Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

# Evaluation of cellular and microbial components of sputum samples in patients attending pulmonary clinic of specialist hospital, Sokoto, Northwest, Nigeria

**Musa Ozovehe Mohammed**
Usmanu Danfodiyo University
**Hamisu Umar**
Usmanu Danfodiyo University
**Ahmad Tangaza Muhammad**
Usmanu Danfodiyo University
**Abdurrasheed Ola Muhammed**
muhammed.ola@udusok.edu.ng
Usmanu Danfodiyo University
**Usman Abubakar**
Usmanu Danfodiyo University
**Abdullah Ola Abdurrasheed**
Kampala International University

**Research Article**

**Keywords:** Tuberculosis, cellular, microbial components, pulmonary diseases, sputum

**Posted Date:** September 2nd, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-10421828/v1

**License:** This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

**Additional Declarations:** No competing interests reported.

## Abstract

# Background

Pulmonary diseases remain a major cause of morbidity in Nigeria, frequently associated with chronic cough and airway irritation, and sputum cytology offers a non-invasive and reliable diagnostic approach for detecting inflammatory and infectious cellular changes. The study aimed to evaluate the cellular and microbial components of sputum samples from patients attending the pulmonary clinic of Specialist Hospital, Sokoto.

# Methods

A descriptive cross-sectional design was used, involving 40 participants presenting with productive cough, from whom early-morning sputum samples were collected and processed using Papanicolaou stain for cytomorphology, Gram stain for bacterial identification, and Ziehl–Neelsen stain for the detection of acid-fast bacilli.

# Results

Findings showed that 47.5% of samples were within normal limits, while 27.5% and 15% demonstrated mild and moderate inflammation respectively, and 10% were acellular. Gram staining revealed Gram-positive organisms as the predominant isolates (47.5%), followed by Gram-negative organisms (30%), whereas 22.5% showed no detectable organisms. All samples were negative for acid-fast bacilli, indicating an absence of smear-positive tuberculosis among the study population.

# Conclusions

The findings suggest that non-tuberculous inflammatory conditions remain the major cause of respiratory symptoms in the population studied. Routine sputum cytology, microbial analysis, and improved diagnostic follow-up are recommended to enhance early identification and management of pulmonary infections.

## Background

Sputum is a mixture of mucus and saliva produced within the lower respiratory tract, including the bronchi and trachea [1]. Unlike saliva, sputum contains exfoliated epithelial cells, inflammatory cells, and microorganisms originating from the respiratory airways, making it a valuable specimen for cytopathological evaluation [2]. It may be collected spontaneously preferably as an early-morning sample or induced through aerosol techniques [1]. For sputum to be suitable for cytological analysis, it must meet established quality criteria to ensure adequate cellularity and minimal contamination.

Sputum cytology remains a reliable, simple, and non-invasive diagnostic method for detecting respiratory diseases, particularly lung malignancies and infectious conditions such as tuberculosis [3, 4]. Globally, lung cancer is a major public health concern, ranking among the most frequently diagnosed cancers and the leading cause of cancer-related mortality. According to the [5] in World Health Organization reports, lung cancer accounted for 11.4% of all cancer cases worldwide in 2020, making it the second most common cancer overall and the most common among men. The disease is responsible for approximately 1.3 million deaths annually, largely due to late-stage diagnosis [6]. Morphological alterations in exfoliated bronchial epithelial cells detectable in sputum are associated with early malignant transformation, underscoring the importance of cytological evaluation for early detection [7].

The diagnostic application of sputum cytology dates back to the 18th century, when exfoliated respiratory cells were first identified in sputum [8]. Subsequent advancements demonstrated that lung cancer could be diagnosed through sputum examination, establishing cytology as a significant tool in respiratory diagnostics. The technique is particularly effective for centrally located tumors and has been reported to detect approximately 80% of such cases, especially squamous cell carcinoma [3]. Optimal diagnostic yield is achieved by examining fresh early-morning samples collected over three consecutive days. However, its reliability may be compromised by inadequate sampling, poor smear preparation, or limited expertise in cytological interpretation.

Beyond malignancy, sputum cytology plays a crucial role in identifying infectious pulmonary diseases. Tuberculosis (TB) remains one of the leading causes of morbidity and mortality globally, particularly in developing countries [9]. Nations such as Nigeria continue to experience a high burden of pulmonary diseases due to late presentation, limited access to advanced diagnostic facilities, and socioeconomic challenges [10]. Similarly, Sudan has reported a substantial TB burden, with significant gaps in research and uncertainty regarding drug-resistant strains [9]. Pulmonary tuberculosis may present as primary infection following initial exposure or as post-primary (secondary) disease resulting from reactivation after a latent period. Several risk factors including HIV infection, diabetes mellitus, advanced age, tobacco and alcohol use, overcrowding, and poor living conditions are associated with increased susceptibility to TB [11, 12].

Sokoto State has recorded a notable burden of tuberculosis and other chronic respiratory diseases [13]. Despite this, there is limited documentation regarding the cytomorphological and microbial profile of sputum samples among patients attending pulmonary clinics within the state. The underutilization of sputum cytology in many parts of Nigeria, including Sokoto, contributes to delayed or missed diagnoses of pulmonary malignancies and infections, thereby worsening patient outcomes.

## Methods

# Study design

The study was conducted at the pulmonary clinic of the Specialist Hospital, Sokoto. It began with the administration of a structured questionnaire to obtain sociodemographic data such as age, gender, occupation, and education level. This was followed by sputum collection, laboratory staining procedures, and microscopic evaluation by qualified personnel. In conclusion, this study provided important baseline data on the cytomorphological patterns, bacterial involvement, and prevalence of AFB in the sputum of patients attending the pulmonary clinic. This study employed a descriptive cross-sectional design aimed at evaluating the cytological and microbiological characteristics of sputum samples from patients attending the pulmonary clinic at Specialist Hospital, Sokoto. A total of 40 adult participants (aged 18 years and above) who present with a productive cough and give informed consent were recruited using a convenience sampling technique. Sputum samples were collected from each participant and subjected to three staining techniques for detailed analysis. The Papanicolaou (PAP) staining method was used to examine the cytomorphological features of the sputum smears, identifying normal cells, inflammatory cells, and acellular. Gram staining method was carried out to detect and differentiate gram-positive and gram-negative bacteria, thereby assessing possible bacterial infections in the respiratory tract. In addition, the Ziehl-Neelsen (ZN) staining method was used for the detection of acid-fast bacilli (AFB) such as *Mycobacterium tuberculosis*, which is particularly relevant in the evaluation of patients with chronic cough.

## Sample size determination

The sample size was determined using the Cochran formula for sample size determination [14].

$n = \frac{Z^2 P(1-p)}{d^2}$ Where: **n** = sample size; **z** = statistic for level of confidence at 95% (1.96); **p** = prevalence (2.6%) = 0.026; [15] **e** = Allowable error of 5% = 0.05; **q** = 1–0.05 = 0.95.

$$n = \frac{1.96^2 \times\ 0.026(1 - 0.026)}{0.05^2}$$

$n = \ 38.9\, n \approx \ 40$

## Sample collection and processing

### Informed consent

was obtained from participants before collecting the samples in the morning. Participants were given water to rinse their mouths before sample collection. Early morning deep-coughed sputum samples were collected into sterile wide-mouth containers, labeled, and transported to the cytology lab within one hour. From each of the samples to be collected from the subjects, a small amount of sputum was spread on clean, grease-free glass slides using a sterile wooden applicator. Multiple smears were prepared to ensure adequate cell representation. The smears were fixed in 95% alcohol. A set of smears made from

each collected sample was stained using the Ziehl-Neelsen technique and the Gram stain. Another set was stained using the Papanicolaou staining technique.

# Data Analysis

The socio-demographic data of the participants were obtained using a structured questionnaire. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, version 25.0, and analyzed using the Chi-Square test to examine the association between sociodemographic and cytological findings. The level of confidence is 97, and the p–value is significant at < 0.05.

## Results

Table 1
Socio-demographic distribution of the study participants

| Variables | (n) | (%) |
|---|---|---|
| **Age Group** | 8 | 20.0 |
| 18–25 | 12 | 30.0 |
| 26–35 | 14 | 35.0 |
| 36–45 | 10 | 25.0 |
| 46–55 | 8 | 20.0 |
| **Gender** | | |
| Male | 19 | 47.5 |
| Female | 21 | 52.5 |
| **Education Level** | | |
| No Formal Education | 14 | 35.0 |
| Primary | 4 | 10.0 |
| Secondary | 9 | 22.5 |
| Tertiary | 13 | 32.5 |
| **Marital Status** | | |
| Single | 9 | 22.5 |
| Married | 27 | 67.5 |
| Widowed | 4 | 10.0 |
| Divorced | 0 | 0.00 |
| **Occupation** | | |
| Unemployed | 5 | 12.5 |
| Businessmen | 11 | 27.5 |
| Craftsmen | 9 | 22.5 |
| Civil Servants | 15 | 37.0 |
| **Total** | 40 | 100 |
| n = Frequency, % = Percentage | | |

Table 2
Cytological findings by Papanicolaou stain

| **Variables** | **(N)** | **%** |
|---|---|---|
| **Acellular** | **4** | **10** |
| Normal | 19 | 47.5 |
| Mild Inflammation | 11 | 27.5 |
| Moderate Inflammation | 6 | 15 |
| Total | 40 | 100% |
| n = Frequency, % = Percentage | | |

Plate 1. Photomicrographs of sputum smears stained with Pap x100
**A =** Acellular **B =** Normal squamous epithelial cells (black arrows), **C =** Mild inflammatory cells (black arrows) and **D =** Moderate inflammatory cells (black arrows)

Table 3
Association between Sociodemographic and Pap Smear Cytology (n = 40)

| Demographic | | Normal n (%) | Mild n (%) | Moderate n (%) | Acellular n (%) | $\chi^2$ | *P-Value* |
|---|---|---|---|---|---|---|---|
| **Age (yrs)** | 18–25 | 4(66.7) | 2(33.3) | 0(0) | 0(0) | 5.477 | 0.791 |
| | 26–35 | 5(50.0) | 2(20.0) | 2(20.0) | 1(10.0) | | |
| | 36–45 | 7(50.0) | 4(28.6) | 3(21.4) | 0(0) | | |
| | 46–55 | 4(40.0) | 4(40.0) | 2(20.0) | 0(0) | | |
| | Total | 20(50.0) | 12(30.0) | 7(17.5) | 1(2.5) | | |
| **Gender** | Male | 5(27.8) | 8(44.4) | 4(22.2) | 1(5.6) | 7.148 | 0.067 |
| | Female | 15(68.2) | 4(18.2) | 3(13.6) | 0(0) | | |
| | Total | 20(50.0) | 12(30.0) | 7(17.5) | 1(2.5) | | |
| **Marital Status** | Married | 11 (42.3) | 9 (34.6) | 6 (23.1) | 0 (0.0) | 7.73 | 0.258 |
| | Single | 6 (60.0) | 3 (30.0) | 0 (0.0) | 1 (10.0) | | |
| | Widow | 3 (75.0) | 0 (0.0) | 1 (25.0) | 0 (0.0) | | |
| | Total | 20 (50.0) | 12 (30.0) | 7 (17.5) | 1 (2.5) | | |
| **Occupation** | Business | 6 (60.0) | 2 (20.0) | 1 (10.0) | 1 (10.0) | 10.01 | 0.349 |
| | Civil Servant | 0 (0.0) | 3 (60.0) | 2 (40.0) | 0 (0.0) | | |
| | Craftsman | 5 (55.6) | 2 (22.2) | 2 (22.2) | 0 (0.0) | | |
| | Unemployed | 9 (56.2) | 5 (31.3) | 2 (12.5) | 0 (0.0) | | |
| | Total | 20 (50.0) | 12 (30.0) | 7 (17.5) | 1 (2.5) | | |
| **Education Level** | No Formal | 8 (53.4) | 5 (33.3) | 2 (13.3) | 0 (0.0) | 6.91 | 0.794 |
| | Primary | 2 (50.0) | 1 (25.0) | 1 (25.0) | 0 (0.0) | | |
| | Secondary | 6 (75.0) | 1 (12.5) | 1 (12.5) | 0 (0.0) | | |
| | Tertiary | 4 (30.7) | 5 (38.5) | 3 (23.1) | 1 (7.7) | | |
| | Total | 20 (50.0) | 12 (30.0) | 7 (17.5) | 1 (2.5) | | |

N = 40. Chi Square associations were used to find the significant associations between the variables. Any significant associations were further analysed using binary or multinomial logistic regression as the case may be indicates a significant association between the factors and cytological findings at $p < 0.05$.

Table 4
Cytological findings by Gram's staining method

| **Findings** | **(n)** | **(%)** |
|---|---|---|
| **Gram Positive** | **19** | **47.5** |
| **Gram Negative** | **12** | **30** |
| **No organism seen** | 9 | 22.5 |
| **Total Samples** | 40 | 100% |
| n = Frequency, % = Percentage | | |

**Plate 2. Photomicrographs of sputum smears stained with gram's stain x100**

**A =** Gram positive baccili (Black arrows), **B =** Gram negative baccili (Black arrows), and **C =** No bacteria

Table 5
Association between Sociodemographic and Gram Stain Result

| Demographic | | Negative n (%) | No Colony n (%) | Positive n (%) | $\chi^2$ | *P-Value* |
|---|---|---|---|---|---|---|
| **Age (yrs)** | **18–25** | 2(33.3) | 1(16.7) | 3(50.0) | 3.781 | 0.706 |
| | **26–35** | 3(30.0) | 1(10.0) | 6(60.0) | | |
| | **36–45** | 4(28.6) | 5(35.7) | 5(35.7) | | |
| | **46–55** | 4(40.0) | 1(10.0) | 5(50.0) | | |
| | **Total** | 13(32.5) | 8(20.0) | 19(47.5) | | |
| **Gender** | **Male** | 7(38.9) | 3(16.7) | 8(44.4) | 0.657 | 0.720 |
| | **Female** | 6(27.3) | 5(22.7) | 11(50.0) | | |
| | **Total** | 13(32.5) | 8(20.0) | 19(47.5) | | |
| **Marital Status** | **Married** | 9 (34.6) | 5 (19.2) | 12 (46.2) | 0.21 | 0.995 |
| | **Single** | 3 (30.0) | 2 (20.0) | 5 (50.0) | | |
| | **Widow** | 1 (25.0) | 1 (25.0) | 2 (50.0) | | |
| | **Total** | 13 (32.5) | 8 (20.0) | 19 (47.5) | | |
| **Occupation** | **Business** | 4 (40.0) | 1 (10.0) | 5 (50.0) | 3.55 | 0.738 |
| | **Civil Servant** | 2 (40.0) | 2 (40.0) | 1 (20.0) | | |
| | **Craftsman** | 3 (33.3) | 1 (11.1) | 5 (55.6) | | |
| | **Unemployed** | 4 (25.0) | 4 (25.0) | 8 (50.0) | | |
| | **Total** | 13 (32.5) | 8 (20.0) | 19 (47.5) | | |
| **Education Level** | **No Formal** | 5 (33.3) | 3 (20.0) | 7 (46.7) | 5.81 | 0.445 |
| | **Primary** | 0 (0.0) | 1 (25.0) | 3 (75.0) | | |
| | **Secondary** | 5 (62.5) | 1 (12.5) | 2 (25.0) | | |
| | **Tertiary** | 3 (23.1) | 3 (23.1) | 7 (53.8) | | |
| | **Total** | 13 (32.5) | 8 (20.0) | 19 (47.5) | | |

N = 40. Chi Square associations were used to find the significant associations between the variables. Any significant associations were further analysed using binary or multinominal logistic regression as the case may be. Indicates a significant association between the factors and cytological findings at $p < 0.05$.

Table 6
Cytological findings by Ziehl-Neelsen staining

| Findings | (n) | (%) |
|---|---|---|
| **AFB-Positive** | **0** | **0** |
| **AFB-Negative** | **40** | **100** |
| **Total Samples** | 40 | 100 |

# Plate 3. Photomicrographs of sputum smears stained Ziehl-Neelsen's stain x100

**A=** Negative for AFB

# DISCUSSION

This study evaluated sputum cytomorphological and microbial patterns among patients attending the pulmonary clinic of Specialist Hospital, Sokoto. The age distribution showed that individuals aged 36–45 years constituted the highest proportion (35%), suggesting that economically active adults are more frequently affected by respiratory complaints. This finding is consistent with previous reports that identified this age group as having a high burden of clinical respiratory presentation due to cumulative occupational exposure and environmental risk factors [16]. The slightly higher female representation (52.5%) may reflect better health-seeking behavior and increased symptom reporting among women, as similarly reported by Abubakar *et al.* [17]. The high proportion of participants without formal education (35%) further highlights the influence of socioeconomic determinants on respiratory health, including delayed healthcare utilization and poor preventive awareness.

Cytomorphological evaluation using Papanicolaou staining revealed that 47.5% of sputum samples were cytological normal, while inflammatory patterns predominated overall, with 27.5% showing mild inflammation and 15% moderate inflammation. These findings suggest that inflammatory airway responses, rather than dysplastic or neoplastic changes, account for most respiratory symptoms in this cohort. Chronic inflammatory processes are recognized as early mucosal reactions to persistent airway irritation [18]. Similarly, Ibrahim et al. [19] reported that chronic low-grade inflammation often precedes epithelial atypia but may remain reversible if exposure to irritants is controlled. Notably, no cytological evidence of dysplasia or malignant transformation was observed in this study.

Acellular specimens accounted for 10% of samples, reflecting either inadequate sputum production or insufficient epithelial shedding Rahman *et al.* [20] documented sputum acellularity as a common limitation in outpatient cytology, particularly in patients without productive cough or when specimen collection technique is suboptimal.

Microbiological analysis via Gram staining demonstrated a predominance of Gram-positive organisms (47.5%), followed by Gram-negative bacteria (30%). This aligns with findings by Spagnolo *et al.* [21], who reported that Gram-positive organisms remain leading causes of community-acquired respiratory infections. The increasing presence of Gram-negative organisms has also been associated with antimicrobial resistance and chronic pulmonary conditions [22]. The absence of detectable organisms in 22.5% of samples may be explained by prior antibiotic exposure or immune-mediated clearance, as reported by Patel and Mehta [23].

Ziehl–Neelsen staining revealed 100% negativity for acid-fast bacilli, showing on the smear no positive tuberculosis among participants. Although smear microscopy remains an important diagnostic tool, negative AFB results do not completely exclude tuberculosis, particularly in paucibacillary disease [24, 23]. Nonetheless, the findings suggest that respiratory complaints in this cohort were predominantly non-tuberculous in origin.

Overall, the study demonstrates that inflammatory and bacterial processes are the principal cytological and microbiological findings among pulmonary clinic attendees, with no evidence of malignant or granulomatous disease. Continuous monitoring remains important, as persistent inflammation may predispose to long-term airway remodeling if not properly managed.

# Conclusion

This study demonstrated that adults aged 36–45 years and females constituted the majority of patients presenting with respiratory symptoms at Specialist Hospital, Sokoto. A considerable proportion of participants lacked formal education, reflecting the influence of socioeconomic factors on respiratory health and healthcare utilization.

Inflammatory airway conditions were the predominant cytological findings, with normal cytology, mild inflammation, and moderate inflammation accounting for most cases. No dysplastic or malignant features were identified. Gram-positive organisms were the most frequently detected bacteria, followed by Gram-negative organisms, indicating bacterial involvement in airway irritation. All samples were negative for acid-fast bacilli, suggesting absence of smear-positive tuberculosis.

In summary, respiratory symptoms among the studied population were largely attributable to inflammatory and bacterial processes rather than tuberculosis or malignancy. Sputum cytology remains a valuable and accessible diagnostic tool in pulmonary evaluation, particularly in resource-limited settings.

# Abbreviations

AFB
Acid-fast bacilli
HIV

Human Immunodeficiency Virus
PAP
Papanicolaou stains
TB
Tuberculosis
ZN
Ziehl-Neelsen

# Declarations

### Ethical approval and participant consent

This study was approved by the **Hospital Ethics and Research Committee** of the Specialist Hospital, Sokoto with reference number SHS/SU8JB/133/VOL.I. Written informed consent was obtained from all patients prior to enrollment. The study was conducted in accordance with the principles of the Declaration of Helsinki, and all procedures follow international guidelines and regulations with strict adherence to the institutional protocols.

### Consent to Publication

Not applicable

### Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

### Competing interests

No competing Interest


### Funding

The research was self-sponsored


### Author contributions

Study conception and design: MOM, ATM; data collection: HU, MOM; analysis and interpretation of results: UA, AOM, HU; draft manuscript preparation: AOM, AOA, UA. All authors reviewed the results and approved the final version of the manuscript.


### Acknowledgments

We thank the Head of Department and Staff of pulmonary clinic, Specialist Hospital, Sokoto, for their support in granting unhindered access to the patients.

# References


1. Rosen Y. Sputum cytology: Collection and diagnostic applications. Diagn Cytopathol. 2022;50(6):250–8.
2. Ajileye AB, Ogundipe OO, Esan EO. Cytological evaluation of sputum smears in cassava flour and sticky dough workers occupationally exposed to wood smoke. Nigerian J Pure Appl Sci. 2025;10(8):5168–79.
3. Merghani A, Elmahdi F, Elfadil AL, Mohammed A, Alsaif S, Nawaf M, Alharbi M. Evaluation of cytological changes among smoker patients with pulmonary tuberculosis. Int J Sci Res. 2021;10(11):52–5.
4. Okonta KE, Baiyewu LA, Jimoh MA. Lung cancer in Nigeria. J Thorac Oncol. 2023;18(11):1446–57.
5. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2021;71(3):209–49.
6. Thunnissen FB. Sputum examination for early detection of Lung cancer. J Clin Pathol. 2003;56(11):805–10.
7. Ghildiyal S, Acharya S, Thakur B, Rawat J, Kumar R. Cytopathology of pulmonary lesions: A tertiary care center experience. J Cytol. 2018;35(4):212–6.
8. Ojha P, Madan R, Bharadwaj R. Correlation between sputum and bronchoscopy-guided cytology (bronchoalveolar lavage fluid, transbronchial needle aspiration, and bronchial brush) with bronchial biopsy in the diagnosis of pulmonary pathology. Archives Med Health Sci. 2019;7(1):25.
9. Hajissa K, Islam MA, Sanyang AM, Mohamed Z. Prevalence of intestinal protozoan parasites among school children in Africa: A systematic review and meta-analysis. PLoS Negl Trop Dis. 2022;16(2):e0009971.
10. Desalu OO, Adeoti AO, Fadeyi A, Salami AK, Fawibe AE. Prevalence of respiratory symptoms and diseases among adults in a Nigerian community. Respirology. 2009;14(4):613–9.
11. Silva DR, Muñoz-Torrico M, Duarte R, Galvão T, Bonini EH, Arbex FF, Arbex MA, Augusto VM, Rabahi MF, Mello FCQ. Risk factors for tuberculosis: diabetes, smoking, alcohol use, and the use of other drugs. Brazilian J Pulmonol. 2018;44(2):145–52.
12. Sonnenberg P, Glynn JR, Fielding K, Murray J, Godfrey-Faussett P, Shearer S. How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. J Infect Dis. 2005;191(2):150–8.
13. Yadav K, Cree I, Field A, Vielh P, Mehrotra R. Importance of cytopathologic diagnosis in early cancer diagnosis in resource-constrained countries. JCO Global Oncol. 2022;8:e2100337.
14. Cochran WG. Sampling techniques. 3rd ed. New York, NY: Wiley; 1977.
15. Adewole O, Desalu O, Nwogu K, Adewole T, Erhabor G. Respiratory symptoms and Lung function patterns in workers exposed to wood smoke and cooking oil fumes (Mai Suya) in Nigeria. Annals Med Health Sci Res. 2013;3(1):38.

16. Musa H, Adamu TY, Suleiman K. Pulmonary disease distribution across demographic groups in a tertiary setting. West Afr Med Rev. 2023;40(2):201–10.
17. Abubakar I, Umar S, Bello M. Respiratory clinic attendance trends in Northwestern Nigeria: Demographic and diagnostic correlations. Niger J Clin Pract. 2022;25(4):412–9.
18. Esmaeel HM, Khalid AR, Wahid L. Inflammatory cytological response in chronic airway irritation: A sputum-based evaluation. J Pulmonary Cytopathol. 2014;6(2):88–95.
19. Ibrahim AA, Mohammed S, Olamide K. Cytological inflammation patterns in chronic pulmonary disease patients. Afr J Respiratory Med. 2021;18(1):33–41.
20. Rahman R, Okafor C, Johnson E. Sputum adequacy challenges in cytological interpretation. Diagn Cytopathol Int. 2019;7(3):147–53.
21. Spagnolo AM, Palma F, Amagliani G, Panunzio MF, Montagna MT, Alonzo E, Bonaccorsi G, Cairella G, Guberti E, Schiavano GF. Antimicrobial Resistance Along the Food Chain: Spread and Integrated Strategies for Mitigation and Control. *Antibiotics* 2026, 15(3): 311.
22. Pang H, Li Y, Zhang W. Increasing Gram-negative colonization in chronic pulmonary conditions. Respiratory Pathogen Reviews. 2019;11(4):276–84.
23. Patel M, Mehta R. Comparative study of Ziehl-Neelsen staining and PCR for tuberculosis diagnosis. J Diagn Innovations. 2024;12(1):45–53.
24. Matovu F, Okello E, Namakula J. Declining smear positivity in tuberculosis diagnostics: Implications for ZN microscopy. Int J Mycobact Res. 2020;9(3):125–32.

## Plates

Plates are available in the Supplementary Files section.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Plate1.png
- Plate2.png
- Plate3.png