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Research Article
Risk Factors and Microbiological Studies on *Streptococcus pneumoniae* Isolated from Pneumonia Patients of Quetta Balochistan

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Abstract

Background and Objective: Pneumococcal diseases remain a major cause of morbidity and mortality worldwide. *Streptococcus Pneumoniae* causing pneumonia in India, Pakistan, Bangladesh and Afghanistan in children under 5 years of age and older adults. Therefore; the present research was design to study the different microbiological aspects of *Streptococcus pneumoniae*. **Materials and Methods:** A total of 480 sputum samples were collected from pneumonia patient at different government hospitals of Quetta. The detail of patient's gender, age, economical status and educational status were taken on performa. Sputum samples were inoculated into selective strep agar *Streptococcus pneumonia* colonies were observed on plates and confirmed through different biochemical tests and PCR. **Results:** Total 480 samples were collected in which 36.6% were *Streptococcus pneumoniae* positive and 63.3% were negative. The sex wise ratio showed that female (24.10%) were more affected with pneumoniae as compare to male (12.50%). The pneumonia infection age wise distribution was 9% in 1-10 years old patients, 16% in 10-20 years old patients and 11% in 20-30 years old patients. The status wise distribution of pneumonia infection showed that lower class (16%) was more affected as compare to middle class and higher class of Quetta. The percentage of pneumonia infection in hazara race was 14%, in Pathan 8.30%, in Punjabi 7.60% and in Baloch 6.60%. It was seen that illiterate patients were more affected with pneumonia infection (28.3%) than literate (8.3%). The *Streptococcus pneumoniae* was confirmed through gram staining, different biochemical tests, different sugar fermentation tests and PCR. Whereas confirmed by PCR showed clear band of 329 kb of ply gene. **Conclusion:** It was concluded that the rate of pneumonia infection was high in female and lower class was more affected with pneumonia.

Key words: Biochemical, sugar fermentation, illiterate patients, pneumonia infection, gram staining, *Streptococcus pneumoniae*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Streptococcus pneumoniae is Gram-positive, capsulated, non-motile, anaerobic, 1-2 µm in diameter, typically present in pairs and short chains[1]. About 93 different serotypes are isolated[2] and it is an important infectious agent causing deadly infection diseases in young and adults[3]. In upper respiratory tract *Streptococcus* is a normal flora part and it causing infection when immune system is become weak and the range of pneumococcal diseases changes in different age groups and populations[4,5].

A pneumococcal infection has a several risk factors including age, race, immunodeficiency, other infection have been reported[6]. The diagnosis is generally made based on collection of sample from sputum, blood and other tissue fluid sites. The culture method needs to incubate at 24-48 h for morphology characteristics[7]. Recently molecular methods developed real-time PCR, for diagnosis of pneumococcal disease[8]. The last three years antimicrobial resistance in *Streptococcus pneumoniae* has increased[9]. The antibiotic-resistant strains are frequently related with multiple serotypes and the rates of antibiotic resistance differs geologically[9,10]. It is the most common bacterial pathogen and reported 550,000 cases of pneumonia annually in global. Pneumonia causes many severe infections in children fewer than 5 years of age and its effect the finical system of undeveloped countries. According to WHO that high rate of death occurs of childrens due to pneumonia in India, Pakistan, Bangladesh and Afghanistan[11,12].

Pneumonia infection is recognized as a major cause of morbidity and mortality in the community. Since an etiological diagnosis of pneumonia infection with susceptibility results is almost never available to assist in the selection of the prompt therapy, necessary for pneumonia infection, the initial approach to the treatment is largely empirical and is ordinarily guided by the risk category of the patient. The knowledge of predominant microbial patterns, however, provides at least as important basis or initial decisions about empirical therapy of pneumonia infection. The issues mentioned highlight the need for continuing the research at national and local level to attain effective therapy of pneumonia infection, as well as to promote rational prescribing of antimicrobials with consequent slowing of the development of resistance to both existing and new agents. In this light, to do away with the lack of authentic data on the etiology of pneumonia infection in Quetta Balochistan, this study aimed at investigating in a prospective study the spectrum and susceptibility of *Streptococcus pneumoniae* of pneumonia infection and establishing associations with known clinical and demographic risk factors in population referred to a distinct hospital of Quetta city.

MATERIALS AND METHODS

Collection of samples: A total of 480 sputum samples were collected from pneumonia patient at different government hospitals of Quetta from 1st March to 30th December, 2017. Samples were collected aseptically in a sterile container and brought to the laboratory for further microbiological process in cool chain condition. The detail of patient's gender, age, economical status and educational status were taken on performa.

Isolation and identification: Sputum samples were inoculated into selective strep agar. Culture plates were incubated into an incubator for 24-48 h at 37°C. After incubation *Streptococcus pneumoniae* colonies were observed on plates and confirmed through different biochemical tests and PCR[13].

Molecular detection of *Streptococcus pneumoniae*: Polymerase chain reaction was used for colonies which were identifies as *Streptococcus pneumoniae.* The entire genomic DNA of *Streptococcus pneumoniae* was extracted through genomic DNA purification kit (Promega, USA). The primer following sequences' F:(TGCAGAGCGTCCTTTGGTCTAT)R :(CTCTTACTCGTGGTTTCCAACTTGA) were design to allow amplification of 329 bp fragment of ply gene. For PCR amplification 25 µL volume reaction mixture was used which contain 12 µL master mix (2x Amp Master™ Taq), 9 µL grade water, 1 µL of each primer (forward, reverse) and 2 µL template DNA. PCR cycling for reaction mixture were: initial melting 95°C for 2 min, denaturing 95°C for 30 sec annealing 59.8°C for 45 sec, extension 72°C for 1.30 min and final extension 72°C for 5 min. The final PCR product was run on 1.5%, agarose gel and observed under UV light[14].

RESULTS

A total of 480 samples were collected from pneumonia patient in which 36.6% were pneumonia positive and 63.3% were negative as shown in Fig. 1.

Gender wise distribution of pneumonia infection showed that the female (24.10%) were more affected as compared to male (12.50%) as shown in Fig. 2.

Status wise distribution of pneumonia infection showed that lower class (16%) was more affected as compare to middle class (11%) and higher class (9%) as shown in Fig. 3.

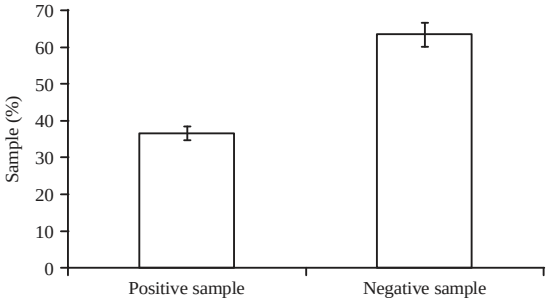

Fig. 1: Positive and negative samples of *Streptococcus pneumoniae* isolated from sputum samples of Quetta city

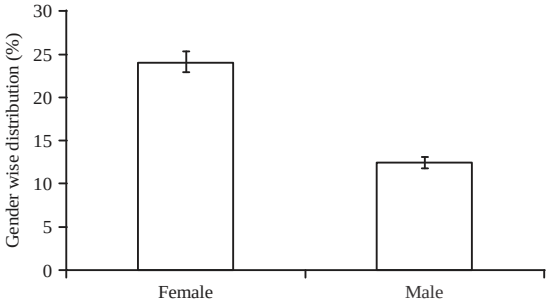

Fig. 2: Gender wise ratio of pneumonia infection in patients admitted in different Hospital of Quetta city Balochistan

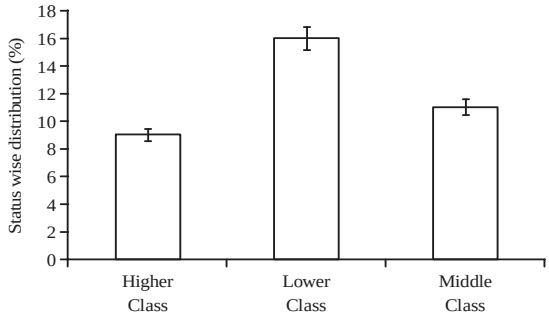

Fig. 3: Status wise ratio showed that pneumonia infection in different economical classes of Balochistan

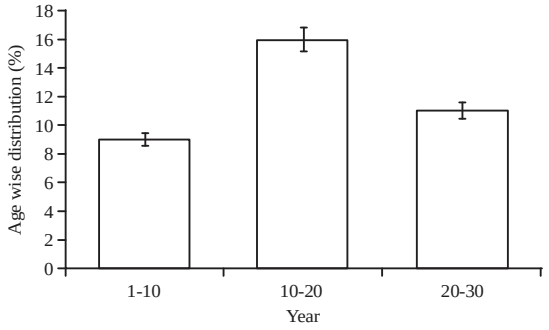

Fig. 4: Age wise rate of infection in patients admitted at different Hospital of Quetta city

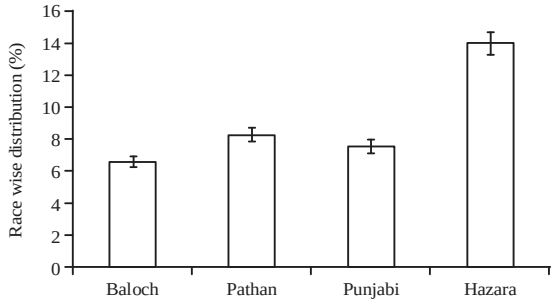

Fig. 5: Race wise ratio of pneumonia infection in patients admitted in different Hospital of Quetta city

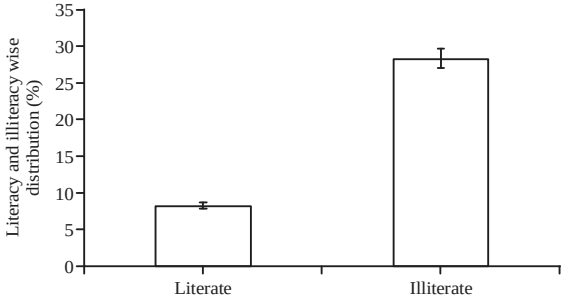

Fig. 6: Prevalence of pneumonia infections in literate and illiterate patients

The pneumonia infection age wise distribution was 9% in 1-10 years old patients, 16% in 10-20 years old patients and 11% in 20-30 years old patients as shown in Fig. 4.

The percentage of pneumonia infection in hazara race was 14%, in Pathan 8.30%, in Punjabi 7.60% and in Baloch 6.60% as shown in Fig. 5.

Literacy and illiteracy wise distribution showed that the illiterate patients (28.3%) were more affected as compared to literate (8.30%) as shown in Fig. 6.

In present study PCR based identification of *Streptococcus pneumoniae* was done through specific ply gene. All isolates produced the specific size of 329 base pair fragment of ply gene as shown in Fig. 7.

The present study was conducted to identify the causal agent causing of life-threatening infection of pneumonia. Routine methods of bacterial culture in selective media, specific colony characters, examination, staining technique, biochemical tests, with positive Methyl red and Citrate test and sugar fermentation tests with positive glucose lactose and dextrose were used for identification of *Streptoccocus pneumoniae* as shown in Table 1.

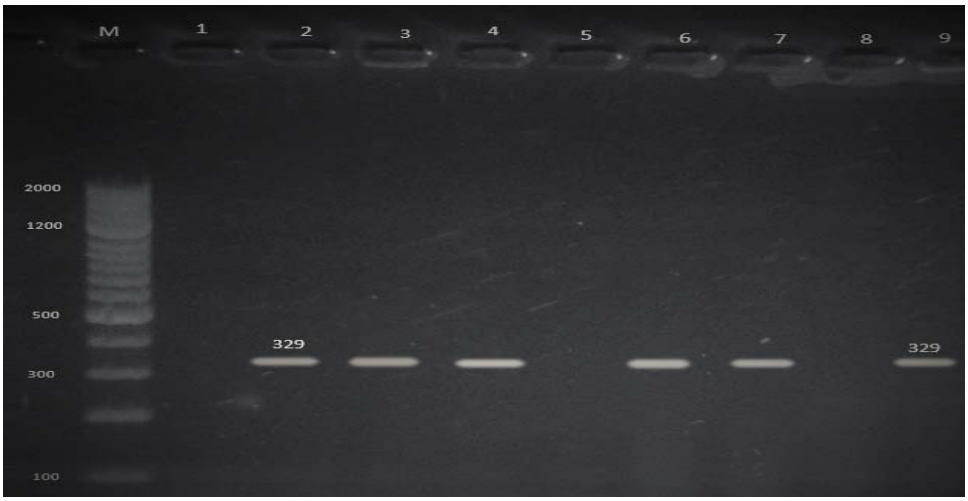

Fig. 7: Molecular identification of *Streptococcus pneumoniae* in children and elder samples by using direct ply gene specific primer. Lane M 100 bp plus DNA ladder, Lane 1 negative control, Lane 2-9 samples

Table 1: Biochemical test and sugar fermentation tests for isolation of *Streptococcus pneumoniae*

Gram staining	Crystal violet colour	
Shape	Slightly pointed cocci, 1-2 µm in diameter	
Biochemical tests	Indole	Negative
	Methyl red	Positive
	Voge- Prokaures	Negative
	Catalase test	Negative
	Urease test	Negative
	Oxidase test	Negative
	Motility test	Negative
	Citrate test	Positive
	Starch test	Variable
Sugar fermentation tests	Glucose	Positive
	Lactose	Positive
	Dextrose	Positive
	Manitol	Negative
	Sorbitol	Negative
	Maltose	Negative
Selective strep agar media	Small (0.5 mm) round and translucent colonies	

DISCUSSION

Streptococcus pneumoniae is a bacterial pathogen that affects children and adults worldwide. Pneumonia causes major morbidity and mortality in children and adult. Total 480 samples were collected out of which 36.3% showed positive growth of *Streptococcus pneumoniae* and 63.6% showed negative results. The sex wise ratio showed that female (24.10%) were more affected with pneumoniae as compare to male (12.50%). On other hand Nejad *et al.*[14], reported that a pneumonia infection is common in female as compared to male. Pneumonia infection age wise was 9% in 1-10 years old patients, 16% in 10-20 years old patients and 11% in 20-30 years old patients, similar findings was found by Ahmad *et al.*[15]. The status wise distribution of pneumonia infection showed that lower class 16% was more affected as compare to middle class and higher class, similar result was found by Bos *et al.*[16]. The percentage of pneumonia infection in hazara race was 14%, in Pathan 8.30%, in Punjabi 7.60%, and in Baloch 6.60%. Iqbal *et al.*[15], reported that the percentage of infectious pneumonia was different in different race group of Pakistan. It was seen that illiterate patients were more affected with pneumonia infection 28.3% than literate 8.3%. The percentage of literacy and illiteracy wise in pneumonia infection were found same Korona-Glowniak *et al.*[17]. The *Streptococcus pneumoniae* was confirmed through gram staining, different biochemical tests, different sugar fermentation tests similar result was found by Dubois *et al.*[18] and Iroha *et al.*[19]. PCR based identification of *Streptococcus pneumoniae* produce specific size of 329 bases ply gene, our finding was similar with the finding of Nejad *et al.*[14].

This work was largely restricted to the research setting, but in future, it will provide large-scale surveillance data regarding the organisms that cause pneumonia, in particular changes in *S. pneumoniae* carriage and disease in the context of vaccination.

CONCLUSION

In present study it was observed that the rate of pneumonia infection is high in female as compare to male while age wise all groups patient were affected with pneumonia infection. It was seen that illiterate patients were more affected with pneumonia infection than literate and the

status wise distribution of pneumonia infection showed that lower class was more affected as compare to middle class and higher class.

SIGNIFICANCE STATEMENT

The results of these and future trials may answer some of the questions surrounding the clinical application of molecular testing in microbial diagnosis and help inform clinical practices regarding their role in the diagnosis and management of pneumonia. With the current significant limitations of diagnostics in pneumonia, the advent of new technologies and the prospect of rapid testing are very exciting. For the clinician, the ability to rapidly diagnose pneumonia and to distinguish at diagnosis the specific etiological agent, whether bacterial, viral, or both, would prove invaluable in directing the appropriate use of antibiotics and is likely to transform the way we deliver care to Balochistan population in future.

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