



**Research Paper**

**Biological Science**

**Isolation of Pathogenic Micro-organisms from Throat Swab and Sputum Samples for Identification of Laboratory Acquired Infections (LAI) of The Working Clinical Laboratory Staffs of Hooghly and Burdwan Districts, West Bengal, India: A Comparative Analysis**

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**ABSTRACT**

Background: Laboratory workers are exposed to a variety of pathogenic microorganisms that may put them at risk of infection. Current available data are limited to retrospective surveys and case reports about selected outbreaks with specific microorganisms. AIM: To assess the risks of laboratory acquired infections investigations were conducted by random sampling among 126 technical personnel in 'Hooghly' and 'Burdwan' districts of West Bengal, India. METHODOLOGY: The cross sectional study was conducted during the period of 2007 to 2012 after getting ethical clearance from 'Institutional Ethics Committee' of Vidyasagar University. The study covered 50% clinical laboratories (both government and private) situated within five kilometer radius surrounding the district/sub-divisional/rural hospitals of said two districts. From 'Hooghly' district out of twenty laboratories, twelve laboratories from government and eight laboratories from private sector were randomly selected. From 'Burdwan' district twenty two laboratories were included, where nine laboratories from government and thirteen laboratories from private sector. From each person one throat swab and one sputum sample were collected. By this way, sixty personnel (34 from government and 26 from private sector laboratories) from 'Hooghly' and sixty six personnel (27 from government and 39 from private sector laboratories) were included from 'Burdwan' district. Freshly collected throat swab and sputum were examined for the pathogenic microorganisms. Growth of insignificant microbial flora of upper respiratory tract was also noted. Results obtained were analyzed statistically. RESULTS: In 'Hooghly' district, pathogen isolated in 52.94% cases and 47.07% cases with insignificant growth out of 34 samples studied for government laboratories. For private laboratories pathogen was isolated in 46.15% cases and 53.85% cases with insignificant growth out of 26 samples. For 'Burdwan' district, among 27 samples from government laboratories, pathogen was reported in 37.04% samples and insignificant growth existed for 62.96% cases. For private laboratories out of said 39 throat swab samples 30.77% cases came with positivity for pathogens and 69.23% cases found with insignificant growth for the same setting. Acid Fast Bacillus positive result was established neither by Z.N. smear test nor by AFB culture for any of the samples from 'Hooghly' and 'Burdwan' district for both of the sectors. CONCLUSION: Lack of knowledge, attitude and practices for infection control and incidence of exposure may cause of spreading of infection among them. LAI amongst the staff may be prevented by good laboratory practice and proper containment facilities against airborne infections.

**KEYWORDS**

Laboratory Acquired Infections; Streptococcus pyogenes; Staphylococcus aureus; throat swab; Hooghly and Burdwan districts

**INTRODUCTION**

Laboratory staffs are exposed more into the infected patient

community to collect their specimen with a possibility of aerosol infection as well as into the microbiological laboratory,

where the positive cultures are handled by them. Considering the easy prone to get streptococcal infection or to sustain as the carrier state amongst the staff leading to spread the infection toward others. The majority of beta-haemolytic streptococci causing infection in man belong to group A and are given the species name of **Streptococcus pyogenes**. This pathogen causes a variety of inflammatory and suppurative conditions such as sore throat, scarlet fever, cellulitis, erysipelas, impetigo, puerperal fever, otitis media, septicaemia, wound infection and necrotizing fasciitis. It is indirectly associated with rheumatic fever, glomerulonephritis and erythema nodosum. It is also found in the throat or nasal cavity in a proportion of apparently healthy persons (Carrier) (1). **Streptococcus** is a genus of 'Coccus (spherical) Gram-positive bacteria' belonging to the phylum 'Firmicutes' (2) and the 'Lactobacillales' (lactic acid bacteria) order. Species of **Streptococcus** are classified based on their hemolytic properties (3). Alpha-hemolytic species cause oxidation of iron in hemoglobin molecules within red blood cells, giving it a greenish colour on blood agar. Beta-hemolytic species cause complete rupture of red blood cells. On blood agar, this appears as wide areas clear of blood cells surrounding bacterial colonies. Gamma-hemolytic species cause no hemolysis. Beta-hemolytic streptococci are further classified by Lancefield grouping, a serotype classification (that is, describing specific carbohydrates present on the bacterial cell wall) (4). The 20 described serotypes are named 'Lancefield groups A to V' (excluding I and J). In the medical setting, the most important groups are the alpha-hemolytic streptococci; **Streptococcus pneumoniae** and **Streptococcus viridans** group, and the beta-hemolytic streptococci of Lancefield groups A and B (also known as "group A strep" and "group B strep"). The only common primary bacterial cause of throat sore is **Streptococcus pyogenes**, which is found in about 30% of cases of pharyngitis, with or without tonsillitis. Its detection is the main purpose of the bacteriological examination of throat swabs, for it's the only common throat pathogen for which antibiotic therapy is clearly indicated. Effective therapy should cause amelioration of symptoms, e.g. within 24 to 48 hours, and prevent serious complications such as otitis media and rheumatic fever (5).

Another targeted throat pathogen of the working laboratory staff in the study was **Staphylococcus aureus**. The **S. aureus** is a gram-positive coccial bacterium, which divide completely in three perpendicular planes to form pairs, tetrads, short chains and characteristics clumps of varying size, fancifully likened to bunch of grapes. It is a member of the 'Firmicutes', and is frequently found in the human respiratory tract and on the skin. It is positive for catalase. Although **S. aureus** is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning. Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of pathogenic **S. aureus** (e.g. MRSA) is a worldwide problem in clinical medicine.

The most important human pathogen **S. aureus**, contains 'protein A' and antiphagocytic virulence factor, covalently incorporated into its cell wall. Most strain also contains 'clumping factors' (bound coagulase) on their outer surface, which bind to fibrinogen, thus causing the organisms to aggregate in plasma. Another coagulase ('free') causes clotting of plasma in a tube test, and distinguishes these species from other human staphylococcus. In medical microbiology, the term 'coagulase-positive staphylococcus' is synonymous with **S. aureus**. Human skin is densely colonized with several of coagulase negative species and to a lesser extent with **S. aureus**, whose preferred habitat is the anterior nares and which is carried there by some 40% of adults (6).

Tuberculosis has long been recognized as a serious hazard to medical laboratory workers (7). Specific considerations in relation to mycobacteria are stressed as in tuberculosis as **M. bovis** and **M. africanum** are established human pathogen and

pose a recognized risk to laboratory staff handling cultures or clinical samples containing them, especially from aerosol formation resulting air borne infection. It has been appreciated that laboratory workers are at increased risk of contacting tuberculosis, with estimated risk from 2 to 5 times that of general population (8).

## MATERIALS AND METHODS

To assess the risks LAI investigations were done for 126 technical personnel in 'Hooghly' and 'Burdwan' districts. The study has covered about 50% clinical laboratories of both government and private sector situated within 5 (five) km. radius surrounding the district/sub-divisional/rural hospitals of 'Hooghly' and 'Burdwan' districts. These laboratories were selected randomly. The data regarding government and private registered laboratories were taken from the 'Directory of Medical Institutions-West Bengal 2004' and from the records of the office of 'Chief Medical Officer of Health' (CMOH), 'Hooghly' and 'Burdwan'.

In 'Hooghly' district out of twenty [20] laboratories, twelve [12] laboratories from government sector and eight [8] laboratories from private sector were selected for LAI study. From 'Burdwan' district twenty two [22] laboratories were included for LAI study, where nine [9] laboratories from government sector and thirteen [13] laboratories from private sector were selected.

This cross-sectional study was conducted during the period of 2007 to 2012. Ethical clearance certificate was issued by 'The Principal and Chairman, Institutional Ethics Committee, Medical College & Hospital', Kolkata, Vide Memo No.- 5105/1(2) dated 08.12.2006 for conducting the research work. Ethical clearance certificate was also obtained from 'Institutional Ethics Committee, 'Vidyasagar University', Midnapore, Vide Memo No.- IEC/4/2/15 Dt. 19.6.15.

Study protocol was explained to the working technologists and assistant category staffs of the selected laboratories. Written informed consent was taken in a pre-standardized proforma from the selected subjects before collection of throat swab and sputum sample to participate in the study. From each person one throat swab and one sputum sample for Z.N. smear examination and AFB culture were collected. By this way, sixty personnel [34 personnel from government sector laboratories and 26 personnel from private sector laboratories] from 'Hooghly' district and 66 personnel [27 personnel from government sector laboratories and 39 personnel from private sector laboratories] were included from 'Burdwan' district as study subjects following random sampling of these laboratories. Specimens of throat swab and sputum were freshly collected from those laboratory personnel. Samples collected from the laboratory workers were brought to the laboratory and examined for the pathogenic microorganisms as follows -

- Throat swab for culture to obtain pathogenic isolate like **Streptococcus pyogenes** and **Staphylococcus aureus** using sheep's blood agar and nutrient agar media. Growth of insignificant microbial flora of upper respiratory tract was also noted.
- Sputum samples for presence of any AFB in ZN stained smear.
- Sputum for AFB culture using LJ media to find out any growth of mycobacteria (Tubercule bacilli).

Collected samples were studied according to standard methods for each test in our laboratory. Throat swab was collected from each individual randomly with sterile cotton swab stick and allowed to preserve it maintaining cold chain to get into laboratory. A blood agar and nutrient agar were inoculated with the swab by streaking technique. Both the media were placed into an incubator where temperature is maintained at 37°C and kept for overnight. On next day morning the plates were observe for the microbial growth. In blood agar media if small pin point translucent colonies with large surround haemolytic areas were observed, further processed with Gram

staining technique. Under the oil immersion lens examined for presence of Gram positive Cocci in long chain, beta-hemolytic *Streptococci* were suggested (9). The isolated pathogen was confirmed by negative 'Catalase test' and positive 'Bacitracin sensitivity test' for *S. pyogenes*. In any culture *S. aureus* was found with its golden colour having approximate size of 1-2 mm, creamy, either haemolytic or non-haemolytic colonies then processed for Gram staining to obtain Gram positive Cocci in cluster. Confirmation for *S. aureus* was done following the biochemical test like 'Catalase positive test' and 'Coagulase positive test' (6). Other than *S. aureus* and *S. pyogenes* the growth of other microbial flora were considered as insignificant in our study (Plate 1).

For AFB staining of smear, sputum provided by the laboratory staff collected in sterile wide mouth screw cap plastic container, which was brought to the laboratory using flame heated sterile nichrome loop a uniform smear (2 cm X 1 cm) with the sputum was prepared onto a new clean and grease free microscopic glass slide. It was fixed by flame heat and stained by ZN technique and examined under oil immersion lens of microscope to obtain any AFB sputum positive result (7) (Plate 2).

For AFB culture standard 'Petrof's method' for concentration was followed and the centrifuged deposit of that treated sputum was inoculated onto egg based LJ media. Incubated up to six weeks with regular three days interval observation for any typical or atypical growth of mycobacteria on the surface of the media (7). Smear prepared from that growth and stained by ZN technique followed by microscopical examination for acid fast bacilli. Reports were recorded as AFB culture positive. Culture was reported as negative in absence of any AFB (Plate 3).

Results obtained were analyzed and compared for significant difference.

## RESULTS

In 'Hooghly' district, pathogen isolated in 52.94% [n=18] cases out of 34 samples studied from government laboratories. Presence of *S. aureus* was found positive for 17.65% [n=6] cases, 35.29% [n=12] cases found with *S. pyogenes* and 47.07% [n=16] cases came with insignificant growth out of said 34 samples. For private laboratories [total cases studied 26] pathogen was isolated in 46.15% [n=12] cases (*S. aureus* and *S. pyogenes* infection were found in 15.39% [n=4] and 30.77% [n=8] cases) and 53.85% [n=14] samples showed insignificant growth for the same setting (Table 1 and Fig. 1).

For 'Burdwan' district, among 27 samples from government laboratories, existence of pathogen was established in 37.04% [n=10] samples, where 18.52% [n=5] cases found with *Staphylococcus aureus*, 18.52% [n=5] cases showed positivity for *Streptococcus pyogenes* and insignificant growth existed for 62.96% [n=17] cases. For private laboratories out of said 39 throat swab samples 30.77% [n=12] cases came with positivity for pathogens. Twelve point eight two percent [n=5] cases came with *S. aureus*, 17.95% [n=7] cases came with *S. pyogenes* and 69.23% cases [n=27] found with insignificant growth for the same setting (Table 2 and Fig. 1).

Acid Fast Bacillus positive result was not established, neither by Z.N. smear test nor by AFB culture (Table 3), was not established for any of the samples out of total 100% [n=34] samples collected from government sector and out of total 100% [n=26] samples collected from private sector, in 'Hooghly' district. In 'Burdwan' district 100% [n=27] sample of sputum from government sector and 100% [n=39] sample from private sector were collected. Samples were tested for AFB by ZN smear as well as culture for AFB. In 'Burdwan' district, similar result like 'Hooghly' district i.e. Z.N. smear test or AFB culture showed 100% negative results for both of the government and private sectors.

## DISCUSSION

In our study the LAI-throat swab reports indicated an alarming rate of pathogen isolated in both the districts with the repre-

sentative pathogenic micro-organisms like *S. pyogenes* and *S. aureus* (Table 1, Table 2, Fig. 1) which echoes the studies of Collee et al. (5). Except those two pathogens we considered the other micro organisms as throat commensals, which are insignificant for upper respiratory tract infection. Still there remain a number of pathogenic micro-organisms (viruses, bacteria, fungi etc.) unexplored in our study, which can cause respiratory infection through aerosol inhalation (9). The staff concerned may be either in carrier or in infectious state. A habit of avoidance of using mask and gloves during their sample handling even apron during working hours may play a great role of this high rate of isolation of the pathogens. Lethargy to practice of proper hand washing in due time during the work interval in the laboratory the environment may get contamination from the clinical sample and culture etc (4). Incident of high rate of isolation of *S. pyogenes* and *S. aureus* may reflect the said fact of bio magnification (10). The *S. pyogenes* may survive for a long time at room temperature (11). As a result of poor sanitation, a good number of laboratories may be the source of large number of air borne Streptococcal disease or carrier state among the staff (12). Isolation rate of *S. aureus* from the throat swab of staff in government and private sector laboratories of both the districts showed no significant difference. In case of isolation rate of *S. pyogenes*, 'Hooghly' district was placed in higher position than 'Burdwan' district for both government and private sector laboratories. This picture may be an effect of maintenance of good personal hygiene and bio-safety practices by the staff in 'Burdwan' district. Perhaps a better awareness among the laboratory staff were developed, disseminated from the medical and paramedical institutes situated in 'Burdwan' district (Table 2 and Table 3).

As our patient community is not aware of spreading of infectious respiratory diseases they mere bother for restricted entry into the laboratory or asepsis of the laboratory environment. Furniture and other articles like door handle, taps of wash basin, towel in the laboratory and reception counter may be contaminated which ultimately enhance the spreading of these pathogens in multiplicative magnitude (6, 10). Clinical sample brought into the laboratory by the patient or their relatives for clinical examination some time not handled properly, which may cause the spread of pathogen or contamination to the environment which may not sub side the high rate of isolation of pathogen (7).

The risk to laboratory workers is approximately 10 times that to the general public (13) and almost 3 times that to other hospital employees about LAI (14). Lack of knowledge, attitude and practices (KAP) for infection control and incidence of exposure may another cause of spreading of infection among them (15).

So, treatment and awareness programmes are essential to prevent the infection and spread of the pathogens to the community. As partial requirement for prevention of 'Laboratory Acquired' respiratory tract infection a regular surveillance of the laboratory staff for significant pathogens is urgently needed. So, their throat swab, naso-pharyngeal washings, sputum or bronco-alveolar lavage (BAL) samples should be examined at a regular interval (16) to exclude their presence either the staff is symptomatic or asymptomatic.

Beside this, serological studies for the detection of antigen or significant antibody level present in the blood sample of the working laboratory staff would be examined at a regular interval which may give some new indications in this parameter. Molecular level study for the presence of pathogenic micro-organisms in the clinical sample of the laboratory staff could be of immense help in this parameter.

Another parameter of LAI bacteriology was performed to obtain any acid fast bacilli through either by ZN staining or AFB culture of sputum samples of the laboratory staffs under study (Table 3). Zero positivity was found in both the test parameters performed with the samples collected from two districts. As the sputum samples tested for AFB were indicative for pul-

monary tuberculosis only, so confirmation of extra pulmonary tuberculosis could not be ruled out for the laboratory staff in this process (17). Perhaps special care for the tubercular patients was taken to handle with clinical samples and personal contact, which may inhibit the spread of infection among the working staff (18). Government's role is appreciable in respect of prevention and control of tuberculosis, a highly infectious disease through launching and monitoring of RNTCP (19). Surveillance programmes for the diagnosis of tuberculosis among the laboratory workers could be performed at a regular interval with other test parameters like 'Mantoux test' (a delayed hyper sensitivity intra-dermal test with tuberculoprotein), Skia-gram of chest, hemogram with ESR and PCR etc (16).

Regular health check-up and treatment for any respiratory infection is essential for the working laboratory staff. Continue medical education for the laboratory staff regarding infectious diseases is also recommended to combat the crisis (6). Stringent follow up of bio-safety rules for the laboratory personnel including supervision by the licensing authority may be helpful. Alongside, good sanitation is essential for the laboratory spaces.

Another suggestion regarding the prevention of LAI is to use of mask is to be mandatory for the patient and his associates entered into the laboratory for investigation. To increase public awareness suitable posters including notifications from the laboratory authorities is to be displayed at suitable places (8). Pioneer role should be cultivated by the Government health authorities in this respect, which may result a cost effective health budget.

Personal bio-safety measures may be followed seriously to handle tuberculosis patients and their specimens considering the severity of the diseases. Special training programme should be continued for the laboratory personnel to alert them about multi drug resistance mycobacterial infection, a worldwide problem (7).

Regarding bacterial pathogens like *S.aureus* and *S.pyogenes* isolated from the throat of the laboratory workers in two districts were alarming. Even the carrier state of the individual of laboratory may be a point of source for nosocomial or hospital acquired infection (20). Personal protection and hygiene as well as following of rules for prevention and control of LAI including therapy of infected staff may help to overcome the problem (21).

**CONCLUSION**

"Prevention is better than cure"- remembering this quotation our suggestion for immunization of the laboratory staff is essential for few fatal diseases, if possible. Prevention of LAI amongst the staff and spreading of respiratory pathogen to the contacted population could be achieved in this way as well as loss of wages of the staff could be managed. These include:

**Careful attention to good laboratory practice.**

Proper containment facilities that protect the operator from airborne infection while containing any released organisms within the safety cabinet.

Prevention of unauthorized access to laboratory areas where cultures or potentially infected samples are being processed.

Proper screening and protection of laboratory medical and technical staff with surveillance.

**Table 1: Prevalence of pathogens (LAI) isolated from throat swab culture in 'Hooghly' district**

'Hooghly' district		
	Government sector (N=34)	Private sector (N=26)
Pathogen isolated	52.94% (18±1.09 <sup>a</sup> )	46.15% (12±1.02 <sup>b</sup> )

Staphylococcus aureus	17.65% (6±0.89 <sup>a</sup> )	15.39% (4±0.68 <sup>b</sup> )
Streptococcus pyogenes	35.29% (12±1.07 <sup>a</sup> )	30.77% (8±0.97 <sup>b</sup> )
Insignificant growth	47.07% (16±1.16 <sup>a</sup> )	53.85% (14±1.14 <sup>a</sup> )
Each horizontal row represents parametric values in percentage (mean± SEM in parenthesis) for each group. Analysis performed by student's two tail 't' test. Values with different superscript (a, b) differ from each other significantly at the level of p<0.05.		

**Table 2: Bacterial isolates from throat swab culture (LAI) in 'Burdwan' district**

'Burdwan' district		
	Government sector (N=27)	Private sector (N=39)
Pathogen isolated	37.04% (10±0.90 <sup>a</sup> )	30.77% (12±0.91 <sup>b</sup> )
Staphylococcus aureus	18.52% (5±0.26 <sup>a</sup> )	12.82 (5±0.26 <sup>a</sup> )
Streptococcus pyogenes	18.52% (5±0.25 <sup>a</sup> )	17.95% (7±0.29 <sup>b</sup> )
Insignificant growth	62.96% (17±1.67 <sup>a</sup> )	69.23% (27±2.13 <sup>b</sup> )

Each horizontal row represents parametric values in percentage (mean± SEM in parenthesis) for each group. Analysis performed by student's two tail 't' test. Values with different superscript (a, b) differ from each other significantly at the level of p<0.05

**Table 3: Intra district comparison considering government and private sectors from the view point of AFB test results under LAI mycobacteriology.**

'Hooghly' district (LAI - Sputum for AFB)					p-value
	Government Sector Laboratories (No. of sample=34)		Private Sector Laboratories (No. of sample=26)		
	AFB Positive	AFB Negative	AFB Positive	AFB Negative	
Z.N. Smear	0	34±1.53 <sup>a</sup> (100%)	0	26±1.16 <sup>b</sup> (100%)	<0.001
AFB Culture	0	34±1.53 <sup>a</sup> (100%)	0	26±1.16 <sup>b</sup> (100%)	<0.001

Each row represents parametric values in mean± SEM (percentage in parenthesis) for each group. Analysis performed by student's two tail 't' test. Values with different superscripts (a, b) differ from each other significantly at the level of p<0.001.

'Burdwan' district (LAI - Sputum for AFB)					p-value
	Government Sector Laboratories (No. of sample= 27)		Private Sector Laboratories (No. of sample=39)		
	AFB Positive	AFB Negative	AFB Positive	AFB Negative	
Z.N. Smear	0	27±1.21 <sup>a</sup> (100%)	0	39±1.74 <sup>b</sup> (100%)	<0.001
AFB Culture	0	27±1.21 <sup>a</sup> (100%)	0	39±1.74 <sup>b</sup> (100%)	<0.001

Each row represents parametric values in mean± SEM (percentage in parenthesis) for each group. Analysis performed by student's two tail 't' test. Values with different superscripts (a, b) differ from each other significantly at the level of p<0.001.



Plate 1: Representative image of microbial growth on 'Blood agar media' from throat swab. [A] Beta-haemolytic colonies of *Streptococcus pyogenes* [B] *Staphylococcus aureus* [C] Insignificant growth



Plate 2: Representative smears prepared from sputum [A] and growth in culture [B] stained by ZN technique

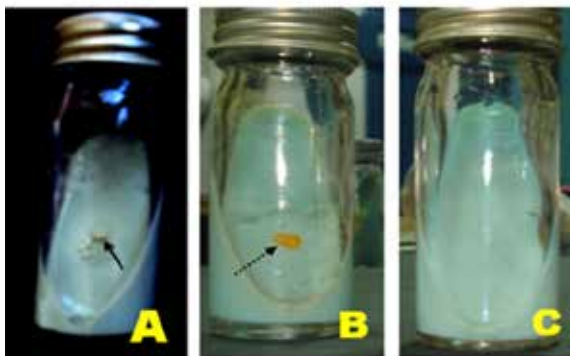


Plate 3: Mycobacterial growth in LJ Media. [A] Typical growth indicated by → [B] Atypical growth indicated by → [C] No growth

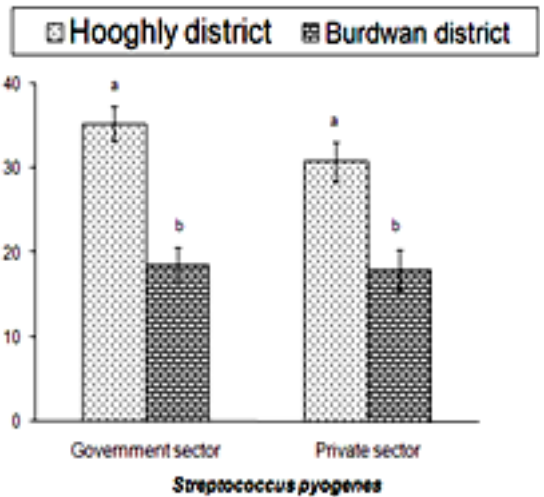
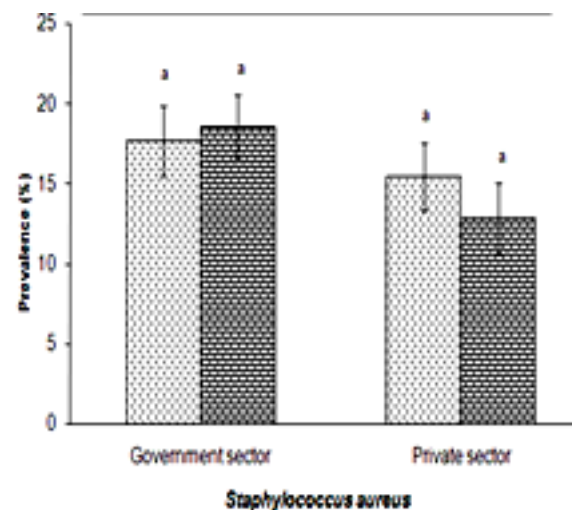


Figure 1: Inter district comparison considering government sector and private sector from the view point of LAI (Throat Swab). Each bar represents mean ± SEM for each group. Analysis performed by two student's two tail 't' test. Values of bars with different superscripts (a, b) differ from each other significantly at the level of p<0.05.

'Burdwan' district (LAI - Sputum for AFB)					p-value
	Government Sector Laboratories (No. of sample= 27)		Private Sector Laboratories (No. of sample=39)		
	AFB Positive	AFB Negative	AFB Positive	AFB Negative	
Z.N. Smear	0	27±1.21 <sup>a</sup> (100%)	0	39±1.74 <sup>b</sup> (100%)	<0.001
AFB Culture	0	27±1.21 <sup>a</sup> (100%)	0	39±1.74 <sup>b</sup> (100%)	<0.001

Each row represents parametric values in mean± SEM (percentage in parenthesis) for each group. Analysis performed by student's two tail 't' test. Values with different superscripts (a, b) differ from each other significantly at the level of p<0.001.

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