

To the Study of Role of Pathogens Isolated from Sputum Samples of Patients at Novus Path Laboratory, India and Nepal and its Associates Hospitals

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Abstract - Advances in antimicrobial treatment in the course of recent many years', pneumonia and other respiratory lot contaminations stay a significant reason for bleakness and mortality. To decide the predominant microorganisms answerable for upper and lower respiratory lot diseases in our nearby organization and the populace profiles in which these life forms happen. This was a review examination of sputum tests shipped off the Novus Path Labs, India and Nepal and its Associates Hospitals, over a time of a year from 2017-2018. Sputum tests were gram stained upon receipt in the research center and surveyed with the Bartlett score. Data analysis was done with EPI INFO version 3.5.1. The mean age of the 155 patients was 36.2+/-18.2 ranging from 4 to 92 years. The predominant organism was *Streptococcus pneumonia* [n = 69 (44.5%)]. Age < 65 was a significant risk for *Streptococcus pneumoniae* infection ($p= 0.00$, O.R=3.72, CI=1.00- 13.77, $X^2=4.34$). It was noted that there was a higher likelihood of *Streptococcus pneumoniae* infections with decreasing age ($p=0.00$ Coefficient= -9.05, Standard error=2.98, F test=9.25). *Streptococcus pneumoniae* remains the prime pathogen associated with respiratory tract infections especially for outpatients in our environment, while Extended Spectrum Beta Lactamase producing *Klebsiella pneumoniae* was seen mainly in inpatients. *Streptococcus pneumonia* and *Klebsiella pneumoniae* were the most prevalent pathogen both among in-patients and outpatients. The findings of this study will help in redesigning empiric therapy for clinicians with respect to respiratory tract infections.

Index Terms - extended spectrum beta lactamases, empiric therapy, *Klebsiella pneumonia*, Respiratory tract infection, *Streptococcus pneumonia*.

INTRODUCTION

Despite advances in antimicrobial therapy over the last few decades, pneumonia and other respiratory tract infections continue to be a major cause of morbidity and mortality. Respiratory tract infections still portend a challenge to several physicians in their practices due to the dilemma of whether to prescribe antimicrobial agents or otherwise for patients who present with features of chronic bronchitis, pharyngitis, chronic obstructive pulmonary disease and other respiratory tract infections (Candan, *et. al.*, 2017).

It is essential that sputum microbial analysis be performed appropriately in order not to generate misleading reports for the physician who may inadvertently treat the patient inappropriately (Egbe CA. *et. al.*, 2011). The incorrect analysis of sputum samples may eventually breed antimicrobial resistance because commensals and oral flora may be reported as pathogens. (Koraei, *et. al.*, 2018).

In several instances, sputum samples sent to the microbiology laboratory for processing are salivary in origin and ultimately give poor correlation between reports and clinical status of the patient. It also wastes precious health resources and experimental time. Moreover, contamination by upper airway resident flora is a limiting factor in analyzing sputum cultures. (Iskhakova, 2018).

Studies performed in the earlier period on respiratory tract infections have had a tendency to focus on pathogens such as *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Staphylococcus aureus* and viruses such as Influenza, Adenovirus and Respiratory Syncytial Virus, still new and emerging pathogens are being frequently implicated in infections (Sharma Vijeta 2015).

Respiratory tract infections are common worldwide, including in developing and underdeveloped countries, where incidences rise during the wet rainy season. The vast majority of consultations in Clinicians' practises concern symptoms of respiratory tract infections, which can be mild or severe. (Shamungum 2013).

The purpose of this study was to identify pathogens that are prevalent in our local environment in order to guide empirical antimicrobial treatment pending the results of culture and susceptibility tests. This is critical because the clinician bases several prescriptions on an educated guess. If left unchecked, this trend may promote the acquisition of multiply resistant pathogens due to high selection pressure. (Sheth *et. al.*, 2012).

No such study has been conduct in our nascent institution and the findings from our analysis will help to establish a baseline for inclination and resistance trends for future studies as well as for surveillance purposes (Sharma, H., *et. al.*, 2019). This study analyzed the sputum samples submitted in the medical microbiology laboratory with the view to identify the prevalent pathogens responsible for respiratory tract infections. The study will therefore identify the patterns of the prevalent isolates in order to guide empirical therapy and to serve as a baseline data of the common microbial agents in our sputum sample (Aktas, *et. al.*, 2012).

METHODOLOGY

Study design and location

The hospital is a 210-bed capacity facility.

Sample size

The average separation rate of bacteria from sputum is approximately 15%

Using the prevalence figure in devious the sample size: $N = Z^2Pq/d^2$

Where Z = Critical value at 95% confidence level set at 1.96

d = is the precision set at 5%

P is the proportion of the population that have positive yield from blood culture.

P is set at 15%

Sample size = $1.96 \times 1.96 \times 0.15 \times 0.85 / 0.05 \times 0.05$
= 195.9.

The sample size for the study was therefore set at 200.
Study population

These were sputum samples from patients aged 4-92 years, who were divided into in- patients and outpatients receiving care for both upper and lower respiratory tract infections (Sheldon *et. al.*, 2010).

Inclusion Criteria

Only mucoid, mucosalivary or mucopurulent sputam from patients were recruited for the study.

Exclusion Criteria

Patients with frankly salivary specimens were excluded from the study

Biochemical identification

The following biochemical tests were used to identify gram positive pathogens–catalase test, coagulase, growth on NaCl agar, susceptibility to Optochin. Gram negative bacilli were subjected to the oxidase test; those that were negative were inoculated onto Microbact 12A/E for identification. Oxidase positive pathogens were inoculated onto Microbact 12A/B for identification (Khanam *et. al.*, 2018).

Statistical analysis

We collected data on age, gender, co-morbid conditions such as hypertension and diabetes mellitus as well as sputum microscopy collected from laboratory records of patients. These were analyzed with EPI-INFO 3.6.1 software version 2008. Cross tabulation of risk factors and univariate/multivariate analysis of data was done. Analysis was done with the Chi square, 95% confidence interval and odd's ratio. The significant level was set at $p < 0.05$ (Millett ERC, *et. al.*, 2013).

RESULTS

Table 1 show that the mean age of patients was 36.2+/- 18.2 years, ranging from 4 – 92 year, with a male to female ratio of 0.7:1. Most patients, n =139 (90.3%) were below the age of 65. There were more outpatients than inpatients in the study n =91 (59.1%) versus n= 63 (40.9%). The AFB positivity rate was found to be 20.6% (15.4% versus 74.6%). ESBL's were detected in 7 of the 41 *Klebsiella pneumonia* isolates. The vast majority of respondents produced mucopurulent sputa for analysis, n = 90 (58.4%) as against 64 (41.6%) mucosalivary specimens. Hypertension was noted in 2

(1.3%) of participants and Diabetes mellitus in 6 (3.9%). Variables such as admission status and age group as well as resistance markers as risk factors with the organisms isolated. Age < 65 was a significant risk factor for acquisition of Streptococcus pneumoniae $p=0.00$, O.R = 3.72, CI = 1.00-13.77, $X^2 = 4.34$. Inpatients

Were more likely to acquire *Klebsiella pneumoniae* infections as opposed to outpatients $p = 0.00$, O.R = 3.16, CI = 1.47- 6.81, $X^2 = 14.84$. ESBL production was strongly linked to *Klebsiella pneumoniae* infection $p = 0.00$, O.R = 21.75, CI = 187.3, $X^2= 14.84$. In addition outpatients were more likely to have *Streptococcus pyogenes* recovered from their sputum $p= 0.00$, O.R = 0.10, CI = 0.03-0.40, $X^2 = 15.11$.

Table 3 shows statistical significance of age in association with organisms using linear regression. It was noted that there was a higher likelihood of Streptococcus pneumoniae infections with decreasing age p value 0.00 Coefficient = -9.05, Standard error = 2.98, F test = 9.25.

The organisms isolated from sputum specimens. The predominant organism isolated from the sputum of patients at our laboratory was *Streptococcus pneumoniae* $n = 69$ (44.5%). This was followed by *Klebsiella pneumoniae* $n = 41$ (26.5%), *Pseudomonas aeruginosa* $n = 15$ (9.7%), *Streptococcus pyogenes* $n = 11$ (7.1%) and *Staphylococcus aureus* $n = 10$ (6.5%). Others were *Escherichia coli* $n = 4$ (2.6%), *Viridans streptococci* $n = 2$ (1,3%). *Candida spp*, *Proteus spp* and *Serratia marsescens* accounted for 1 isolate each (0.6%).

DISCUSSION

The macroscopic and microscopic examinations as well as the culture of expectorated sputum till date

Table 2: Variables with statistical significance in relation to isolates

Variable	95% CI			Organism N(%) X^2	p-value	Odds	ratio
Age Category		S pneumoniae					
		Yes	No	0.04	3.72	1.00-13.77	4.34
	< 65	67(48.2)	72(51.8)				
	> 65	3(20)	12(80)				
Gender	Female	40(43.5)	52(56.5)	0.61	0.85	0.45-1.61	0.26
	Male	30(47.6)	33(52.4)				
Patient Category	Adult	54(43.5)	70(56.5)	0.42	0.72	0.33-1.60	0.65
	Pediatric	10(40)	15(60)				
Type of Infection	LRTI	54(47.8)	59(52.2)	0.28	1.49	0.72-3.07	1.62
	URTI	16(38.1)	26(61.9)				
Tuberculosis	AFB +	2(28.6)	5(71.4)	0.28	0.40	0.06	1.05
	AFB -	12(50)	12(50)				
		K pneumoniae					

remain the main- stay of the laboratory evaluation of chest infections such as pneumonia and other respiratory tract infections such as chronic bronchitis, despite on-going controversy concerning their sensitivity and specificity by different experts in the field of infectious pulmonology.

Table 1: Frequency distribution of variables associated with sputum specimens

Variable		Frequency (N)	Percentage (%)
Gender	Male	63	40.6
	Female	92	59.4
Age Strata	< 65	139	90.3
	> 65	15	9.7
Patient group	Adult	124	80
	Paediatric	31	20
Type of Infection	LRTI	113	72.9
	URTI	42	27.1
Macroscopy	Mucopurulent	90	58.4
	Mucosalivary	64	41.6
Macroscopy*	Bloody	11	7.7
	Mucoid	131	92.3
Admission status	Inpatient	63	40.9
	Outpatient	91	59.1
Tuberculosis	AFB +	24	15.4
	AFB -	131	74.6
HIV	Positive	11	61.1
	Negative	7	38.9
ESBL	Positive	7	4.5
	Negative	148	95.5
Hypertension	Yes	2	1.3
	No	153	98.7
Diabetes Mellitus	Yes	6	3.9
	No	149	96.2
Mean age (years)		36.2+/-18.2	
Age range (years)		4-92	

* 2nd Macroscopy view, LRTI – Lower respiratory tract infection, URTI – Upper respiratory tract infection

		Yes	No				
Admission Status	Inpatient	23(36.5)	40(63.5)	0.00	3.16	1.47-6.81	9.10
	Outpatient	14(15.4)	77(84.6)				
Type of Infection	LRTI	32(28.3)	81(71.7)	0.07	2.37	0.91-6.17	3.26
	URTI	6(14.3)	36(85.7)				
ESBL production	Positive	6(15.8)	32(84.2)	0.00	21.75	2.53-187.3	14.84
	Negative	1(0.9)	116(99.1)				
		S aureus					
		Yes	No				
Admission status	Inpatient	1(1.6)	62(98.4)	0.09	0.19	0.02-1.61	2.82
	Outpatient	7(7.7)					
S pyogenes		84(92.3)					
		Yes	No				
Admission status	Inpatient	3(2.7)	110(97.3)	0.00	0.10	0.03-0.40	15.11
	Outpatient	9(21.4)	33(78.6)			1.00-13.77	

*AFB – Acid fast Bacilli, ESBL – Extended spectrum beta lactamase

Table 3: Statistical significance between age and isolates using linear regression

Organism	Coefficient	Standard Error	F test	p-value
<i>Streptococcus pneumoniae</i>	-9.05	2.98	9.25	0.00
<i>Klebsiella pneumoniae</i>	3.95	3.53	1.25	0.26
<i>Streptococcus pyogenes</i>	-4.11	5.68	0.52	0.47
<i>Pseudomonas aeruginosa</i>	10.10	5.42	3.46	0.06
<i>Staphylococcus aureus</i>	-3.34	6.87	0.24	0.63
<i>Escherichia coli</i>	15.10	9.51	2.52	0.11

CONCLUSION

Streptococcus pneumoniae was the most prevalent pathogen both among in-patients and outpatients, this was followed by *Klebsiella pneumoniae* as a frequent cause of inpatient respiratory tract infections. The findings of this study have provided a baseline of prevalent pathogens will help in redesigning empiric therapy for clinicians with respect to respiratory tract infections.

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