INTRODUCTION

The infections of lower respiratory tract are foremost cause of diseases and have severe consequence on the human health throughout the human race universally. Among lower respiratory infections, pneumonia and bronchitis are most frequent clinical conditions. Bacteria behave critical in succession of acute and chronic diseases in order to check the life sustainability (Noecker et al., 2017). Chronic obstructive pulmonary disease positioned central caused by bacterial
infections that leads to great mortality and morbidity among all age groups especially children and old age individuals. Pneumonia is a major infectious disease both in brood and elders (Sridhar et al., 2015). The infections of trachea and bronchial tree (tracheitis, bronchitis and broncholitis) or the infections of lung tissues (alveolitis and pneumonia) are categorized as lower respiratory tract infections. *Streptococcus pneumoniae*, *Klebsiella pneumoniae* or *Legionella pneumophilia* are responsible for the consolidation of a single lobe of lung. *Pseudomonas aeruginosa* and *Serratia marcescens* are linked with a severe necrotizing pneumonia in immune-compromised patients (Taubenberger et al., 2008). Lung abscess altering into cavitary lesions are seen with numerous species of anaerobes and *Staphylococcus aureus* (Yazbeck et al., 2014). Septic pulmonary emboli are seen in patients that have IV drug history use or contaminated indwelling devices (right-sided prosthetic heart valves, pacemaker wires and central venous catheters). Physical examination is supposed to comprise an assessment of signs of endocarditis and assessment of contaminated indwelling devices. In patients with IV drug use, the probable pathogenic agent is *Staphylococcus aureus*. *Mycoplasma pneumoniae* and *Bordetella pertussis* play a significant role are posing lower respiratory tract infections in children and old age population also. Pneumonia is ranked fourth in diseases causing death in the world. WHO reported in the top 10 causes of death, by year 2018 that Pakistan poisoned third number where pneumonia is most cause of deaths in children of fifteen countries having high death rate alarmingly. Approximately, 71,000 children die because of pneumonia in Pakistan every year according to in Dawn news paper 13th November 2014. The un-encapsulated strains of bacteria are imperative cause of lower respiratory tract infections in patients with cystic fibrosis reported by Centers for Disease Control and Prevention, in "Q, fevers Symptoms, Diagnosis, and Treatment, 2013. The etiological diagnosis in infections of lower respiratory tract may be valuable for both prognostic and therapeutic purposes. Invasive collecting techniques may be obligatory to diagnose pulmonary infections in patients not capable of producing sputum (Dheda et al., 2016). Processing bronchoscopy to gain specimens is superior to other conventional methods. Specimens that can be obtained include bronchial washings, bronchial brushings, bronchio-alveolar lavage fluid and trans-bronchial biopsy specimens. Bronchoscopy has been recommended as a technique of establishing nosocomial pneumonia diagnosis and to investigate the microbial infections (Dheda et al., 2016). Published sensitivity and specificity rates for the protected brushes use, broncho alveolar lavage and washings have reached 80-90%. The objectives of this research study were to detect pathogenic bacteria from respiratory tree and to determine their antibiotic sensitivity in order to provide better diagnostic technique for optimal treatment as well as searching reality of hospital acquired infection contamination for population recruited for the treatment of common disease.

**MATERIALS AND METHODS**

This study was carried out on the patients visiting Jinnah hospital Lahore Pakistan and processed in Microbiology laboratory of Allama Iqbal Medical College Lahore. This research was conducted from March 2019 to December 2019. Over the course of study, total of 128 samples were collected as shown in Table 1.
Antibiotic Sensitivity of Bronchial Wash Pathogens

Table 1: Showing age and gender wise distribution of study participants

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Males</th>
<th>%</th>
<th>Females</th>
<th>%</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>10-20</td>
<td>04</td>
<td>05.56</td>
<td>12</td>
<td>22.23</td>
<td>16</td>
<td>12.70</td>
</tr>
<tr>
<td>21-30</td>
<td>13</td>
<td>18.05</td>
<td>10</td>
<td>18.52</td>
<td>23</td>
<td>18.25</td>
</tr>
<tr>
<td>31-40</td>
<td>08</td>
<td>11.12</td>
<td>09</td>
<td>16.67</td>
<td>17</td>
<td>13.49</td>
</tr>
<tr>
<td>41-50</td>
<td>07</td>
<td>09.73</td>
<td>04</td>
<td>07.41</td>
<td>11</td>
<td>08.73</td>
</tr>
<tr>
<td>51-60</td>
<td>21</td>
<td>29.17</td>
<td>12</td>
<td>22.23</td>
<td>33</td>
<td>26.19</td>
</tr>
<tr>
<td>61-70</td>
<td>11</td>
<td>15.27</td>
<td>03</td>
<td>05.56</td>
<td>14</td>
<td>11.12</td>
</tr>
<tr>
<td>&gt;71</td>
<td>08</td>
<td>11.12</td>
<td>04</td>
<td>07.41</td>
<td>12</td>
<td>09.52</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>100</td>
<td>54</td>
<td>100</td>
<td>126</td>
<td>100</td>
</tr>
</tbody>
</table>

Collection of Bronchial Washings
Bronchial washings were collected during bronchoscopy in Tuberculosis, Chest, Medicine, ENT, and Gynae wards of Jinnah Hospital Lahore. A flexible fiberoptic bronchoscope was used to take the bronchial washings. About 30-50 ml of saline induced into the bronchioles and at least 5 ml of bronchial washings was collected in a sterile container. Bronchial washings were sent immediately to the Microbiology Laboratory Allama Iqbal Medical College Lahore for further processing.

Media Preparation
The media used were Nutrient Agar (NA), Chocolate Agar, MacConkey Agar, Blood Agar and Sabouraud dextrose Agar growth media as described in Monica Cheesbrough Microbiology book 2006. Other media were prepared according to the manufacturer’s instructions (Cheesbrough, 2006).

Sample Culturing
The collected bronchial washings were processed for culture and sensitivity test finally reported according to standard method procedure and protocol. In order to perform visual examination and calibrated Loop Streak method was performed for bronchial washings culture. The culture plates were incubated at 35 to 37 °C for 24-48 hours. Following the well appeared growth and isolated bacterial colonies were subjected to Gram staining, biochemical tests and antibiotic sensitivity test (Cheesbrough 2006).

Identification of Microorganisms
All isolated bacterial pathogens were identified and confirmed on the basis of colonial morphology, cellular morphology, Gram stain, Catalase test, Oxidase tests and DNAs test parameters. From the outcome of first round identification tests the gram negative bacteria were processed following regular biochemical tests such as Indole production, Citrate utilization Motility and Urease production tests as well as Triple sugar iron reaction (Cheesbrough, 2006).

Antibiotic Sensitivity
The entire isolated pathogens were processed for a variety of antibacterial agents in vitro, antibiotic susceptibility was tested in laboratory by disc diffusion assay on Muller Hinton Agar, modified by Kirby-Bauer method (Bartlett, 1994), using the following antibiotic discs, Augmentin, Penicillin, Sulzone, Cefotaxime, Cefoperazone, Amikacin, Gentamicin, Ciprofloxacin,
Antibiotic Sensitivity of Bronchial Wash Pathogens

Imipenum, Novobiocin, Tazocin, Erythromycin, and Tetracycline.

Statistical Analysis
The data was subjected to statistical analysis using SPSS 22 software and results were obtained as frequencies. Evaluations were conceded out at 95% assurance level and \( P < 0.05 \) value was measured statistically considerably.

RESULTS
Out of 126 specimens of bronchial washings 44 (34.92%) specimens revealed no bacterial pathogen. Microbial etiology was determined in 82(65.08%) of bronchial washings. These Eighty two specimens revealed Pseudomonas aeruginosa 25(30.48%), Acinetobacter, 17 (20.73%), Escherichia coli, 07(8.54%), Haemophilus influenzae 3(3.66%), Proteus mirabiles 2 (2.44%), Klebsiella Pneumonia 3(3.66%); B-haemolytic streptococci 6(7.32%), Streplococcus pneumonia 7(8.54%), Staphylococcus aureus, 6(7.32%); Neisseria meningitidis 3(3.66%); Candida species 3(3.66%) pathogenic microbial recover from male samples are more as compared to females as shown in Table 2.

The results of antibiotic sensitivity concealed great variations for isolated bacterial pathogenic strains; most of bacteria have adapted resistance against various commonly used antimicrobial agents especially recruited for this study. In this research, it was observed that the antibiotic sensitivity of the isolated pathogens against tested antibiotics differed with the species to species. Pseudomonas aeruginosa presented resistance against Augmentin, and Cefoperazone. Acinetobacter species were resistant against Augmentin, Cefoperazone, Gentamicin, and Tazocin. Escherichia coli have shown less sensitivity against Augmentin, Cefotaxime, Cefoperazone and Gentamicin. Haemophilus influenzae was not much sensitive against Amikacin, Sulzone and Tazocin. Proteus mirabilis was resistant against Tazocin at great extent. Klebsiella pneumonia sensitivity was low for Cefotaxime. All isolate of gram negative bacteria were not commonly sensitive to all tested antibiotic as shown in Table 3.

\[
\begin{array}{|c|c|c|c|c|}
\hline
\text{Isolated Pathogens} & \text{Males} & \% \text{age} & \text{Females} & \% \text{age} & \text{Total} & \% \text{age} \\
\hline
\text{Gram Negative Bacteria} & & & & & & \\
\hline
\text{Pseudomonas aeruginosa} & 14 & 29.78 & 11 & 31.43 & 25 & 30.48 \\
\text{Acinetobacter} & 08 & 17.02 & 09 & 25.71 & 17 & 20.73 \\
\text{Escherichia coli} & 03 & 06.38 & 04 & 11.43 & 07 & 08.54 \\
\text{Haemophilus influenzae} & 01 & 02.13 & 02 & 05.71 & 03 & 03.66 \\
\text{Proteus mirabiles} & 01 & 02.13 & 01 & 02.86 & 02 & 02.44 \\
\text{Klebsiella Pneumonia} & 02 & 04.26 & 01 & 02.86 & 03 & 03.66 \\
\hline
\text{Gram Positive Bacteria} & & & & & & \\
\hline
\text{B-haemolytic Streptococci} & 04 & 08.51 & 02 & 05.71 & 06 & 07.32 \\
\text{Streptococcus pneumoniae} & 05 & 10.64 & 02 & 05.71 & 07 & 08.54 \\
\text{Staphylococcus aureus} & 05 & 10.64 & 01 & 02.86 & 06 & 07.32 \\
\text{Neisseria meningitidis} & 02 & 04.26 & 01 & 02.86 & 03 & 03.66 \\
\hline
\text{Fungi} & & & & & & \\
\hline
\text{Candida species} & 02 & 04.26 & 01 & 02.86 & 03 & 03.66 \\
\hline
\text{Total} & 47 & 100 & 35 & 100 & 82 & 100 \\
\hline
\end{array}
\]
Antibiotic Sensitivity of Bronchial Wash Pathogens

Table 3: Percentage susceptibility of Gram negative isolates of bronchial washings:

<table>
<thead>
<tr>
<th>Organisms</th>
<th>AMC%</th>
<th>AK%</th>
<th>CIP%</th>
<th>CTX%</th>
<th>CPZ%</th>
<th>GEN%</th>
<th>IMP%</th>
<th>SCF%</th>
<th>TZP%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>S=43</td>
<td>S=72</td>
<td>S=89</td>
<td>S=70</td>
<td>S=40</td>
<td>S=92</td>
<td>S=90</td>
<td>S=78</td>
<td>S=73</td>
</tr>
<tr>
<td></td>
<td>R=57</td>
<td>R=12</td>
<td>R=11</td>
<td>R=30</td>
<td>R=60</td>
<td>R=80</td>
<td>R=10</td>
<td>R=12</td>
<td>R=27</td>
</tr>
<tr>
<td>Acinetobacter species</td>
<td>S=21</td>
<td>S=91</td>
<td>S=42</td>
<td>S=87</td>
<td>S=31</td>
<td>S=30</td>
<td>S=79</td>
<td>S=83</td>
<td>S=20</td>
</tr>
<tr>
<td></td>
<td>R=79</td>
<td>R=09</td>
<td>R=58</td>
<td>R=13</td>
<td>R=69</td>
<td>R=70</td>
<td>R=21</td>
<td>R=17</td>
<td>R=80</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>S=43</td>
<td>S=85</td>
<td>S=93</td>
<td>S=60</td>
<td>S=51</td>
<td>S=39</td>
<td>S=77</td>
<td>S=79</td>
<td>S=73</td>
</tr>
<tr>
<td></td>
<td>R=67</td>
<td>R=15</td>
<td>R=07</td>
<td>R=40</td>
<td>R=49</td>
<td>R=51</td>
<td>R=33</td>
<td>R=21</td>
<td>R=17</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>S=65</td>
<td>S=33</td>
<td>S=83</td>
<td>S=71</td>
<td>S=60</td>
<td>S=64</td>
<td>S=82</td>
<td>S=41</td>
<td>S=23</td>
</tr>
<tr>
<td></td>
<td>R=35</td>
<td>R=77</td>
<td>R=17</td>
<td>R=29</td>
<td>R=40</td>
<td>R=36</td>
<td>S=82</td>
<td>R=18</td>
<td>R=77</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>S=70</td>
<td>S=83</td>
<td>S=77</td>
<td>S=63</td>
<td>S=86</td>
<td>S=96</td>
<td>S=93</td>
<td>S=72</td>
<td>S=30</td>
</tr>
<tr>
<td></td>
<td>R=30</td>
<td>R=17</td>
<td>R=27</td>
<td>R=37</td>
<td>R=14</td>
<td>R=14</td>
<td>R=07</td>
<td>R=28</td>
<td>R=70</td>
</tr>
<tr>
<td>Klebsiella Pneumonia</td>
<td>S=93</td>
<td>S=94</td>
<td>S=71</td>
<td>S=30</td>
<td>S=84</td>
<td>S=67</td>
<td>S=93</td>
<td>S=77</td>
<td>S=68</td>
</tr>
<tr>
<td></td>
<td>R=07</td>
<td>R=06</td>
<td>R=29</td>
<td>R=70</td>
<td>R=16</td>
<td>R=33</td>
<td>R=07</td>
<td>R=30</td>
<td>R=32</td>
</tr>
</tbody>
</table>

S: Sensitive, R: Resistant
AMC-Augmentin, AK-Amikacin, CIP-Ciprofloxacin, CTX-Cefotaxime, CPZ-Cefoperazone, GEN-Gentamicin, IMP-Imipenum, SCF-Sulzone, TZP-Tazocin

Streptococcus pneumonia has not expressed considerable sensitivity against Cefotaxime, Tetracycline and Imipenum. Haemolytic streptococi not showed high susceptibility against Augmentin, Tetracycline and Imipenum. Staphylococcus aureus was not among highly susceptible to isolates against Cefotaxime. Neisseria meningitidis is not consider able sensitive against Augmentin, Erythromycin, Tetracycline and Imipenum. There was no commonly tested antibiotic for all isolated Gram positive bacteria also as shown in Table 4.

Table 4 Percentage susceptibility of Gram positive isolates of UTIs

<table>
<thead>
<tr>
<th>Organisms</th>
<th>AMC%</th>
<th>CIP%</th>
<th>CTX%</th>
<th>CPZ%</th>
<th>E%</th>
<th>GEN%</th>
<th>P%</th>
<th>TE%</th>
<th>IMP%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumonia</td>
<td>S=24</td>
<td>S=96</td>
<td>S=40</td>
<td>S=70</td>
<td>S=80</td>
<td>S=78</td>
<td>S=71</td>
<td>S=67</td>
<td>S=49</td>
</tr>
<tr>
<td></td>
<td>R=76</td>
<td>R=14</td>
<td>R=60</td>
<td>R=30</td>
<td>R=20</td>
<td>R=12</td>
<td>R=29</td>
<td>R=33</td>
<td>R=51</td>
</tr>
<tr>
<td>B-haemolytic Streptococci</td>
<td>S=44</td>
<td>S=70</td>
<td>S=75</td>
<td>S=61</td>
<td>S=77</td>
<td>S=85</td>
<td>S=72</td>
<td>S=54</td>
<td>S=60</td>
</tr>
<tr>
<td></td>
<td>R=66</td>
<td>R=30</td>
<td>R=25</td>
<td>R=39</td>
<td>R=33</td>
<td>R=15</td>
<td>R=18</td>
<td>R=46</td>
<td>R=40</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>S=91</td>
<td>S=73</td>
<td>S=47</td>
<td>S=77</td>
<td>S=81</td>
<td>S=70</td>
<td>S=60</td>
<td>S=73</td>
<td>S=71</td>
</tr>
<tr>
<td></td>
<td>R=09</td>
<td>R=17</td>
<td>R=53</td>
<td>R=33</td>
<td>R=29</td>
<td>R=30</td>
<td>S=40</td>
<td>R=17</td>
<td>S=29</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>S=60</td>
<td>S=75</td>
<td>S=99</td>
<td>S=77</td>
<td>S=34</td>
<td>S=71</td>
<td>S=88</td>
<td>S=40</td>
<td>S=55</td>
</tr>
<tr>
<td></td>
<td>R=40</td>
<td>R=25</td>
<td>R=01</td>
<td>R=13</td>
<td>R=66</td>
<td>R=29</td>
<td>R=12</td>
<td>R=60</td>
<td>R=45</td>
</tr>
</tbody>
</table>

S: Sensitive, R: Resistant
AMC-Augmentin, CIP-Ciprofloxacin, CTX- Cefotaxime, CPZ-Cefoperazone, E-Erythromycin, GEN-Gentamicin, P- Penicillin and TE-Tetracycline, IMP-Imipenum
DISCUSSION

Pneumonia and influenza are among major causes of morbidity and mortality in the most developed nations and developing countries worldwide (Kochanek et al., 2016). Adults about 65 years old and children <5 years, pregnant women and individuals with chronic clinical conditions are at high risk for superimposed infections and other complications that may lead hospitalization resulting many death each year (Lee et al., 2010). According to a report only influenza responsible for estimated 710,000 cases of hospitalization and 56,000 deaths in the United States each year (Rolfes et al., 2016). The pneumonia and influenza mortality rate was predicted at 18/100,000 in American population. According to Centers for Disease Control and Prevention NC for HS, Compressed Mortality File 1999-2015, on CDC WONDER Online Database, CMF 1999–2015, Series 20, No 2U. 2016 collectively Influenza mortality rate was 18/100,000 residents of all age groups 102/100,000 in older than 65 years by the year 2015. Lower respiratory tract infections cause high mortality rate in third world countries as well as damage pose effects on international community. A research conducted by Ruiz et al to investigate etiology of community acquired infections they processed 395 specimens of bronchial washings and investigated that from those 395 specimens 182 (46%) were positive for pathogens and 227 pathogens were detected in that study (Ruiz et al., 1999). The five leading pathogenic microorganisms were determined as 65 (29%) Streptococcus pneumoniae, 25 (11%) Haemophilus influenza, 23 (23%), influenza virus A and B, 12 cases Pseudomonas aeruginosa 5% cases were of pneumonia. Our results are consistent as at greater extent with the results of Ruiz and his coworkers. Fine and his coworkers (Fine et al., 1996), conducted various researches which were based on bronchial washings they determined Streptococcus responsible for community acquired pneumonia. Approximate 2/3 of all cases were bacteriomic pneumonia. Our study also showed consistent results with Fine and his coworkers (Fine et al., 1996). Lindsay also conducted investigated 2,011 patients ill with pneumonia, 94 cases were intubated and 84 were processed for tracheal aspirate, of those 84 cases 47 (56%) possessed pulmonary pathogen identified by tracheal aspirate culture, 80 specimen were also performed for blood cultures and 71 undergone Pneumococcal and Legionella urinary antigen testing. A microbiological analysis was performed in 55 cases by any suitable diagnostic methodology, in 39 cases the tracheal aspirate culture test was the only positive showing exclusive microbiological diagnosis in patients who would have otherwise been classified as “culture negative (Lindsay et al., 2016). Our instigative experiments detected leading isolates from bronchial washing were Pseudomonas- aeruginosa 25(30.48%), Acinetobacter,17 (20.73%), Escherichia coli, 07(8.54%), Haemophilus influenzae 3(3.66%), Proteus mirabiles 2 (2.44%), Klebsiella Pneumonia 3(3.66%); B- haemolytic streptococci 6(7.32%), Streptococcus pneumonia 7(8.54%), Staphylococcus aureus, 6(7.32%); Neisseria meningitidis 3(3.66%); Candida species 3(3.66%). Sputum is the most common specimen obtained specimen but it is the most problematic. Bronchoalveolar washings by bronchoscopy have been recommended as a technique of making identification of lower respiratory tract infections as well as a
Antibiotic Sensitivity of Bronchial Wash Pathogens

way to determine the accurate and easiest microbiological diagnosis (Lindsay et al., 2016).

CONCLUSION
From our current study it is concluded that Broncho-alveolar washings for isolation and identification of etiological micro-organisms in the lower respiratory tract infections are superior and most reliable sample technique which is significant to determine single pathogenic organism to authenticate accurate urgent treatment. Isolation of respiratory tract infections pathogenic organisms via bronchial washings is the way to investigate treatment of superimposed infections regarding pulmonary specimens.

ACKNOWLEDGEMENT
I am very thankful to Microbiology Laboratory Allama Iqbal Medical College Lahore.

REFERENCES


Antibiotic Sensitivity of Bronchial Wash Pathogens