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

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Abstract:

Background: *Streptococcus pneumonia (S. pneumoniae) is the most frequent etiologic agent of bacterial pneumonia which develops when encapsulated "virulent" S. pneumonia 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 106males 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. pneumoniaeisolates 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 pneumonia*e 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 from April of 2014 to march 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 Gramstain 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 Farlandturbidity 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%).

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%

According to age groups, the highest frequency rate of *S. pneumoniae* isolates 7(33.3%)were in the age range 51-60 years(Table 2).

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

Age (years)	*S.pneumoniae* isolated		
	Positive	Negative	Total
10-20	2 (5.1%)	12 (9.3%)	14 (9.3%)
21-30	4 (19.0%)	18 (12%)	22 (14.7%)
31-40	2 (9.5%)	31 (21%)	33 (22.0%)
41-50	6 (28.6%)	36 (27.9%)	42 (28.0%)
51-60	7(33.3%)	32(21.3%)	39(26.0%)
Total	21 (14.0%)	129 (86.0%)	150 (100.0%)

Out of the 101 tuberculosis patients9 patients (42.9%) were resident inKassala 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 (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 City	Within residence	12 (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. pneumonia* isolates

Antibiotics	Sensitive		Resistant	
	No.	Percent	No.	Percent
Ciprofloxacin		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 pneumonia* isolates in this study was 21(14%). This result fellwithin 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. pneumonia* 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. While 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* 7(33.3%) was found in patients aged(51-60 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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