WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 3, 1007-1018. Research Article ISSN 2277– 7105

# A SURVEILLANCE OF VIRULENT BACTERIAL ISOLATES AMONG THE RESPIRATORY INFECTED COTTON INDUSTRY LABOURS, TAMIL NADU

**Hemalatha P[1*], Martin P[2], Senthilkumar B[3], Senbagam D[3], Rajasekarapandian M[4]**

[1]Department of Zoology, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

[2]Department of Zoology Government Arts College for Men (Autonomous) Nandanam, Chennai - 600 035, Tamil Nadu, India.

[3]Department of Biotechnology, Muthayammal College of Arts and Science, Rasipuram - 637 408, Tamil Nadu, India.

[4]Department of Zoology, Arignar Anna Government Arts College, Namakkal-637001, Tamil Nadu, India.

Article Received on
15 Dec 2014,

Revised on 09 Jan 2015,
Accepted on 03 Feb 2015

***Correspondence for Author**
**Hemalatha P**
Department of Zoology, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

## ABSTRACT

This study is aimed to isolate and identify the most common bacterial pathogens causing upper respiratory tract infection among the people working in cotton industries. A total of 72 cotton industry labours clinical specimen were (nasal samples 20, throat samples 25 and sputum samples 27) collected in Salem District, Tamil Nadu. Of these 85% of nasal and sputum samples and 80% throat samples were found to be positive for upper respiratory tract infections (URTIs) by culture positive. The isolates were screened and identified on the basis of phenotypic characterization *as Staphylococcus aureus* (34.7%) *Pseudomonas aeruginosa* (33.3%), *Streptococcus* species (25%), *Klebsiella pneumoniae* (18%) and *Escherichia coli* (16.6%). Virulent bacterial isolates were determined by their ability to produce slime (biofilm), beta lactamase, protease, hydrolysis of starch and tween 80.

**KEYWORDS:** Respiratory tract infection, virulence factors, cotton industry labours, bacterial pathogens.

## INTRODUCTION

Respiratory tract (RT) is the part of the human system that plays a fundamental role in breathing mechanism. The RT is constantly exposed to microbes due to the extensive surface area, for instance, the lungs have an exposed internal surface area of approximately 500 $m^2$. [1] Respiratory tract infections (RTIs) refer to the presence of microorganisms in the respiratory tract, viz. the pharynx, trachea, bronchi and lungs. Upper respiratory tract (URT) infection is an acute infection which involves the nose, sinuses, pharynx or larynx. This commonly includes: tonsillitis, pharyngitis, laryngitis, sinusitis, ottis media and the common cold. [2] The human URT is the reservoir of a diverse community of commensals and potential pathogens, including *Streptococcus pneumoniae, Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus*, [3] which occasionally turns into pathogens causing infectious diseases. To cause respiratory disease, bacteria first need to colonize the nasopharyngeal niche and it is a dynamic process: acquisition and elimination of species, interactions among microbes and between microbes and the host, and interference by environmental factors are suggested to cause a dynamic and complex microbial interplay. [4] Moreover, imbalance in this respiratory microbial community can also contribute to acquisition of a new bacterial or viral pathogen, carriage of multiple potential pathogenic bacteria, or a viral co-infection. [5]

Among cotton workers, the increasing incidence of byssinosis and chest tightness is about 24% and 23%, respectively, and is significantly more common in smokers than non-smokers. A high proportion of symptoms are found to be intermittent, rather than persistent. Among silk workers, no typical byssinosis is identified; the incidence of chest tightness is about 10%. Chronic bronchitis, cough, and dyspnoea are more common and persistent among cotton workers than silk workers. Significantly lower odds ratios for symptoms are observed in cotton workers who left the cotton mills; risk is also related to years since last worked. Multivariate analysis indicated a trend for higher increasing exposure to endotoxin in relation to a higher risk for byssinosis. [6]

Respiratory tract infection is the most common infection reported of all human infections. Generally most of these infections are mild, transient lasting and sometimes self-limiting. However, respiratory infections are a common and important cause of morbidity and mortality worldwide. For example, in USA alone, about 62 million persons suffer from cold annually, [7] while in the UK, about 8 million people are infected by some forms of chronic

lung diseases. [8] Surveillance of RT infections especially acute cases in defined populations is required to monitor prevailing pathogens while the determination population groups at risk is important for implementing strategies. [9, 10] Therefore, this study focuses cotton mill workers who are suffering from respiratory diseases to reveal the risk and outcome of respiratory disease of the virulent bacterial isolates.

## MATERIALS AND METHODS

### *Study population*

In the present study, a total of 72 clinical samples were collected from patients of cotton industry who attended the various hospitals and clinical wards in Salem, Tamil Nadu State, India. All patients had clinical evidence of respiratory tract infections, as determined by the treating Physicians.

### *Specimen collection*

The specimens were collected aseptically from 72 (20 nasal swab , 25 throat swab, 27 sputum)) patients. All patients were instructed on how to collect the sputum samples aseptically and taken to the laboratory immediately for analysis. The sputum samples were collected into well-labelled, sterilized, wide mouthed screw capped glass bottles. Using a sterile cotton swab, the inner surface of the infected nose and throat were swabbed gently and the swabs were transported to the laboratory. For a collection of throat specimens, the handle of a spoon was used to depress the tongue to examine the mouth for the presence of inflammed membrane, exudates or pus.

### *Bacteriology*

Each sample was inoculated on nutrient agar, MacConkey agar, mannital salt agar, EMB (Eosin Methylene Blue agar), citramide agar and blood agar. The inoculum on the plate was streaked out for discrete colonies with a sterile wire loop. The culture plates were incubated at 37°C for 24 h and observed for growth of bacterial colonies. All the bacteria were isolated and identified using morphological, and biochemical tests following the standard procedures described by Senthilkumar *et al*. (2014a). [11]

### *Virulence factors*

#### Slime Production Assay (Biofilm)

Brain heart infusion agar supplemented with 5% sucrose and congo red (0.08 g/l) was prepared and autoclaved at 121 °C for 15 min. The isolates were inoculated and the plates

were incubated aerobically for 24 to 48 h. Biofilm production was indicated by black colonies with a dry crystalline consistency whereas biofilm non-producers remain pink, though occasional darkening at the center of the colony was also observed. [11]

**Beta lactamase production Assay**

Beta lactamase production was assayed by the method of Lateef (2004). [12] Broth culture of the test organism was spot inoculated onto Mueller-Hinton agar added with 1% starch and incubated overnight at 37 °C. The plates were flooded with freshly prepared phosphate buffered saline containing potassium iodide, iodine and penicillin. The presence of clear colourless zone around the bacterial growth is an indication of beta lactamase production. Beta lactamase converts penicillin to penicilloic acid, which reduces iodine to iodide and was monitored via decolourisation of the starch iodine complex. All the bacterial isolates were tested for the production of beta lactamase.

**Protease enzyme production**

Protease activity was qualitatively assayed by spreading the test organism on nutrient agar containing 1.5 % skim milk powder. After 72 h incubation at 30 $^{o}$C, the production of protease was observed by the formation of a clear zone around the colonies, due to casein degradation.[11]

**Starch hydrolysis test**

The enzyme amylase was excreted out of the cells into the surrounding media, catalyzing the breakdown of starch into sugars. At first, a starch agar plate was picked up and divided into half. Then the isolates were inoculated on the plate either by a straight line or a zig-zag and the plate was incubated overnight at 37°C. After incubation the plate was flooded with iodine and the appearance of yellow or gold zone around the growth indicated positive result. [11]

**Hydrolysis of Tween 80**

Tween 80 (1% v/v) was incorporated in nutrient agar (Oxoid), and the organism to be tested was spread over an area approximately 1 cm in diameter. Plates were incubated at 37°C for 2-3 days. A positive test was denoted by the appearance of a halo of fatty acids around the inoculum.

## RESULTS AND DISCUSSION

Constantly diverse sequence of microbial communities inhabits a variety of points of the respiratory tract, with community composition dictated by distance from colonization sources, colonization rates, and extinction rates. Ecology and evolution theory developed in the context of biogeography is relevant to clinical microbiology and could reframe the interpretation of recent studies comparing communities from lung explant samples, sputum samples and / or pharyngeal swabs. [13]

Therefore in this study, a total of 72 samples (Nasal samples 20, throat samples 25 and sputum samples 27) were subjected to microbial identification. Among them 85% of nasal and sputum samples and 80% of throat samples had bacterial isolates. Five different bacterial isolates have been identified as *S. aureus, Streptococcus* sp., *K. pneumoniae, P. aeruginosa* and *E. coli* (Tables 1 and 2). Among them *S. aureus* (35%) was found to be predominant isolate of respiratory origin and was followed by *P. aeruginosa* (33%) and *Streptococcus* sp. (25%) (Fig. 1). The present result is concurrent with the study of El-Mahmood *et al*. (2010) [14] in Yola. In terms of frequency of occurrence, the results were in accordance with those conducted in other countries. But Taura *et al*. (2013) [15] reported that *S. Pneumoniae* (25.6%) was found to be most frequent pathogen of respiratory origin and was followed by *K. Pneumoniae* (20.9%), *E. coli* (20.9%), *S. aureus* (16.3%), *Proteus* sp. (4.7%) and *P. aeruginosa* (4.7%).

**Table-1 Morphological and Biochemical tests of bacterial isolates**

| Preliminary tests | | | | Biochemical tests | | | | | | | | | | | | | | | | | Bacterial isolates |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Sugar fermentation | | | | | IMVIC | | | | Other tests | | | | | | | | |
| Gram Staining | Motility | Catalae | Oxidase | Glucose | Suurose | Lactose | Mannitol | Maltose | Indole | MR | VP | Citrate | Coagulase | Urease | Starch | Gelatin | Nitrate | Lipase | $H_2S$ | TSI | |
| + cocci | - | + | - | A | A | A | A | A | - | + | + | - | + | + | - | + | + | + | - | NA | *Staphylococcus aureus* |
| + cocci | - | - | - | - | A | A | - | A | - | + | - | - | - | - | - | - | - | - | - | NA | *Streptococcus* sp. |
| - rods | + | + | - | AG | d | AG | AG | AG | + | + | - | - | - | - | - | - | + | - | - | A/AG | *Escherichia coli* |
| - rods | + | + | - | AG | AG | AG | AG | AG | - | - | + | + | - | + | - | - | + | - | - | A/AG | *Klebsiella pneumoniae* |
| - rods | + | + | + | A | - | - | - | A | - | - | - | + | - | - | + | - | + | + | - | AK/A | *Pseudomonas aeruginosa* |

**Table 2 Cultural characteristic of bacterial isolates**

| Culture media | Characteristic | Bacterial isolates |
|---|---|---|
| EMB agar | Metallic green sheen indicating vigorous fermentation of lactose and acid production which precipitates the green metallic pigment | *E. coli* |
| Mannitol Salt Agar | Colonies that fermented mannitol and appeared golden yellow | *S. aureus* |
| Nutrient agar | Green color pigment | *P. aeruginosa* |
| MacConkey | Organisms that ferment lactose and produce the pink colour colony | *K. pneumoniae* |
| Blood Agar | Beta haemolytic colonies | *Streptococcus* sp. |

Nasal swabs and tissue samples were screened for the presence of microbial pathogens by cultural and biochemical characteristics. Out of 144 isolates, 57 isolates were found to be Gram positive pathogens and identified as *Staphylococcus* sp. (43 isolates) and *Streptococcus* sp. (14 isolates) on the basis of Gram staining, cultural and biochemical features. [16] This result is correlated with the present study result showed the dominant *Staphylococcus aureus* isolates.

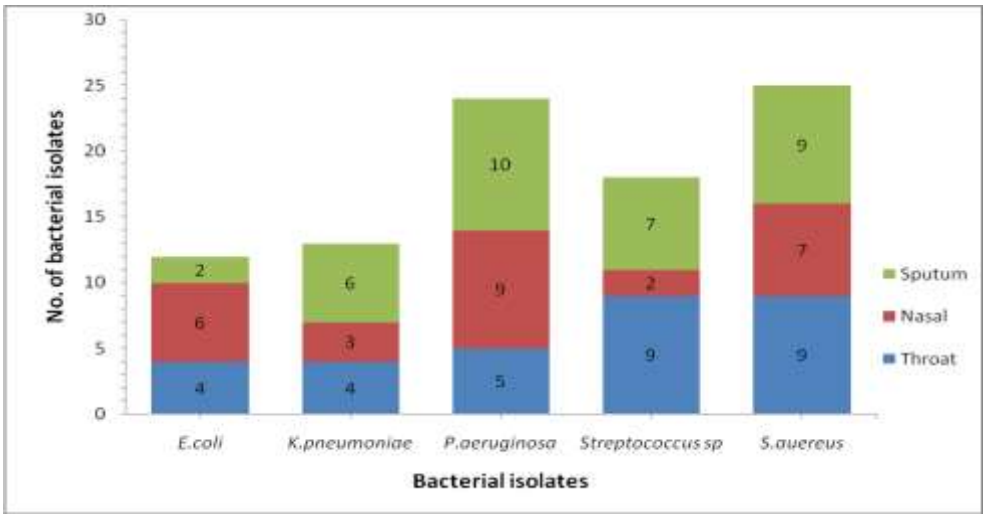


**Figure 1. Prevalence of bacterial isolates from different samples of RT infected patients**

Group A beta-hemolytic streptococci causes 5% to 10% cases of pharyngitis in adults. [17] Other less common causes of bacterial pharyngitis include group C beta-hemolytic streptococci, *Corynebacterium diphtheria* and *Neisseria gonorrhoeae*, *Arcanobacterium haemolyticum*, *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and herpes simplex virus. *S. pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* are the most common organisms that cause the bacterial super infection of viral acute rhinosinusitis. [18]

Less than 10% of cases of acute tracheobronchitis are caused by *Bordetella pertussis*, *B. parapertussis*, *M. pneumoniae*. [19]

In this study, highest occurrence of bacterial pathogens was observed from male samples (78.1%) than female samples (69%). According to age, highest occurrence was found in the age group of 19 to 30 (94.4%), and was followed by 51 to 70 age group (69.4%) and 41 to 50 age group people (67%) (Fig. 2). These findings are in agreement with the work of Taura *et al*. (2013) [15] who reported that people under the age range of 20-29 was found to be the highest percentage of occurrence of pathogens and was followed by the people under the age 30-39.

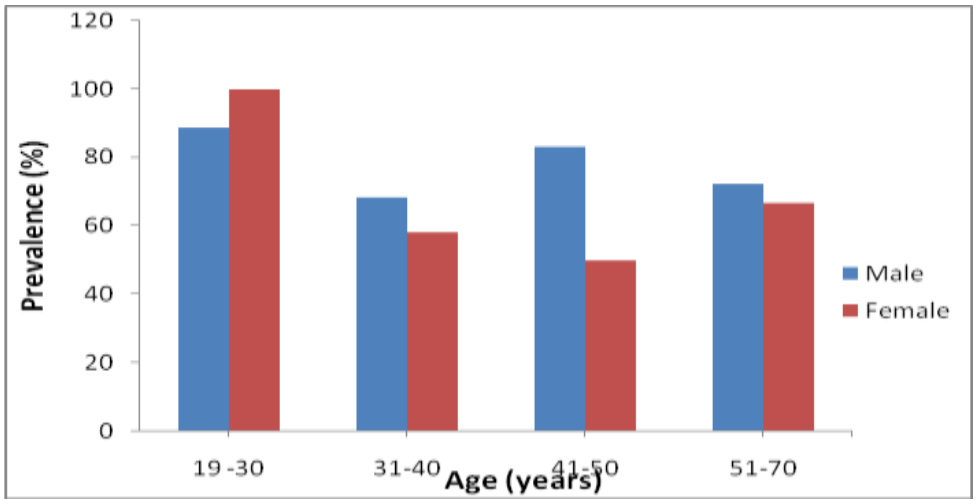


**Figure 2. Sex and Age wise prevalence rate of bacterial pathogens among the cotton industry workers**

In this present study, the nasal-bacterial isolates (27) including *P. aeruginosa* (53%), *S. aureus* (45.3%) and *E. coli* (23%), *K. pneumoniae* (11%) and *Streptococcus* sp (23%) were screened and identified. According to the age group, highest number of isolates was obtained from 19 to 30 and 41 to 50 age groups (Table 3and Fig.3).The throat - bacterial isolates (31) were observed including *E. coli* (13.4%), *S. aureus* (28%), *P. aeruginosa* (9%), *Streptococcus* sp.(28%) and *K. pneumoniae* (21%). In case of age wise result, occurrence was highly observed in 19 to 30 age groups (100%) and was followed by 31 to 40 age groups of male samples ((Table 4 and Fig.4)). The sputum - bacterial isolates (34) were observed were including *E. coli* (3.6%), *S. aureus* (33%), *P. aeruginosa* (39.5%),*Streptococcus* sp.(21%) and *K. pneumonia* (28.1%). Among the 5 types of age groups, the highest occurrence was in 51 to 70 (Table 5 and Fig.5). The overall results of the current study showed that among the 5 bacterial pathogens, *S. aureus* was found to be a predominant pathogen than others.

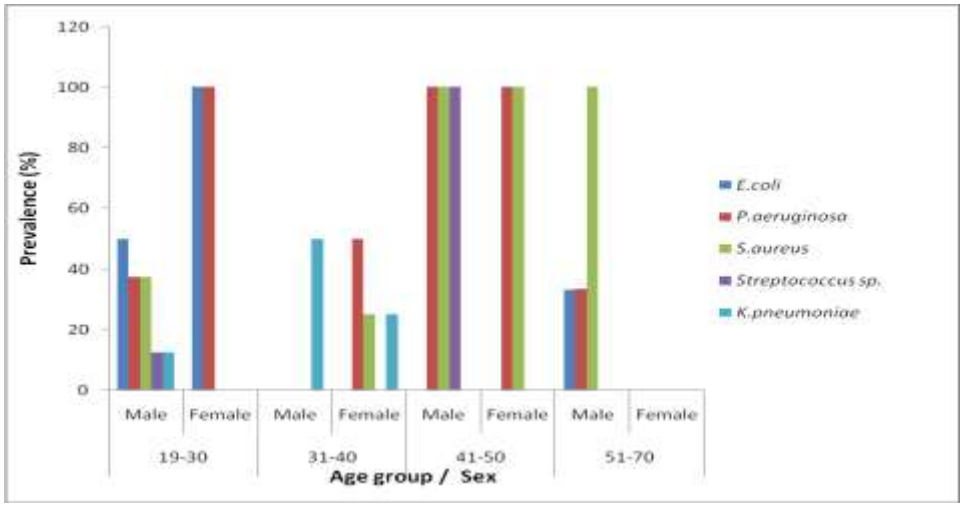


**Figure 3 Prevalence of bacterial pathogens from Nasal samples of different age group people**

**Table 3 Prevalence of bacterial pathogens from nasal sample**

| S.No | Sex | Age groups | | | |
|---|---|---|---|---|---|
| | | 19-30 | 31-40 | 41-50 | 51-70 |
| 1. | Male | 8/8 | 1/2 | 1/1 | 2/3 |
| 2. | Female | 1/1 | 3/4 | 1/1 | 0 |

**Table 4 Prevalence of bacterial pathogens from throat sample**

| S.No | Sex | Age groups | | | |
|---|---|---|---|---|---|
| | | 19-30 | 31-40 | 41-50 | 51-70 |
| 1. | Male | 8/8 | 4/5 | ½ | ½ |
| 2. | Female | 4/4 | 0/1 | ½ | 1/1 |

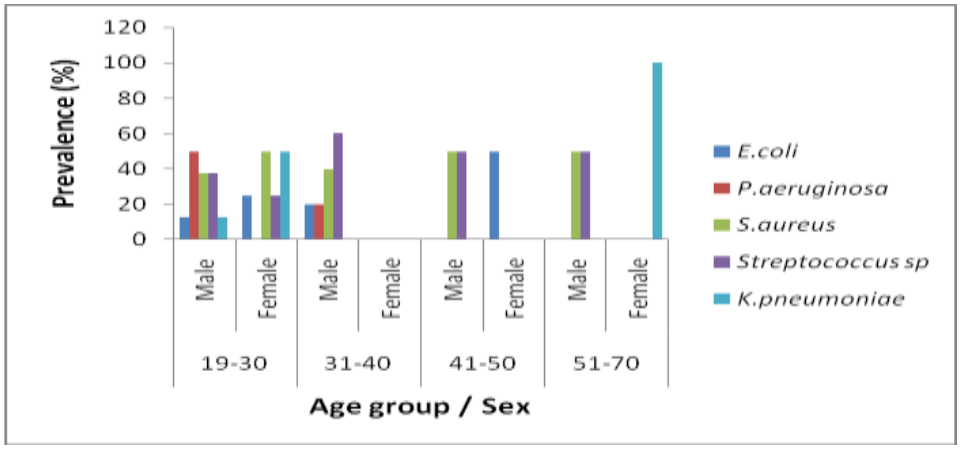


**Figure 4 Prevalence of bacterial pathogens from throat samples of different age group people.**

**Table 5 Prevalence of bacterial pathogens from sputum sample**

| S.No | Sex | Age groups | | | |
|---|---|---|---|---|---|
| | | 19-30 | 31-40 | 41-50 | 51-70 |
| 1. | Male | 4/6 | 6/8 | 2/2 | 1/1 |
| 2. | Female | 4/4 | 4/4 | 0/1 | 1/1 |

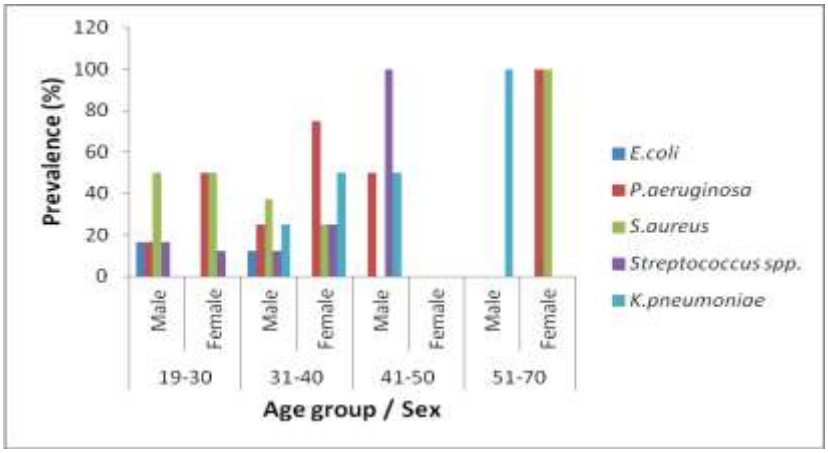


**Figure 5 Prevalence of bacterial pathogens from sputum samples of different age group people**

*S. aureus* (68% 73.07%, 96%, 96%, 80%), *K. pneumoniae* (46.15%, 30.76%, 30.76%, 15.38%), *P. aeruginosa* (20.83%, 20.83%, 25%, 20.83%, 12.5%), *E. coli* (58.33%, 33.33%, 58.33%, 41.66, 33.33%), *Streptococcus* sp. (16.66%, 16.66%, 22.22%, 11.11%, 16.66%) were found to be positive for biofilm production, beta lactamase production, protease production, starch hydrolysis and tween-80 hydrolysis respectively (Table 6). Of all the bacterial isolates, *S. aureus* were found to be the most dominant in prevalence and virulence characteristics.

**Table - 6 Detection of Virulence factors among the bacterial pathogens**

| Bacterial pathogens | Virulence factors | | | | |
|---|---|---|---|---|---|
| | Biofilm | Beta lactamase | Protease | Starch hydrolysis | Tween-80 hydrolysis |
| *S. aureus* | 17/25 (68%) | 19/25 (73.07%) | 24/25 (96%) | 24/25 (96%) | 20/25 (80%) |
| *K. pneumoniae* | 3/13 (23.07%) | 6/13 (46.15%) | 4/13 (30.76%) | 4/13 (30.76%) | 2/13 (15.38%) |
| *P. aeruginosa* | 5/24 (20.83%) | 5/24 (20.83%) | 6/24 (25%) | 5/24 (20.83%) | 3/24 (12.5%) |
| *E. coli* | 7/12 (58.33%) | 4/12 (33.33%) | 7/12 (58.33%) | 5/12 (41.66) | 4/14 (33.33%) |
| *Streptococcus* sp. | 3/18 (16.66%) | 3/18 (16.66 %) | 4/18 (22.22%) | 2/18 (11.11%) | 3/18 (16.66%) |

Moderate to high *in vitro* biofilm-forming capacity was detected in 9 out of 12 patients with CRS/NP (mean [SD] optical density values between 0.284 [0.017] and 3.337 [0.029]). The microorganisms isolated were *Staphylococcus* (5 patients), *Streptococcus viridans, Pseudomonas aeruginosa, Enterococcus faecalis* and *Streptococcus viridians/ Corynebacterium*. Biofilms were demonstrated *in vivo* in 2 patients and no biofilm structures were evident in any of the control.[20] *In vivo* virulence determination of the bacterial isolates was carried out in mice. [21] However, in this study virulence of bacterial isolates was determined by *in vitro* studies only.

The overall mortality rate due to respiratory disease was 56%, and the median survival time was 10 days. All of the deaths were attributed to *S. aureus* infection and were secondary to refractory shock and/or respiratory failure. Fatal outcome was associated with classical severity factors, such as the need of mechanical ventilation or inotrope support, and with onset of the acute respiratory distress syndrome. Airway bleeding was strongly associated with fatal outcome (*P* =0 .002). Patients who had focal staphylococcal infection before the onset of pneumonia had a significantly lower mortality rate (*P* = 0.002). The main biological feature associated with death was leukopenia (*P* <t; .001). In multivariate analysis, leukopenia and erythroderma occurring within the first 24 h after admission to the hospital were independently associated with fatal outcome. Erythroderma was not associated with toxic shock syndrome toxin (Yves *et al*., 1990). [22]

Air borne allergens with sensitized mast cells in the conjunctivae and nasal mucosa to induce the release of pharmacologically active mediators from mast cells. This result in localized vasodilation and increased capillary permeability and symptoms include watery exudation of conjunctivae, nasal mucosa, URT, sneezing and coughing (Rajasekarapandian and Senthilkumar, 2007). [23] Therefore, there are more chances for the persons who are working in cotton industries to dust, cottons pieces and other contributes for the symptoms of allergy as well as upper respiratory tract infection. This study also concludes that more percentage of labours in the cotton industry may be easily suffered from upper respiratory infections. Their treatment requires to adequate medication to get rid of respiratory illness.

**REFERENCES**

1. Underwood. General and systemic pathology published by Churchill living stone, New York 1992; 317.
2. Eccles MP, et al. Applying psychological theories to evidence-based clinical practice: Identifying factors predictive of managing upper respiratory tract infections without antibiotics". *Implement Sci*, 2007; 2**(**26). doi:10.1186/1748-5908: 2-26.
3. Watson KBH et al. Upper respiratory tract bacterial carriage in aboriginal and non-aboriginal children in a semi-arid area of Western Australia. *Pediatr Infect Dis J*, 2006; 25(9): 782-790.
4. Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol*, 2009; 7(12): 887-894.
5. Murphy T et al. Microbial interactions in the respiratory tract. *Pediatr Infect Dis J*, 2009; 28(10): S121–S126.
6. Wang XR et al. Respiratory symptoms and cotton dust exposure; results of a 15 year follow up observation. *Occup Environ Med*, 2003; 60(12): 935-41.
7. National Institute of Allergy and Infectious Diseases. Common cold (Overview). Bethesda, USA: National Institute of Allergy and Infectious Diseases. 2010 [Online].
8. British Lung Foundation. Facts about respiratory disease. London: British Lung Foundation. 2010 [Online].
9. Papenburg J et al. House hold transmission of the 2009 pandemicA/ H1N1 influenza virus: Elevated laboratory- conformed secondary attack rates and evidence of asymptomatic infections. *Clin infectious dis*, 2010; 51: 1033-1041.
10. Savage R et al. Assessing secondary attack rates among house hold contacts at the beginning of the influenza A (H1N1) pandemic in Ontario, Canada, April-June 2009: A prospective, observational study. *BMC Public Health*, 2011; 11: 234.
11. Senthilkumar B et al. Practical Microbiology- *A Laboratory Manual*, Panima Publishing Corporation, New Delhi, India. 2014a; DOI: 10.13140/2.1.2667.6163.
12. Lateef A et al. Antimicrobial resistance of bacterial strains isolated from orange juice products. *Afr. J. Biotechnol*, 2004; 3(6): 334-338.
13. Whiteson KL et al. The upper respiratory tract as a microbial source for pulmonary infections in Cystic Fibrosis: Parallels from Island Biogeography. *Am J Respir Crit Care Med*, 2014 [Epub ahead of print].

14. El-Mahmood AM et al. Antimicrobial susceptibility of some respiratory tract pathogens to commonly used antibiotics at the specialist hospital, Yola, Adamawa, Nigeria. *J. Clin. Med. Res*, 2010; 2(8): 135-142.
15. Taura DW et al. Bacterial isolates of the respiratory tract infection and their current sensitivity pattern among patients attending Aminu Kano Teaching Hospital Kano-Nigeria. *Int J Microbiol Res*, 2013; 4(9): 226-231.
16. Tushar KA et al. Molecular detection of virulence genes associated with pathogenicity of *Gram positive* isolates obtained from respiratory tract of apparently healthy as well as sick goats. *Vet World*, 2012; 5(11): 676-681.
17. Wessels MR. Clinical practice: streptococcal pharyngitis. *N Engl J Med*, 2011; 364: 648–655.
18. Wilson JF. In the clinic: acute sinusitis. *Ann Intern Med*, 2010; 153: ITC3-2–ITC3-14.
19. Wenzel RP, Fowler AA. Clinical practice. Acute bronchitis. *N Engl J Med*, 2006; 355(20): 2125-2130.
20. Zernotti ME et al. Evidence of Bacterial Biofilms in Nasal Polyposis. *J Investig Allergol Clin Immunol*, 2010; 20(5): 380-385.
21. Senthilkumar B, et al. Induction of deletion mutation on *omp*R gene of *Salmonella enterica serovar Typhi* isolates from asymptomatic typhoid carriers to evolve attenuated strains for vaccine development. *APJTM*, 2014b; 7(12): 933-939.
22. Yves G et al. Factors Predicting Mortality in Necrotizing Community-Acquired Pneumonia Caused by *Staphylococcus aureus* Containing Panton-Valentine Leukocidin. *Clin Infect Dis*, 2007 1; 45(3): 315-21
23. Rajasekarapandian M, Senthilkumar B. *Immunology & Immunotechnology*. Panima Publishing Corporation, New Delhi, India. 2007; DOI: 10.13140/2.1.5033.0565.