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# Isolation and Phenotyping of *Streptococcus pneumoniae* associated with Pulmonary Tuberculosis in Patients attending Kassala Teaching Hospital, Sudan

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## Abstract

**Background**: *Streptococcus pneumonia*e (*S. pneumoniae*) is the most frequent etiologic agent of bacterial pneumonia which develops when encapsulated "virulent" *S. pneumoniae* is inhaled into the alveoli of susceptible hosts.

**Objective:** To isolate and phenotype *Streptococcus pneumoniae* associated with pulmonary tuberculosis in patients attending Kassala Teaching Hospital, Sudan.

**Materials and methods**: 150 sputum specimens were collected from patients suffering from pulmonary tuberculosis. *S.pneumoniae* was isolated by culture on chocolate agar medium and biochemical tests were employed for identification. Kirby Bour method was used to detect susceptibility of isolates to antibacterial agents.

**Results:** Out of the 150 sputum specimens 21 (14%) specimens were found culture positive for *S.pneumoniae*. The frequency rate of *S. pneumoniae* among 44 females was (7/15.9%) and among 106 males was (14/13.2%). 101of the patients investigated were residents of Kassala City and 9 (42.9%) of them were harboring *S. pneumoniae;* while 49 of the patients studied were residents outside Kassala City and 12 (57.1%) of them were harboring *S. pneumoniae.* According to age groups, most of *S. pneumoniae* isolates were in the age range 41-60 years (frequency rate 28.6%).

**Conclusion**: Some of the isolated bacteria were multi-drug resistant; and females were more susceptible to pulmonary tuberculosis than males.

**Key words**: *Streptococcus pneumoniae,* Phenotyping, Pulmonary tuberculosis.

## Introduction:

In most developing countries, the problem of inability to find out the exact cause of infection before commencement of treatment is highly important especially in remote rural areas. This could be due to lack of good health facilities with standard diagnostic equipment and qualified

laboratory staff. This has promoted over-dependence on presumptive diagnosis and treatment of infectious agents based on clinical findings. In 2014, the World Health Organization (WHO) and partners announced a post-2015 tuberculosis strategy and accompanying targets with the goal of ending the global tuberculosis epidemic[1].

*Streptococcus pneumoniae* is the most frequent etiologic agent of bacterial pneumonia[1]. Unlike other endemic countries of the world where three-quarters of global pneumonia deaths occurs, risk factors that have been identified for invasive pneumococcal diseases (IPD) due to *S. pneumoniae* in Nigeria include air pollution, overcrowding, naso-pharyngeal carriage and high level transmission of the pathogen as well as the presence of co-morbidities such as HIV/AIDS and sickle cell anemia[2].

Deaths resulting from *S. pneumoniae* infections have been attributed to its capsular polysaccharide cell wall that gives rise to over 90 serotypes, and protein factors such as autolysin (lytA) and pneumolysin (ply) that are involved in invasion, disease progression, and protection from host mediated opsonization and phagocytic killing[3].

Furthermore, the country is also plagued by an inadequate health system with sub-optimal vaccine coverage that is presently at 72% for non-pneumococcal vaccines[4], disease surveillance and health system research in the last 20 years[5].

On the other hand, the pulmonary form of tubercle bacilli after inhalation multiplies within the lower respiratory tract. There is an inflammation of lung tissues leading to the initial formation of exudative lesions; these lesions contain the *mycobacteria*, phagocytic leukocytes and an area nonspecific inflammation[6].

Pneumonia often occurs as a complication of secondary infection which typically occurs when an individual's is run down and his or her physiological state depresses the effectiveness of the immune response system. This may happen after surgery or during the course of treatment of another disease[2]. Immunodeficiency can result in activation latent tuberculosis and over 5% of AIDS patients have developed active tuberculosis[6].

Both *S.pneumoniae* and *Mycobacterium tuberculosis* produce infection that affects the lobes of the lung producing lobar pneumonia and pneumonitis respectively. When there is co-infection pneumonia and tuberculosis in patients with *S.pneumoniae* pneumonia as the underlying disease, undiagnosed underlying *S.pneumoniae pneumonia* could pose health management problems if tuberculosis a lone is diagnosed. This may be true in rural settings where poverty, illiteracy, and cultural beliefs scare people away from few available hospital services, and those who manage to go to the hospital expects quick recovery.

No published data was encountered in Sudan regarding the association of *S.pneumoniae* with pulmonary tuberculosis. Hence the object of this study was to isolate and phenotype *Streptococcus pneumoniae* associated with pulmonary tuberculosis in patients attending Kassala Teaching Hospital (Sudan).The study also determined the frequency rate of pulmonary tuberculosis according to gender and age incidence.

## Materials and methods

This was a qualitative, descriptive, cross-sectional, case finding study. The study population was patients suffering from pulmonary tuberculosis and attending Kassala Teaching Hospital (Sudan). The study was carried out during the period 2014-2016. Sampling was a randomized,

convenience type; and the sample size investigated was 150 male and female patients. Data were collected as per a structural questionnaire. Approval to run the study was taken from Al Neelain University (Khartoum, Sudan). Permission to collect the specimens was granted by the authorities of Kassala Teaching Hospital. Verbal consent was obtained from all patients studied. Positive and negative results were handed to all patients included in this study for proper treatment at Kassala Teaching Hospital (Sudan).
All 150 patients were tuberculosis positive by the Mantoux test and by the routine acid and alcohol fast Ziehl-Neelsen stain. Direct examination of sputum samples was done by the Gram stain to select samples for *S.pneumoniae* culture. Criteria for selection were presence of at least 15-25 white blood cells and less than 10 epithelial cells per high-power field[7]. The SPSS computer program was used for the statistical analysis of results.

All samples collected for processing were inoculated on blood and chocolate agar media and smears were prepared for Gram staining prior to transportation to ALYarmouk Collage Laboratory for further processing. Sputum specimens were homogenized gently with 2 ml sterile normal saline. The sputum saline mixture was refluxed in a small syringe. Homogenized samples were streak-stabbed onto freshly prepared blood agar medium for the determination of streptococcal hemolysis. The plates were incubated in a candle extinction jar. Alpha hemolytic isolates consistent with *S. pneumoniae* were picked and identified using standard bacteriological techniques including bile solubility and optochin sensitivity test.
Antibiotics used in susceptibility testing were ciprofloxacin, gentamycin, erythromycin, clindamycin, tetracycline, vancomycin, penicillin, tobramycin, and oxacillin. To obtain reproducible results, a standard number of bacteria ($1.5x10^8$ bacterial per ml) were used. It was prepared by direct touching of a colony with sterile loop and the growth was adjusted by using Mac Farland turbidity standard. The antimicrobial discs were placed on the inoculated plates using a sterile forceps. Each disc was pressed gently down to ensure even contact with medium, and plates were placed inverted in an incubator at 37° C. After overnight incubation the diameter of each zone was measured and recorded in mm, using the ruler on the under surface of the plate. The diameters of the zones were recorded to the nearest millimeter. The zone margin was taken as the area showing no obvious growth that was detected with unaided eye. The result of the zone inhibition was interpreted according to the critical diameters given in the most recent NCCS guidelines, showing the test organism as either susceptible or resistant to the antibiotic that had been tested.

## Results

As shown in Table (1), out of the 150 sputum samples, 21 samples (14%) were found positive for *S.pneumoniae*. The frequency rate of *S. pneumoniae* among 44 females was (7/15.9%) and among 106 males was (14/13.2%).

According to age groups, the highest frequency rate of *S. pneumoniae* isolates (28.6%) were in the age range 41-60 years (Table 2).

Out of the 101 tuberculosis patients 9 patients (42.9%) were resident in Kassala City and were found infected with *S. pneumoniae*. While among 49 patients living outside Kassala City,

12 tuberculous patients (57.1%) were found infected with *S. pneumoniae* (Table 3).

Table (1): Distribution of *S.pneumoniae* infection according to gender

| Gender | Frequency rate | *S.pneumoniae* isolated | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Male | Within gender | 14 (13.2%) | 92 (86.8%) | 106 (100.0%) |
| | Within isolation | 66.7% | 71.3% | 70.7% |
| Female | Within gender | 7 (15.9%) | 37 (84.1%) | 44 (100.0%) |
| | Within isolation | 33.3% | 28.7% | 29.3% |
| Total | Within gender | 21(14.0%) | 129 (86.0%) | 150 (100.0%) |
| | Within isolation | 100.0% | 100.0% | 100.0% |

Table (2): Distribution of *S.pneumoniae* infection according to age incidence

| Age (years) | *S.pneumoniae* isolated | | Total |
|---|---|---|---|
| | Positive | Negative | |
| 10-20 | 2 (5.1%) | 12 (9.3%) | 14 (9.3%) |
| 21-30 | (23.8%) | 7 (13.2%) | 22 (14.7%) |
| 31-40 | 2 (9.5%) | 37 (28.7%) | 9 (26.0%) |
| 41-50 | (28.6%) | 36 (27.9%) | 42 (28.0%) |
| Total | 1 (14.0%) | 29 (86.0%) | 50 (100.0%) |

Table (3): Distribution of *S.pneumoniae* infection according to residence

| Residence | Frequency rate | *S.pneumoniae* isolated | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Inside Kasala City | Within residence | 9 (8.9%) | 92 (91.1%) | 101(100.0%) |
| | Within isolation | 42.9% | 71.3% | 67.3% |
| Outside Kasala | Within residence | (24.5%) | 37 (75.5%) | 49 (100.0%) |
| | Within isolation | 57.1% | 28.7% | 32.7% |
| Total | Within residence | 21(14.0%) | 129 (86.0%) | 150 (100.0%) |
| | Within isolation | 100.0% | 100.0% | 100.0% |

According to occupation, the highest frequency rate of *S. pneumoniae* isolates 12(57.1%) was among entrepreneurs (Table 4).

Table (4): Distribution of *S.pneumoniae* infection according to occupation

| Occupation | *S.pneumoniae* isolated | | |
|---|---|---|---|
| | Negative | Positive | Total |
| Entrepreneurs | 50 (38.8%) | 12 (57.1%) | 62 (41.3%) |
| House-wives | 51 (39.5%) | 5 (23.8%) | 56 (37.3%) |
| Labourers | 6 (4.7%) | 2 (9.5%) | 8 (5.3%) |
| Farmers | 13 (10.1%) | 0 (0.0%) | 13 (8.7%) |
| Employers | 4 (3.1%) | 1 (4.8%) | 5 (3.3%) |
| Retirers | 5 (3.9%) | 1 (4.8%) | 6 (4.0%) |
| Total | 129 (86%) | 21 (14%) | 150 (100%) |

As shown in Table (5), the antibiotic susceptibility pattern revealed that all *S. pneumoniae* isolates (21/100%) were sensitive to vancomycin, penicillin, tobramycin, and oxacillin. Also *S. pneumoniae* isolates were found resistant to erythromycin (9/42.9%), tetracycline (6/28.6%), ciprofloxacin (5/23.8%), gentamycin (4/19.0%), and clindamycin (2/9.5%).

Table (5): Antibiotic sensitivity pattern of *S. pneumoniae* isolates

| Antibiotics | Sensitive | | Resistant | |
|---|---|---|---|---|
| | No. | Percent | No. | Percent |
| Ciprofloxacin | 16 | 76.2 | 5 | 23.8 |
| Gentamycin | 17 | 81.0 | 4 | 19.0 |
| Erythromycin | 12 | 57.1 | 9 | 42.9 |
| Clindamycin | 19 | 90.5 | 2 | 9.5 |
| Tetracycline | 15 | 71.4 | 6 | 28.6 |
| Vancomycin | 21 | 100.0 | 0 | 0 |
| Penicillin | 21 | 100.0 | 0 | 0 |
| Tobramycin | 21 | 100.0 | 0 | 0 |
| Oxacillin | 21 | 100.0 | 0 | 0 |

## Discussion:

The frequency rate of *Streptococcus pneumoniae* isolates in this study was 21(14%). This result fell within the frequency range 5-75% reported by Uneke and his colleagues [6]. Also our finding was similar to that reported (8.5%) by Lentino[8], and slightly different from the 21% frequency rate reported elsewhere[9]. This may be due to differences in geographical locations.

Furthermore, the frequency rate reported in this context was different from that found (10%) in Malaysia[10].
According to Katz and Morrens[11], the carriage rate was higher and associated with infection during the cold months and might also have contributed to the low frequency rate obtained in our study which was conducted in the dry months.
On the other hand, this study demonstrated that males were more susceptible (66.7%) to infection of *S. pneumoniae* than females (33.3%). This may be explained by the fact that males are more than the females in most developing African countries where the males have dominated the population of camps, prisons, construction sites, and factories. This finding agrees with Agwu and his co-workers[12] who reported a frequency rate of 10.4% among males and a frequency rate of 3.2% among females. It may be difficult to determine the trend of both pneumococcal and tuberculous infections in developing countries due to concomitant diseases such as HIV and sickle cell diseases.
In this study the highest frequency rate of *S. pneumoniae* (28.6%) was found in patients aged 41-50 years. This result was different from that observed by Agwu and his co-workers[12] who found a 100% frequency rate of *S. pneumoniae* infection in the age group 1-9 years. Furthermore the highest frequency rate of *S. pneumoniae* (57.1%) was found among entrepreneurs while Agwu and his co-workers[12] reported a high frequency rate of *S. pneumoniae* infection among farmers. This discrepancy may be due to the sample size investigated and the climate in which the study was conducted.

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