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Original Research Article

Processing sputum specimen without biosafety cabinet; how safe are we?

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Abstract

Background: Sputum specimen is a commonly submitted specimen in routine diagnostics. Patient presenting with signs and symptoms of respiratory tract infection in many cases may have pulmonary tuberculosis as a differential diagnosis owing to its widespread prevalence and vague presentation. When sputum specimens of such patients are submitted for Acid Fast Staining (AFB), primary smear preparation is done in biosafety cabinet, but sputum specimen of the same patient when submitted for Gram's staining and culture sensitivity, in our and many other setups, is not processed in biosafety cabinet.

Objectives: This study aims to show that the sputum specimens we process without biosafety cabinet may contain tubercle bacilli.

Methods: Laboratory data of total sputum specimens submitted from 14th April 2013 to 13th April 2014 for microscopy and culture sensitivity and those submitted for AFB were collected retrospectively. Head to head comparison was done to reveal the specimen from same patient submitted simultaneously for microscopy and culture in one hand and for AFB on other.

Results: A total of 1235 sputum specimens were submitted for microscopy and culture in that period. Among them 307 were simultaneously submitted for AFB. Out of 307 sputum specimens, in 29 specimens AFB were found to be present in various loads, ranging from few number of AFB seen to a grade of 3+ in 16 of the specimens.

Conclusion: This study shows that every sputum specimen should be processed under biosafety cabinet considering the likeliness of presence of tubercle bacilli imposing threat to laboratory workers.

Keywords: Sputum specimen, Acid fast bacilli (AFB), Biosafety cabinet.

1. Introduction

Respiratory tract infections are the major cause of mortality and morbidity from infectious origin worldwide and are the foremost amongst any cause in low income countries. [1]

Clinical spectrum ranges from simplest of common cold to gravest of pneumonia and pulmonary tuberculosis. Lower respiratory tract infections have killed 3 million peoples worldwide in 2016, while Pulmonary tuberculosis had a mortality of 1.3 million during the same period, and had a crude death rate of 35/100000 population in low income countries. [1]

These infections remain one of the major clinical entities that need medical attention and extensive investigations, making sputum as a commonly submitted specimen for laboratory analysis.

Handling and processing biological specimen itself imposes various health threat to the health care personnel. Besides other threats, transmission of infectious agents from specimen to health care worker is the foremost concern. Studies have found a 3-9 times greater incidence of tuberculosis among laboratory personnel compared with the general population. [2,3]

Study has also revealed that laboratory technicians claimed for sickness benefit for pulmonary tuberculosis more than other medical auxiliaries. [3]

To minimize such hazards, processing of clinical specimens requires stringent protocol and adherence to it,

which ensures validity of the laboratory report as well as safety of the concerned. WHO and other similar national and international bodies have developed such protocol giving emphasis on biosafety aspects. [4,5]

Ideally every sputum specimen should be processed under BSL-2 facility and those likely to have tubercle bacilli under BSL-3 facility preferably in Class II, Type A2 biosafety cabinet. [4]

Whenever a patient presents to health care facility with features of lower respiratory tract infection, in many cases pulmonary tuberculosis remains one of the differentials owing to its widespread prevalence and vague presentation. When sputum specimens of such patients are submitted for Acid Fast Staining (AFB), primary smear preparation is done in biosafety cabinet, considering the probable risk of aerosol generation and transfer of infectious agent to laboratory workers. But sputum specimen of the same patient when submitted for Gram's staining and culture sensitivity in bacteriology laboratory, in our and many other setups with limited resources, is not processed in biosafety cabinet. So this study aims to show that the sputum specimens we process for microscopy and culture sensitivity without biosafety cabinet may contain tubercle bacilli imposing threat to health care workers.

2. Methodology

2.1 Study settings

This study was conducted in Department of Microbiology, BPKIHS, a tertiary care hospital serving the population of Eastern Nepal.

2.2 Study design:

A retrospective study

2.3 Data Collection

Laboratory data of total sputum specimens submitted from 14th April 2013 to 13th April 2014 for microscopy and culture sensitivity and those submitted to Tuberculosis research laboratory, BPKIHS, Dharan for AFB were collected.

2.4 Data Analysis

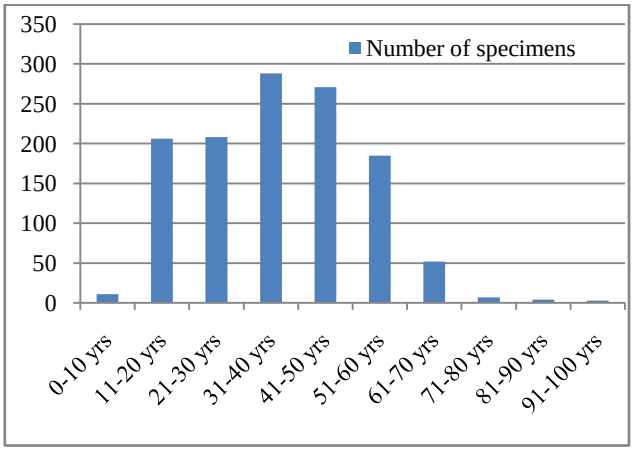
Data were entered in Microsoft excel and analyzed. Head to head comparison was done to reveal the specimen from same patient submitted simultaneously for microscopy and culture in one hand and for AFB on other.

3. Results

A total of 1235 sputum specimens were submitted to microscopy and culture in bacteriology laboratory during the period.

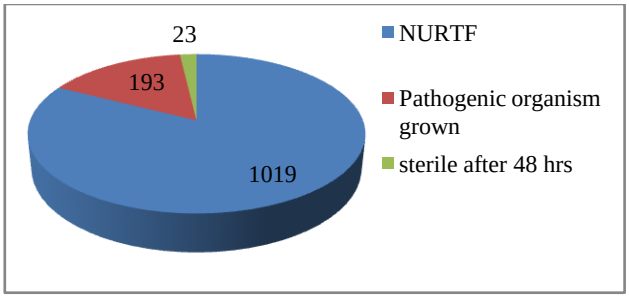
Maximum of the specimens were from age group 31-40 years, followed by 41-50 years as shown in Figure 3.1

Figure 3.1: Age wise distribution of the specimens



Almost equal numbers of specimens were submitted by male (629) and female (606), with male to female ratio of 1.038. In culture, most of the specimens yielded growth of Normal upper respiratory tract flora (NURTF) as shown in figure3.2.

Figure 3.2 Outcome of Sputum Culture



Out of 193 specimen that yielded growth of pathogenic organism, 28 yielded polymicrobial growth whilst 85.49% were monomicrobial.

The most common organism isolated was *Klebsiella pneumoniae*, followed by *Acinetobacter anitratus* as shown in Table 3.1

Table 3.1 Frequency of isolation of pathogenic organism

S.N	Organism Isolated	Frequency of Isolation
1.	*Acinetobacter anitratus*	44
2.	*Citrobacter species*	07
3.	*Enterococcus species*	09
4.	*Escherichia coli*	41
5.	*Klebsiella pneumoniae*	49
6.	*Pseudomonas aeruginosa*	38
7.	*Staphylococcus aureus*	39
	Total	227

Among these 1235 sputum specimens, 307(24.85%) specimens were simultaneously submitted for AFB in Tuberculosis Research laboratory. Out of 307 specimens, 169 were from male and 138 were from female with a male to female ratio of 1.22.

Among those 307 sputum specimens, in 29 (9.45%) specimens, AFB were found to be present in various loads, with a 3+ grading in 16 specimens as shown in table 3.2.

Table 3.2 Showing AFB grading in Z-N stained specimens

AFB grading	Number of Specimen
AFB not seen	278
1-10 AFB seen	7
1+	4
2+	2
3+	16
Total	307

Head on comparison revealed that out of 29 specimens that showed AFB in Microscopy, 1 was culture sterile after 48 hours, 22 yielded NURTF and pathogenic organisms were grown in 6 of the specimens.

4. Discussion

This study been conducted in a tertiary care hospital tends to show the relative risk of processing sputum specimen without biosafety cabinet. In various resource limited set ups, including ours, though a tertiary care center, processing of clinical specimen in bacteriology laboratory is not done under biosafety cabinet likely due to financial constraints. It has been clearly stated by WHO and other governing authorities about the health threats by likelihood of transfer of infectious agents during processing of clinical specimens specially while handling, pipetting, flaming the wire loop and other such activities that leads to aerosol generation.[6]

In this study we have found that a total of 1235 sputum specimens were submitted in bacteriology laboratory for culture sensitivity during one year period. It was seen that 193(15.63%) of the specimen had yielded growth of pathogenic organisms (Table 3.1). This finding was much less than other studies from Nepal and abroad where rate of isolation were much higher. [7-11]

It might probably be due to use of antimicrobials prior to submission of specimen and if we could have isolated fastidious organisms such as *H. influenzae* and *S. pneumoniae* that makes a larger portion of the respiratory tract isolates, our rate of isolation might have been comparable to those findings.

Among 193 specimens, most (165, 85.49%) showed monomicrobial growth, while 28 yielded polymicrobial growth. The yield of polymicrobial growth was higher than that observed by Sherchan J B *et al*, where 12.5% of growth in sputum specimen were polymicrobial and further less polymicrobial growth (8.7%) was observed in a study from Tribhuvan University Teaching Hospital.[12, 7]

As polymicrobial growth may vary from 3 to 40%, depending upon the laboratory technique used, our polymicrobial yield was within the range observed elsewhere. [13] Most of the isolates were Gram Negative bacteria (179/227) while only 21.14% were Gram positive. These findings were almost similar to a study from western Nepal where 22.39% of the lower respiratory tract isolates were Gram positive in nature, but a much lower yield (5.72%) of Gram positive bacteria was observed in a study from the capital city. [12,14]

In our study, the most frequently isolated organism was *Klebsiella Pneumoniae*(21.58%), and the most common amongst Gram Positive was *Staphylococcus aureus.* This finding is similar to a study from India but in other studies *H.influenzae* and *S. Pneumoniae* were the most frequently isolated Gram negative and Gram Positive organism respectively. [7, 14,15]

Furthermore we have found that out of 1235 specimens, almost 25% of the sputum specimens were simultaneously submitted for Acid fast Bacilli in Tuberculosis laboratory. So about 1/4th of the specimen that we processed without biosafety cabinet in bacteriology laboratory may have tubercle bacilli. Archived data from tuberculosis laboratory showed that 29 out of 307 had shown AFB in Microscopic observation. So it can be seen that every 42nd sputum specimen that we processed without biosafety cabinet harbored tubercle bacilli. It is a baseline finding depending on AFB Microscopy alone which requires 5000-10000 bacilli/ml of sputum specimen to be revealed. [16]

If those sputum specimens were cultured for *M. tuberculosis* there was likelihood of higher yield. It should also be noted that AFB were present in various loads in those specimens (Table 3.2), with 16 specimens having a grade of 3+. With an ID_{50} of <10 bacilli, the risk of laboratory workers to acquire tuberculosis while processing sputum specimen with such high load of tubercle bacilli would be much higher. [17]

Thus based on our findings we would like to conclude that as stated in literature, clinical specimens must be processed under suitable biosafety cabinets as recommended, to minimize the likelihood of transmission of infectious agents to laboratory personnel.

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