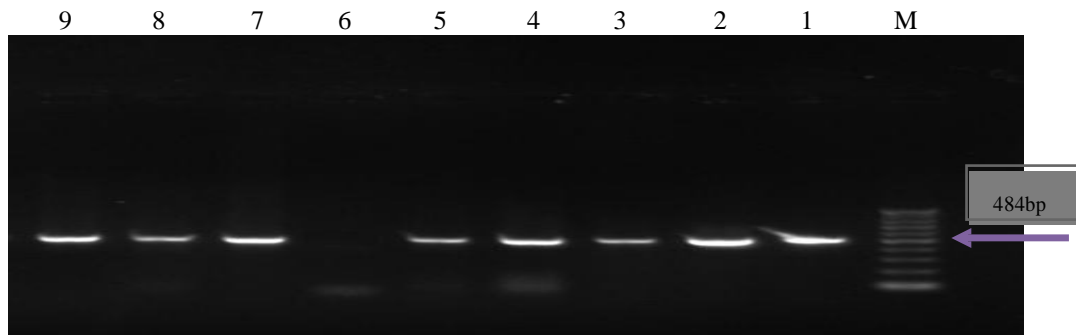
significant relationship between gender and risk for each bacterial pneumonia, ( $P > 0.05$ ). Of 124 samples 45 samples on blood agar and catalase and optochin disk sensitivity test were positive for *S. pneumoniae*. Of 63 *S. pneumoniae* positive samples by PCR, only 45 samples were culture positive. That may be because it has not met the conditions necessary for the cultivation or use of antibiotics before the biopsy taken.

culture (9.7%). In present study, 63 samples were positive by PCR isolation 45 cases had a positive culture that reflects the high sensitivity of PCR than culture for detecting *S. pneumoniae*. Lack of isolation of bacteria from media can be used of antibiotics that have influenced the growth of bacteria, and can interfere with the results of bacteriological tests. Some studies demonstrated that PCR analysis based on the amplification of *cpsA* or other pneumococcal genes has been shown to be sensitive and efficient in

**Figure 1:** The relative frequency



**Figure 2:** Electrophoresis samples on the gel for the bacteria *S. pneumoniae*. No M: Marker 100 bp, No1: positive control, No 6: negative control samples NOs 2, 3, 4, 5, 7, 8 and 9 clinical specimens positive for *Streptococcus* (Product bp 484) in infected samples



**Table 3.** distribution of patients and age rang

average age	0-20	21-40	41-60	61-80	0
Number	12	24	64	23	3
(%)	9.5	19.1	50.8	18.2	2.4

**Discussion**

In this study, PCR was used to detect pneumococci among 126 total cases. Of the total samples, 126 underwent simultaneous bacterial culture and PCR analysis, with a concordance of 79%. The detection rate of pneumococcal strains by PCR (21.4%) was higher than that by bacterial

detecting pneumococcus in clinical samples [13][14][15]. Another study revealed that 18% of middle ear fluid specimens to be positive by culture and 30% by culture and PCR [14]. It has been shown by other [13] study that sequential PCR of 127 culture-negative cerebrospinal fluid samples yielded serotypes for 51 additional cases. Our results are consistent with those of previous studies in this field. In the present study, although 2.6% of samples showed positive results in culture but negative by PCR, these are thought to be the result of operator

error and will likely be reduced by increased operator experience. It can be concluded that molecular methods, especially PCR, is suitable for detection of *S. pneumoniae* and can be easily diagnosed the bacteria from clinical samples with high speed and high sensitivity. As the test sera expensive and can only existing various regions of the province to check for pollution, but results will obtain in less than a day and can be achieved within 15 hours. To prevent the spread of disease the antibiotic resistance is very important. Due to difference in treatment of pneumonia factors, in spite of clinical symptoms, similar relative and also side effects that may have, so a lot of testing to identify the main cause of the disease and in preventing antibiotic resistance and decrease the duration of therapy and patient's satisfaction, and prevent the spread of disease in society. Therefore all these factors can explain that, despite being more expensive cost of treatment and hospitalization PCR may be more useful than the other sign total. To that end, our PCR system can be used as an accurate and rapid way of isolating and serotyping *S. pneumoniae* compared to conventional microbiology culturing. However, our study had an important limitation; 26% of total isolates were non-typeable because only 35 primer sets for pneumococcal serotyping were used in the protocol.

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