Lower respiratory tract infections (LRTI) are one of the major causes of morbidity and mortality caused due to infectious diseases among patients. According to a report of WHO, LRTI cause 5.2% of total deaths worldwide that makes it fourth leading cause of deaths (WHO Global health estimates, 2016). The incidences of deaths due to LRTI are higher in South-Eastern region of Asia (i.e. 5.7% of total deaths) as compared to global average. LRTI consist of a group of diseases differing in epidemiology, pathogenesis and clinical manifestations viz. bronchitis and pneumonia. The etiological agents of LRTI differ from area to area. The microbial agents responsible for LRTI include virus, bacteria, mycoplasma, fungi etc. Among bacteria, both Gram positive (viz. Staphylococcus aureus, Streptococcus pneumonia etc.) and negative bacteria (viz. H. influenzae, Pseudomonas, and Klebsiella species etc.) have been isolated from LRTI. Three quarter of all antibiotic are consumed for the treatment of respiratory tract infections (Basnet et al., 2013). Risk factors for LRTI in adults include active and passive smoking, cancer, major trauma or surgical intervention, cystic fibrosis, heavy alcohol intake, chronic obstructive pulmonary disease (COPD) and ageing. Pneumonia is the most common LRTI that is classified into three groups: Community-acquired pneumonia, hospital acquired (nosocomial) pneumonia and pneumonia in patients with impaired immunity (Biscevic-Tokic et al., 2013). The common bacterial cause of community acquired pneumonia is Streptococcus pneumoniae followed by Haemophilus influenzae. The other bacterial agents of pneumonia include Klebsiella pneumoniae, Chlamydia pneumoniae, Mycoplasma pneumoniae, Staphylococcus aureus, Moraxella catarrhalis, Pseudomonas aeruginosa, etc. (Eddens and Kolls, 2012; Biscevic-Tokic et al., 2013).

Tuberculosis (TB) stands tenth leading causes of deaths from infections that occur in the respiratory tract (WHO Global health estimates, 2016). India has the highest burden of tuberculosis (i.e. 27% of all cases of the world); however, TB affects all countries and all age groups. In 2017, TB killed approximately 1.3 million HIV-negative people, and 3, 00,000 HIV-positive people globally (WHO Global tuberculosis report, 2018). Pulmonary tuberculosis is an airborne disease, caused by Mycobacterium tuberculosis. This is the bacterial infection which deteriorates the lung tissue resulting in coughing up blood, fever, weight loss, problem in breathing and chest pain (Fogel, 2015). This infection can be lethal, if not treated properly.

Estimation of the burden of the disease and associated predisposing factors in an endemic population is very important for planning out public health strategies to encounter infectious disorders. In the current study, we identified the causative agents of LRTI, prevalent in Northern region of India in certain districts of Punjab near Mohali. Also, incidences of pulmonary tuberculosis were examined. Trends of age and sex wise distribution of LRTI among patients were also observed.

Material and Methods

The media and chemicals used were obtained from HiMedia Laboratories Pvt. Ltd. Mumbai, India.

Sample

The present study was undertaken in the Polo Labs, Kharar, Punjab, India. Study of sputum and pleural fluid
along with tuberculosis section was performed in the labs of molecular diagnosis and microbiology department. The samples were taken from the areas near Mohali, Punjab including Ambala, Amritsar, Ropar, Mohali, Kharar, Nangal, Chandigarh, Hoshairpur, Panchkulla, Ludhiana, Malerkotla, Khanna etc. Samples were collected for a period of January 2018 up to April 2018. The samples from patients of all age group and sex were taken. The samples of sputum and pleural fluid were further categorized on the basis of age group, areas and the sex of patient along with the most occurring bacterial infection in the diagnosis.

### Bacterial Isolation and Characterization

Mac Conkey Agar was used for sputum and pleural fluid culture. The collected samples were incubated on Mac Conkey Agar at 37°C for 48 hrs. After incubation period, the isolated colonies were subjected to identification.

### Bacterial identification

Identification of bacteria was done with the help of VITEK2 (BioMérieux, USA) system using Gram positive (GP ID REF21342) and Gram negative (GN ID REF21341) identification cards. All the assays were performed according to the manufacturer’s instructions.

Identification of *Mycobacterium tuberculosis* in the sample was performed with the help of Real time PCR (Biorad, USA). Briefly, DNA from the sputum or pleural fluid was extracted with Genefinder™ EX-MATE 32 (Neon Diagnostics, UK) as per manufacturer’s protocol. Further, isolated DNA was subjected to Real Time PCR with appropriate primers for identification by using Real Time PCR TB identification assay kit.

### Results

Figure 1 represents the prevalence of bacteria obtained from sputum culture and their age wise distribution among patients. The bacteria identified in the sputum culture in present study were *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Candida lusitaniae*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella enterica*. Out of 1000 patients, *P. aeruginosa* was detected in highest (59.9%) individuals followed by *K. pneumoniae* (22.3%), *A. baumanii* (13%), *Salmonella enterica* (2.2%), *E. coli* (2.1%) and *C. lusitaniae* (0.5%).

*P. aeruginosa* was most prevalent among the patients of age 61-75 years. Incidences of other infections were also prevalent among different age groups i.e. *K. pneumoniae* (61-75 yrs.), *A. baumanii* (46-60 yrs.), *Salmonella enterica* (61-75 yrs.), *E. coli* (61-75 yrs.) and *C. lusitaniae* (61-75 yrs.).

All of the bacteria infected males more than females as represented in Fig. 2.

Further, we observed the status of infection in pleural fluid of 399 patients was examined. Among these, 347 patients did not have any infection. However, 52 patients were found to be infected with *Escherichia coli* (2/52), *Staphylococcus aureus* (20/52) and *Streptococcus pneumonia* (30/52). The age wise distribution is given in Fig. 3. Males were more affected with these infections as compared to females (Fig. 4).

Thus, as illustrated in figure 5, among all the LRTI found, 94.77% were caused by Gram negative bacteria whereas 4.75% infections were caused Gram positive bacteria. Fungi accounted for only 0.48% of total LRTI in the present study.
Further, our results suggest a significantly high number of males (61.5%) infected with LRTI as compared to females (38.5%) as observed in sputum as well as pleural fluid samples collectively (Fig. 6).

For the detection of pulmonary tuberculosis, pleural fluid of 139 patients and sputum sample of 250 patients were taken and diagnosed with real time PCR. Out of these, only 7 and 6 were found positive in pleural fluid and sputum sample respectively. The age wise distribution is given in Fig. 7. The total number of TB cases were 9 (30.76%) among males and 4 (69.24%) among females as shown in figure 8.

The primary goal of present study was to assess the current prevalence of bacteria accounting for LTRI in Northern part of India and analyze its distribution among patients of different age and gender. There are very few reports that have analyzed the bacteriological profile of microorganisms causing LRTI in this area of Punjab (Oberoi and Aggarwal, 2006). We found that the major bacteria causing LRTI are Pseudomonas aeruginosa, Acinetobacater baumanii, Klebsiella pneumoniae, Staphylococcus aureus and Streptoccocus pneumoniae. Majority of pathogens were recovered from sputum sample (95%) than pleural fluid (5%). Males are more vulnerable to LRTI as well as pulmonary tuberculosis as compared to females, which is in agreement with a previous study (Vijay and Dalela, 2016). Moreover, people of age more than 45 years are more at risk of having LRTI and TB as compared to younger ones.

Pseudomonas aeruginosa is a Gram negative rod shaped bacterium that can cause multiple infections in humans from local to systemic level. Since the last few decades, it is known to be one of the most frequent organisms to cause hospital acquired infections (HAI) that may lead to notable mortality (Klockgether and Tümmler, 2017). Its infections are difficult to treat because of its natural resistance to several antibiotics and presence of several multidrug- and pan-drug-resistant strains globally (Moradali et al., 2017). It accounts for 20% of cases of hospital acquired pneumonia with ~30% mortality rate (Berube et al., 2016). In our study, P. aeruginosa has been found to be most prevalent with maximum patients from 61-75 age group, which is in accordance with Shah et al. (2010) and Oberoi and Aggarwal (2006).

Second most prevalent pathogen in the present study was found to be Klebsiella pneumoniae, affecting 21.2% of total 1052 positive samples. In previous studies conducted in India, it was found to be most prevalent bacterial isolate (Shailaja et al., 2004). Klebsiella pneumoniae is a Gram negative, encapsulated rod shaped bacterium which serves as normal flora of mouth and intestine. It can become pathogenic if inhaled and can lead to destruction of lungs.

Acinetobacater baumanii is another Gram negative, rod shaped, pleomorphic, aerobic and non-motile bacteria (Howard et al., 2012). It is an opportunistic pathogen majorly associated with HAI. It has been frequently recovered from skin, respiratory and oropharynx secretions of infected persons (Sebeny et al., 2008). This organism can cause hospital- as well as community-acquired pneumonia with
mortality rate between 40% - 60%. Moreover, it has been designated “red alert” pathogen because of its diverse antibiotic resistivity patterns (Cerqueira and Peleg, 2011). *Acinetobacter baumannii* was estimated to infect 12.4% of LRTI in the present study.

Out of 383 patients screened, 13 (3.4%) were found infected with pulmonary tuberculosis. Among these, males (69.24%) were higher infected with TB than females (30.76%). It is similar to WHO report (WHO tuberculosis report, 2004) which says that in low income countries the ratio of male to female incidences of TB are 2:1. Another study in India also reported the same (Rao, 2009).

*Streptococcus pneumonia* is considered as the most common cause of LRTI (Biscevic-Tokic et al., 2013). However, in current study, Gram negative bacteria were highly prevalent as compared to Gram positive bacteria with very little involvement of fungi in causing LRTI. The prevalence of such infections may be related to poor hygienic conditions and overpopulation. In a previous comprehensive report regarding bacteriological profile of community acquired pneumonia among hospitalized patients in Srinagar, Kashmir, India; Gram negative bacteria (19/29) were found to be the commonest cause followed by Gram positive bacteria (10/29), which is in agreement with our study (Shah et al., 2010). Moreover, similar patterns of LRTI were observed in other studies conducted in India (Oberoi and Aggarwal, 2006; Chaudhury et al., 2016).

In conclusion, the current study suggests the prevalence of Gram-negative bacteria *i.e.* *P. aeruginosa, K. pneumoniae* and *A. baumannii* over Gram positive bacteria in LRTI in Punjab state of India with individuals of age more than 45 at high risk. Males are more vulnerable to these diseases as compared to females.

**References**


