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ORIGINAL ARTICLE

Biofilm Producing Pseudomonas Aeruginosa in Patients with Lower Respiratory Tract Infections

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ABSTRACT

Background and Aim: Pseudomonas aeruginosa is a vital pathogen causing major life-threatening infections among respiratory disease patients that leads to higher morbidity and mortality. Lower respiratory tract infections (LRTIs) are amongst these infections commonly found in hospitalized patients. Antibiotic tolerant biofilms formation and bacterium high intrinsic resistance to antibiotics make these infections difficult to treat. The present study aimed to assess the biofilm producing pseudomonas aeruginosa in lower respiratory tract infections patients.

Methodology: This cross-sectional study was carried out on 246 Lower respiratory tract infections patients admitted to ICUs of Sharif Medical and Dental College Lahore and Mohi-ud-Din Teaching Hospital, Mirpur Azad Kashmir for the duration from June 2021 to December 2021. The signs and symptoms of LRTIs were investigated in each patient. Prior to study conduction, ethical approval was obtained from the institutes research and ethical committee. Each individual provided written informed consent. Identification of isolated organisms and antimicrobial sensitivity was done. Microtiter method was used for all the isolated, to imperil the isolates of P. aeruginosa for biofilm detection. SPSS version 25 was used for the data analysis.

Results: Of the total 246 specimens consisted of sputum 198 (80.5%), pleural fluid 19 (7.7%), suction tube 16 (6.5%), and tracheostomy tip specimens 13 (5.3%). The incidence of positive culture was 78 (31.7%). Out of 78 positive cultures, sputum 56 (71.8%) was the major factor followed by pleural fluid 3 (3.8%), suction tube 12 (15.4%) and tracheostomy tip 7 (9.0%). Of the 78 positive specimens, the prevalence of single bacterium and poly-microbial infection was 58 (74.4%) and 20 (25.6%) respectively. The poly-microbial infection consisted of sputum 10 (50%), pleural fluid 1 (5.0%), suction tube 6 (30.0%), and tracheostomy tube 3 (15%). Out of 78 positive culture specimens, isolated gram-negative bacteria were 102, out of which K. pneumonia and P. aeruginosa were present in 46 (45.1%) and 24 (23.5%) respectively. Regarding the resistance pattern of gram-negative respiratory pathogens, Cefepime (86.9%) and ceftriaxone (91.9%) were given higher resistance by K. pneumoniae. Likewise, higher numbers of Acinetobacter spp provided resistance to cefepime (86.7%) and ceftriaxone (87%). Out of 24 P. aeruginosa isolates, biofilm producers were found in 16 isolates, in which the incidence of strong, moderate, and weak biofilm producers was 3 (18.7%), 5 (31.3%), and 8 (50%) respectively. Sputum in 11 (68.8%) was the prevalent biofilm producer isolates followed by suction tube 4 (25%) and tracheostomy tube 1 (6.3%). The incidence of inpatients and outpatients were 15 (93.8%) and 1 (6.2%) respectively.

Conclusion: Our study concluded that gram-negative bacteria was the predominant and had increased resistance to antibiotics in Lower respiratory tract infections (LRTIs) patients. In LRTIs, the most common organism was K. pneumonia. Also, P. aeruginosa isolated in LRTIs patients were susceptible to producing biofilm. Biofilm producers had higher resistance than biofilm non-producers.

Keywords: Biofilm producing P. aeruginosa, K. pneumonia, Lower respiratory tract infections

INTRODUCTION

Lower respiratory tract infections (LRTIs) are the most prevalent infections caused by highly susceptibility to nosocomial infections among patients admitted to intensive care units (ICU) [1, 2]. Lack of mobility, prolonged hospitalization, and various medication's exposure cause these nosocomial infections. The prevalence of nosocomial infections varies from 2.3% to 49.2% in developing countries [3, 4]. The increasing mortality of LRTIs are significantly associated with Pseudomonas specimens causing nosocomial infections. P. aeruginosa among Pseudomonas specimens is considered as the most prevalent species related to severe LRTIs among ICUs admitted patients [5]. Ventilator-associated pneumonia is the second most common pathogen that causes severe LRTIs [6]. A China-based study revealed that P. aeruginosa was the most prevalent isolated pathogen of LRTIs that accounts for 13.4% cases [7]. In US, a multicenter cross-sectional study reported that P. aeruginosa (36.2%) was the most prevalent isolated gram-negative organism in LRTIs patients admitted to ICUs [8].

The etiologies of respiratory infections play a vital role in the diagnostic process. LRTIs might be caused by various organisms. The most common bacteria that causes lower respiratory tract infections were gram-negative bacteria [9]. LRTIs complications could be minimized by microbiological investigations. P. aeruginosa inhabits and impaired the defense mechanism of the host by forming a biofilm [10]. P. aeruginosa survival mainly relies on biofilm formation mechanisms that provide protection against antibiotic therapy and hot immune system, thus antibiotic resistance is rendered and supports the infection's chronicity [11]. Biofilm formation might lead to respiratory failure and deterioration of lung function that comes from the Pseudomonas aeruginosa pathogenesis [12]. Tube test is the most frequently used method for determining the biofilm formation [13], where bacterial film forms lines on culture tubes stained with cationic dye and visually scaled. Microtiter-plate test is used for determining the biofilm formation by spectrophotometrically measuring the optical density of stained bacterial film [14]. The aim of the present study was to assess the biofilm producing pseudomonas aeruginosa in lower respiratory tract infections patients.

METHODOLOGY

This cross-sectional study was carried out on 246 Lower respiratory tract infections patients admitted to ICUs of Sharif Medical and Dental College Lahore and Mohi-ud-Din Teaching Hospital, Mirpur Azad Kashmir for the duration from June 2021 to December 2021. The signs and symptoms of LRTIs were investigated in each patient. Prior to study conduction, ethical approval from the institute research and ethical committee. Each individual provided written informed consent. Identification of isolated organisms and antimicrobial sensitivity was done. Microtiter method was used for all the isolated, to imperil the isolates of P. aeruginosa for biofilm detection. Non-duplicate

respiratory specimens such as sputum, tracheostomy tube, suction tube, and pleural fluid were examined for the symptoms of lower respiratory tract infections. All the specimens with visible contaminations and not properly labeled were excluded. Blood Agar and Mac Conkey Agar medium were used for incubating the specimens at 37°C for duration of 24 hours to 48 48 hours. Colony morphology, microscopy, and biochemical tests were used for the identification of bacterial isolates. Of the total isolates, biofilm formation was detected in P. aeruginosa isolated using microtiter plate method. The strong, moderator, and weak bio-film producer were distinguished based on optical densities as follows; i) Non-biofilm producer OD ≤ ODc (≤0.658), strong biofilm producer 4× ODc < OD (>2.632), moderate biofilm producer 2× ODc < OD ≤ 4 × ODc (>1.316-2.632), and weak biofilm producer ODc < OD ≤ 2× ODc (>0.658-1.361).

SPSS version 25 was used for data analysis. Quantitative variables were described as mean and standard deviation. Qualitative variables were expressed as frequency and percentage. Chi-square test was used for comparing different specimens and their resistance to antibiotic using 95% confidence interval and 5% level of significance.

RESULTS

Of the total 246 specimens consisted of sputum 198 (80.5%), pleural fluid 19 (7.7%), suction tube 16 (6.5%), and tracheostomy tip specimens 13 (5.3%). The incidence of positive culture was 78 (31.7%). Out of 78 positive cultures, sputum 56 (71.8%) was the major factor followed by pleural fluid 3 (3.8%), suction tube 12 (15.4%) and tracheostomy tip 7 (9.0%). Of the 78 positive specimens, the prevalence of single bacterium and poly-microbial infection was 58 (74.4%) and 20 (25.6%) respectively. The poly-microbial infection consisted of sputum 10 (50%), pleural fluid 1 (5.0%), suction tube 6 (30.0%), and tracheostomy tube 3 (15%). Out of 78 positive culture specimens, isolated gram-negative bacteria were 102, out of which K. pneumonia and P. aeruginosa were present in 46 (45.1%) and 24 (23.5%) respectively. Regarding the resistance pattern of gram-negative respiratory pathogens, Cefepime (86.9%) and ceftriaxone (91.9%) were given higher resistance by K. pneumoniae. Likewise, higher numbers of Acinetobacter spp provided resistance to cefepime (86.7%) and ceftriaxone (87%).

Table-1: distribution of sputum, pleural fluid, tracheostomy tube, and suction tube in all the specimens (n=246)

Specimens	Frequency N	Percentage %
Sputum	198	80.5
Pleural fluid	19	7.7
Suction tube	16	6.5
Tracheostomy tube	13	5.3
Total	246	100

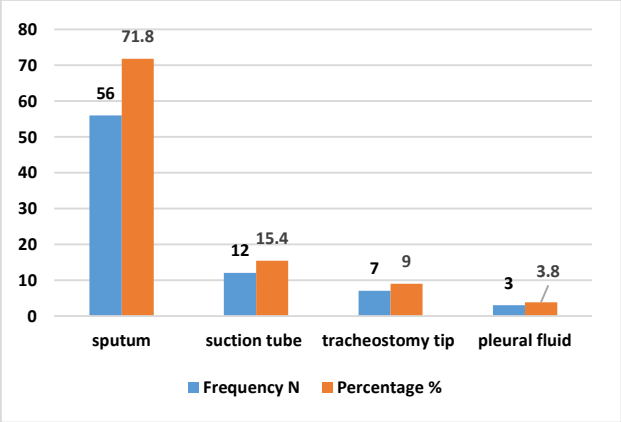

Figure-1: incidence of sputum, pleural fluid, tracheostomy tip, and suction tube among positive culture specimens (n=78)

Out of 24 P. aeruginosa isolates, biofilm producers were found in 16 isolates, in which the incidence of strong, moderate, and weak biofilm producers was 3 (18.7%), 5 (31.3%), and 8 (50%) respectively. Sputum in 11 (68.8%) was the prevalent biofilm producer isolates followed by suction tube 4 (25%) and tracheostomy tube 1 (6.3%).The incidence of inpatients and outpatients were 15 (93.8%) and 1 (6.2%) respectively. Table-I shows the distribution of sputum, pleural fluid, tracheostomy tube, and suction tube in all the specimens. The incidence of sputum, pleural fluid, tracheostomy tip, and suction tube among positive culture specimens (78) are depicted in Figure-1. Figure-2 illustrates the prevalence of single bacterium and poly-microbial infections. Distribution of bacterial isolates are shown in Figure-3. Table-II represents the distribution of poly-microbial infections. Gram-negative respiratory pathogens resistance pattern is illustrated in Figure-4. Figure-5 demonstrate the prevalence of strong, moderate, and weak biofilm producers in P. aeruginosa 24 isolates.

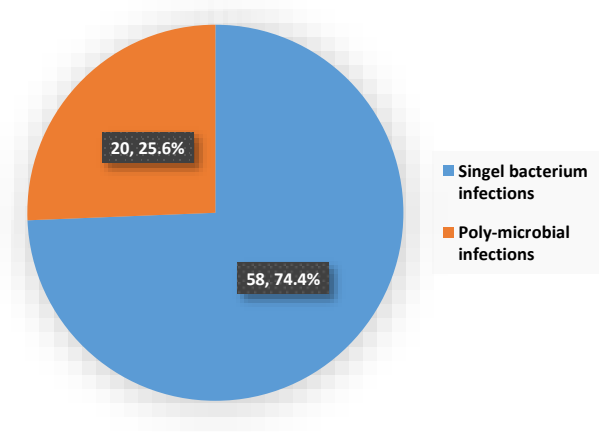

Figure-2: prevalence of single bacterium and poly-microbial infections in gram-positive isolates (n=78)

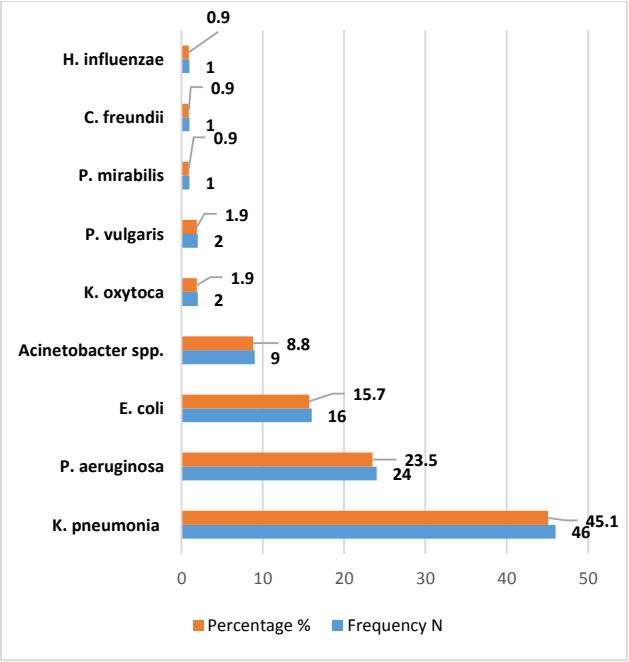

Figure-3: Distribution of gram-negative bacterial isolates (n=102)

Table-2: distribution of single bacterium and poly-microbial infections (n=20)

Specimens	Mono-microbial growth N (%)	Poly-microbial infections N (%)
Sputum	46 (79.3)	10 (50)
Pleural fluid	11 (19)	1 (5)
Suction tube	1 (1.7)	6 (30)
Tracheostomy tube	0 (0)	3 (15)
Total	58 (100)	20 (100)

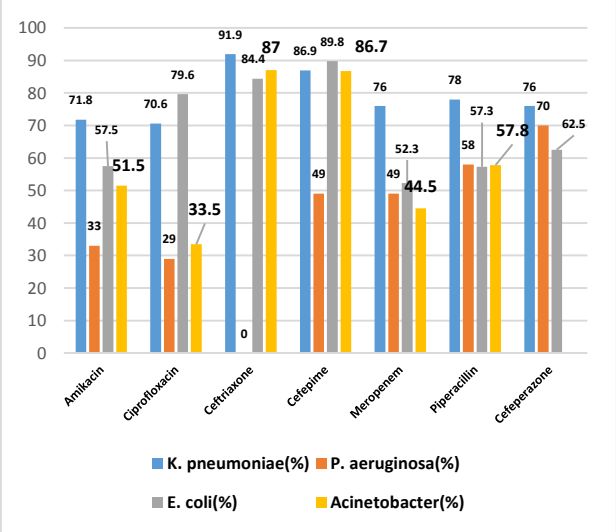

Figure-4: pattern of resistance to Gram-negative respiratory pathogens

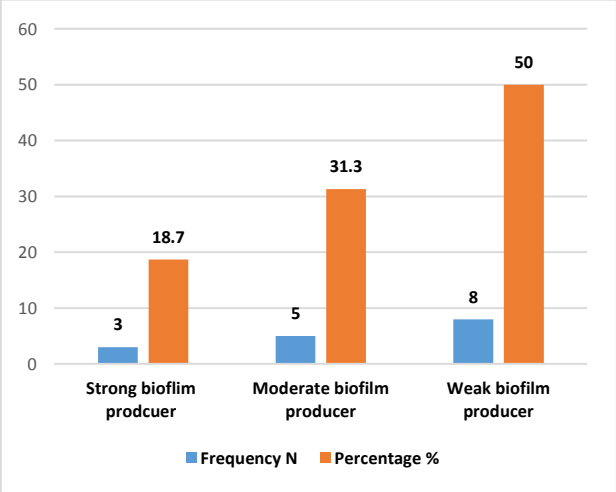

Figure-5: prevalence of strong, moderate, and weak biofilm producers in P. aeruginosa 24 isolates

DISCUSSION

The present study mainly focused on the biofilm producing pseudomonas aeruginosa in lower respiratory tract infections patients and found that gram-negative bacteria was the predominant and had increased resistance to antibiotics in Lower respiratory tract infections (LRTIs) patients. In LRTIs, the most common organism was K. pneumonia. Also, P. aeruginosa isolated in LRTIs patients were susceptible to producing biofilm. Respiratory tract infections are the second most prevalent cause for hospital assimilated infections. Medical management fundamental part included detailed assessment and history taking, comprehensive clinical examination, and underlying comorbidities. Elderly patients with cystic fibrosis, respiratory infections, diabetes, immunocompromised hosts, cardiac comorbidities, and HIV are more susceptible to infections [15]. The majority of cases are caused by bacterial infections, which necessitate antibiotic therapy as the primary treatment. The microbiological diagnosis of LRTIs is frequently difficult because specimen collection for the investigation involves the risk of contamination by microbes that live in the upper respiratory tract, necessitating the use of sophisticated invasive procedures [16].

P. aeruginosa as a multidrug-resistant has been found globally and recognized as the major cause for health associated infections in LRTIs patients [17]. The LRTIs patients' sputum originated the biofilm formation capacity. The possible specimen contamination caused by upper respiratory tract microorganisms is made perplexing the microbiological diagnosis of lower tract infection [18]. The important parameters that play a key role are appropriate clinical specimens and techniques. In the present study, P. aeruginosa in Pseudomonas strains were found in 23.5% isolates. The incidence of P. aeruginosa was significantly higher in our study compared to 13.9% prevalence reported in a study by Nepal et al. [19]. However, the prevalence was higher 24% and 26.8% in a study done in Pakistan and India respectively [20, 21]. Another study conducted in China reported 13.4% prevalence of P. aeruginosa in LRTIs patients [22].

K. pneumoniae and Gram-negative bacteria were the most isolated pathogens from LTRIs. The pattern of antibiotic resistance to these pathogens on a routine basis increased as found in the present study. Bacterial pathogens are the major reasons for huge health issues in managing LRTIs patients. Additionally, it has been found that P. aeruginosa isolated are more susceptible to producing biofilms in LRTIs patients. Comparing the biofilm non-producers, biofilm producers were more resistive which added the antimicrobial therapy challenges to treat these infections. The LRTIs incidence was reported 31.7% higher than a reported 24.6% in a previous study [23]. Kunwar et al [24] and Ieven et al [25] reported that incidence of culture positivity was 44.4% and 59% respectively. The lower prevalence of LRTIs was due to the atypical bacteria, antibiotic prior use, and viral bacteria exclusion. The incidence of inpatients and outpatients were 15 (93.8%) and 1 (6.2%) respectively in the present study. Mohammadi et al [26] reported the similar findings. The weak immune system of LRTIs patients are susceptible to infections due to steroid and medication use along with hospital longer stay [27].

The incidence of poly-microbial infections was 25.6% in the current study. In contrast, numerous studies reported lower incidence of poly-microbial infections 9%, 15.36%, and 20% [28-30]. The poly-microbial infection identification is a key factor in treatment strategies as it might not be managed by antibiotics.

Of the LRTIs different bacterial etiological factors, Enterobacteriaceae members are predominant pathogens. K. pneumonia with prevalence 45.1% was the most prevalent organism followed by P. aeruginosa in the present study. Similar findings were reported in various studies [31, 32]. Nosocomial infections and presence of ubiquitous were the reasons for higher incidence of K. pneumonia [33].

Yekani et al [34] found that biofilm producers were mostly seen in P. aeruginosa isolates. The incidence of P. aeruginosa as the biofilm producer was present in 77 LRTIs patients as reported in a study conducted by Lima et al [35]. Majority of biofilm producers were isolated from sputum, pleural fluid, tracheostomy tube, and suction tube. A significantly higher resistance of biofilm-producing P. aeruginosa was shown to cefoperazone/ sulbactam. Saha et al [36] reported that biofilm-producing P. aeruginosa showed different patterns of antibiotic-resistant. Biofilm producers make antibiotic treatment ineffective, thereby promoting chronic infectious diseases. Because biofilm production not only helps pathogens adapt to different environmental niches, but it also helps them resist many antimicrobial agents. The pathogens had colonised the majority of hospital equipment. As a result, it is critical to routinely screen bacterial pathogens for biofilm production using their antibiogram patterns.

CONCLUSION

Our study concluded that gram-negative bacteria was the predominant and had increased resistance to antibiotics in Lower respiratory tract infections (LRTIs) patients. In LRTIs, the most common organism was K. pneumonia. Also, P. aeruginosa isolated in LRTIs patients were susceptible to producing biofilm. Biofilm producers had higher resistance than biofilm non-producers.

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