

Study on Antimicrobial Susceptibility Patterns of Respiratory Bacteria in a Tertiary Care Hospital in Delhi NCR

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ABSTRACT

Bacterial respiratory infections, Diagnosis and Sensitivity Testing for Antibiotics using Automated VITEK 2 Compact System (bioMérieux, Benisongheny, France). Bacterial respiratory tract infections are an important health problem worldwide affecting children, the elderly and patients with chronic disease. This can involve the upper or lower respiratory tract, ranging from mild such as cold to severe such as pneumonia. The most common bacteria include *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The management of such infections has become increasingly challenging in recent years due to the emergence of issues related to antibiotic resistance caused by over-prescribing or misuse of antibiotics. A total of 460 samples like sputum, throat swabs and tracheal fluids were collected using aseptic techniques from October, 2024 to March, 2025 at Diagnostic Laboratory, Gurgaon, Haryana. Basic tests like gram staining and simple biochemical methods like catalase, oxidase and coagulase tests were done for presumptive identification of bacteria. For isolation and identification of bacteria, sensitivity testing to various antibiotics was carried out using the VITEK® 2 Compact system (bioMérieux, France), following the CLSI guidelines. Out of 460 samples, bacterial growth was observed at 41.52%. The most frequently isolated bacteria were *Pseudomonas aeruginosa* (30.9%), *Escherichia coli* (25.1%), *Staphylococcus aureus* (18.3%), *Klebsiella pneumoniae* (17.8%), *Acinetobacter baumannii* (4.2%), *Enterococcus faecalis* (1.6%), *Streptococcus pyogenes* (1.0%), and *Streptococcus pneumoniae* (1.0%). Among the *S. aureus* isolates, 65.7% were methicillin resistant (MRSA). In general, linezolid and daptomycin showed the most effective antimicrobial effects, and cephalosporins, fluoroquinolones, and amoxicillin-clavulanic acid were associated with high-resistance rates of the isolates.

Keywords: Respiratory infections, antibiotic resistance, bacterial pathogens, pneumonia, CLSI

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Introduction

Respiratory tract infections (RTIs) involve both upper and lower airways, and are among the most common infectious diseases worldwide and carry a high morbidity and mortality rate in both developed and developing countries (Miriti et al., 2018). Respiratory infections (both acute and chronic) are among the most common diseases in people of all ages and are responsible for a large health burden worldwide due to the high morbidity, mortality and economic costs incurred (Niederman et al., 2022). Respiratory tract infections (RTIs) are among the most common acute illnesses in children, ranging from mild, self-limiting diseases such as the common cold to severe, potentially lethal illnesses such as pneumonia and epiglottitis (Vashishtha et al., 2010). RTIs are commonly classified into upper & lower respiratory tract infections. Upper airway involves infection of nasal

passages, pharynx, tonsils and epiglottitis. The clinical spectrum of URTIs includes pharyngitis, rhinosinusitis, nasopharyngitis, tonsillitis and epiglottitis. LRTIs involve the bronchi and lungs and include infections such as pneumonia, bronchitis, bronchiolitis and pleuritis (Vashishtha et al., 2010).

Acute respiratory illness (ARIs) are generally divided according to the site of infection into upper and lower respiratory tract infections. The upper respiratory tract includes the airways from the nostrils to the vocal cords in the larynx, including the paranasal sinuses and the middle ear, whereas the lower respiratory tract is the continuation of the airways from the trachea and bronchi to the bronchioles and alveoli (Grüber et al. 2025).

In India, they are among the most common causes of outpatient visits and hospitalization, especially among children, the elderly, and those with

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underlying diseases (Kumar et al., 2020). *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* are common bacterial respiratory pathogens associated with a range of respiratory diseases ranging from mild upper respiratory infection to severe diseases, such as pneumonia (Prina et al., 2015). The symptoms of infection in the respiratory tract can vary from mild to chronic. The form of infection, the organism that caused it, the patient's age, and any underlying medical issues will all affect the severity and kind of infection. Common Symptoms of RTIs are cough, fever, sore throat, runny or stuffy nose, sneezing, headache, fatigue, wheezing, chest pain or discomfort, Shortness of breath, dizziness, sputum production, nausea, vomiting and diarrhoea (NHS, 2024; Centers for Disease Control and Prevention, 2025). Particularly in children under five, infections of the respiratory system rank among the top causes of disease and mortality globally. The age group under five years of age is most frequently affected by RTIs, with a hospitalization rate ranging from 20% to 40% and an admission rate of 30% to 50% (Grüber et al. 2025).

According to a recent World Health Organization report, approximately 700,000 people die each year as a result of an infection that can no longer be treated with standard therapies (World Health Organization, 2023). Globally, bacterial AMR was directly responsible for 1.27 million deaths in 2019 and indirectly for an additional 4.95 million deaths (Antimicrobial Resistance Collaborators, 2022). The Centers for Disease Control Prevention (CDC) claims that the issue of antimicrobial resistance (AMR) is critical to the world population health because it causes a significant burden of death and increased difficulties in the treatment of common diseases (Centers for Disease Control and Prevention, 2025). The MDROs increase the costs of treatment, prolong hospitalization, and aggravate the illness and mortality conditions, leading to various infections and creating a significant burden on the healthcare system (Yimer et al., 2025). The emergence of very pathogenic strains such as methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) has exacerbated this issue. Due to the resistance mechanisms that developed with the help of these bacteria even to our strongest antibiotics, treating these infections is becoming increasingly difficult, which increases the chances of complications and mortality (Habboush et al., 2025). The use of antibiotics has been abused for a long period of time. As a result, they are

no longer effective, and sometimes they no longer work at all. This has led to an escalating worldwide health crisis that is outpacing the available solutions to the problem (Sangita et al., 2021). In India, studies have shown high resistance in gram-negative bacilli isolated from lower respiratory tract infections, which underlines the importance of continuous surveillance and local data to guide empirical therapy and reported an incidence of LRTI of 248.3 per 1000 person-years among older adults, which was higher in those aged 75 years and above in North India (Kumar et al., 2021).

Delhi, India's capital has a high rate of respiratory diseases due to a number of variables, including densely populated, automobile emissions along with seasonal pollution peaks. While there is a paucity of comprehensive data specific to bacterial respiratory infections in Delhi NCR, there are several studies that have shed light on the bigger picture of respiratory diseases in the region. Considering above, on present investigation aims to assess the antibiotic resistance profiles of pathogenic bacteria obtained via respiratory specimens acquired from a hospital in Delhi NCR.

Methodology

Research methodology and sample acquisition

The investigation was carried out from October, 2024 to March, 2025 at a Diagnostic Laboratory, Gurgaon Haryana and 460 samples were obtained for this investigation. All the respiratory samples were collected from OPDs (Out Patient Department) and patients admitted in HPDs (Hospital Inpatient Department). Sputum was collected in a marked, clean container with a wide mouth (figure 1). Every patient was instructed to collect sputum in an aseptic manner and to bring the sample to the lab right away. A medical officer picked oral samples with a moistened saline sterile cotton swab. BAL samples were collected in the lungs with the use of instillation of sterile saline with the help of bronchoscope and the subsequent aspiration. All samples were immediately taken to the laboratory to be analyzed.



Figure 1:- Respiratory samples

Microbiological Processing

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Culture & Identification: - Gram staining was performed on purulent components of the samples to determine the main intracellular bacteria (Figure 2). Sputum samples were inoculated onto MacConkey agar, blood agar, and chocolate agar and incubated for 18-24 hours at 37°C. At 24 hours, all plates were checked for growth. Plates that showed no growth were incubated for up to 48 hours. Colonies of varying size, shape, and colour were chosen from various plates and sub cultured further. Following the establishment of pure colonies, typical microbiological procedures such as gram staining, colony morphology identification, and biochemical assays were used (Figure 3-5).

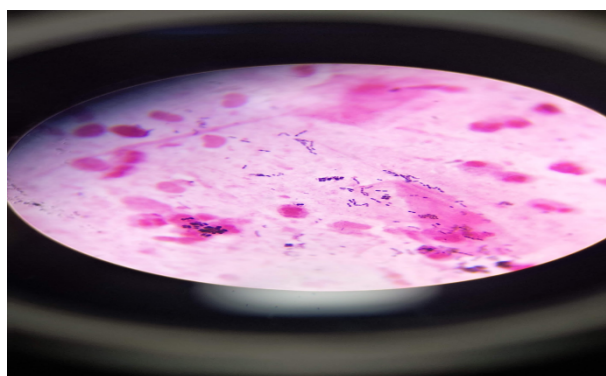


Figure 2:- Gram staining of sample

Automated Identification & Susceptibility Testing:

- Isolated bacterial cultures were identified and evaluated for antibiotic sensitivity using Vitek 2 Compact system (BioMérieux, France), which included GP ID and GN ID (Figure 6). This system uses colorimetric reagent cards specific to gram positive or gram negative bacteria to identify the organism and determine the minimal inhibitory concentrations (MICs) of several antibiotics. Antibiotic sensitivity testing was performed using AST-P628 (for Gram-positive bacteria) and AST-N405 & AST-406 (for Gram-negative bacteria) cards and the results were interpreted with Vitek 2 Compact software version 07. The antibiotic susceptibility test was performed as per the CLSI, 2022 (Alaidarous et al., 2017).

Quality control

Quality control strains included *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* 49619 and *Pseudomonas aeruginosa* 27853 were used as a control to ensure the accuracy and reliability of the susceptibility testing (Alaidarous et al., 2017).

Results

In this study, a total number of 460 respiratory specimens were collected from patients with the

clinical symptoms of respiratory infections in the form of sputum, throat swabs, tracheal aspirates and bronchoalveolar lavage (BAL). From the total number of 460 samples, 191 (41.52%) revealed positive bacterial growth (figure 2). From these samples it has been found that 78.01% were gram negative bacteria while 21.99% were reported as gram positive bacteria (figure 3). A variety of bacterial pathogens were isolated in the study, with *Pseudomonas aeruginosa* (30.9%), followed by *Escherichia coli* (25.1%), *Staphylococcus aureus* (18.3%), *Klebsiella pneumoniae* (17.8%), *Acinetobacter baumannii* (4.2%), *Enterococcus faecalis* (1.6%) and *Streptococcus pyogenes* (1.0%), and *Streptococcus pneumoniae* (1.0%). The isolates were identified by standard microbiological techniques like colony morphology and biochemical tests (figure 4, 5).

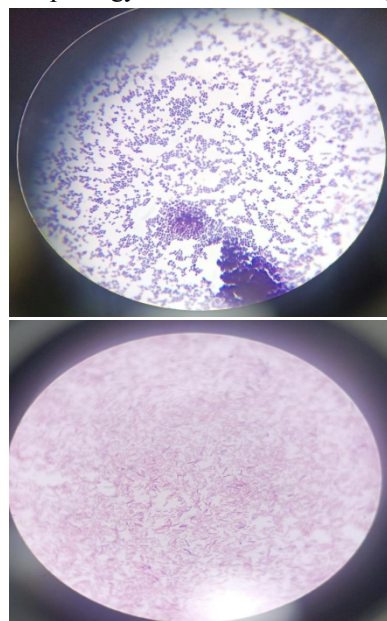


Figure 3:- Microscopic view of gram staining Gram positive isolate (left side) and Gram negative isolate (right side)

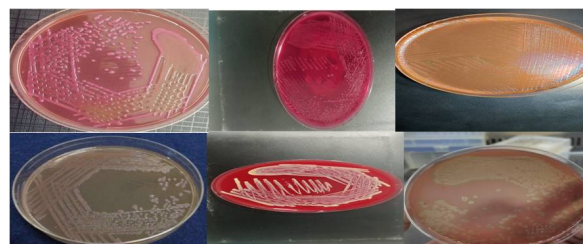


Figure 4:- Isolation of different bacterial isolates on the bases of culture and morphology

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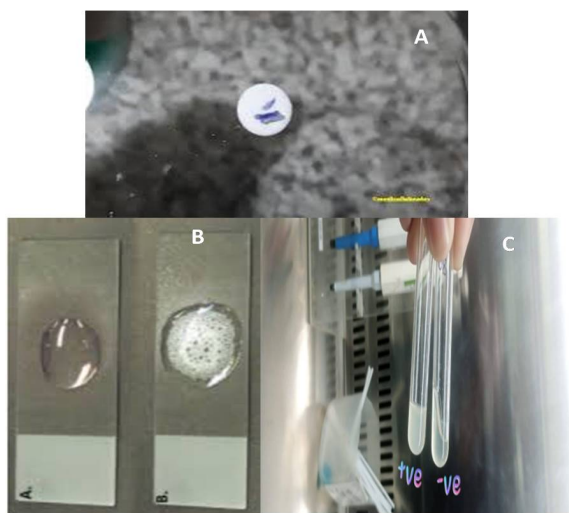


Figure 5:- Biochemical Tests A) Oxidase test B) Catalase test C) Coagulase test

Automated Identification & Susceptibility Testing:

- Isolated colonies of bacterial cultures were identified and tested for antimicrobial susceptibility using VITEK 2 Compact system (bioMérieux, France), i.e., GP ID (for Gram-positive bacteria) and GN ID (for Gram-negative bacteria) cards (Figure 6).



Figure 6 :- Vitek 2 Compact Machine & cassette loaded cards

Antimicrobial susceptibility patterns

Susceptibility testing in the isolates, revealed high level resistance to various antibiotics. *Escherichia coli* was resistant to ceftriaxone (86%), cefuroxime (84%), amoxicillin/clavulanic acid (78%), cefepime (72%), ciprofloxacin (72%) and levofloxacin (70%). Piperacillin/tazobactam and gentamicin had moderate resistance (32% each), and carbapenems (28-30% each) and amikacin (26%) had lower resistance, respectively. Colistin (80%), tigecycline (78%), aminoglycosides (64–70%) and carbapenems (66–68%), had the highest susceptibility and low intermediate response (0-4) with a low monthly prevalence (Figure 7). Equally, *Klebsiella pneumoniae* was particularly resistant to cephalosporins (76-90%), amoxicillin/clavulanic acid (84%), and fluoroquinolones (72-74%), and cotrimoxazole (70%). The resistance to piperacillin/tazobactam, carbapenems and aminoglycosides was moderate (34-38%). Colistin was the most sensitive (78%), then aminoglycosides, tigecycline, and carbapenems (58-62%), and low

intermediate susceptibility (0-10%) (Figure 8). The rate of resistance according to the case of *Pseudomonas aeruginosa* included lower rates of resistance to piperacillin/tazobactam (14%), carbapenems (18-20%), and aminoglycosides (16-22%), moderate resistance rate was encountered with cephalosporins (22-32%), and aztreonam (34%). The resistance was more conspicuous to fluoroquinolones (44-46%). Colistin (84%), followed by piperacillin/tazobactam (82%), amikacin (80%), and carbapenems (76-78%) were found to be the most susceptible with very few intermediate responses (4%) (Figure 9). The resistance to ciprofloxacin (100%), carbapenems (87.5%), cotrimoxazole (87.5%), and ceftazidime (87.5%) was very high with *Acinetobacter baumannii*. Cefepime, aminoglycosides, and levofloxacin were also noted to be of high resistance (75%). Piperacillin/tazobactam and colistin were found to have moderate resistance (62.5% each). Minocycline had a 50:50 ratios of resistance and susceptibility, whereas tigecycline had only intermediate response (100% completely) (Figure 10). In the case of *Streptococcus pneumoniae*, maximum resistance was detected to gentamicin, ciprofloxacin, cotrimoxazole, benzylpenicillin, erythromycin and Clindamycin (100 percent). Conversely, levofloxacin, linezolid and vancomycin had full susceptibility (100%), whereas tetracycline exhibited equal resistance and susceptibility (50%) (Figure 11). The isolates of *Staphylococcus aureus* were highly resistant to benzylpenicillin (90%), erythromycin (88%), cotrimoxazole (82%), ciprofloxacin (72%), and clindamycin (74%). As per cefoxitin and oxacillin, the prevalence of methicillin resistance was found in about 65.7 percent of the isolates. Levofloxacin and gentamicin had moderate resistance (64 and 48% respectively). The greatest susceptibility was observed by linezolid and daptomycin (100%), then teicoplanin (94%), tigecycline (68%) with low response intermediate (0-6%), (Figure 12). *Streptococcus pyogenes* was found to be resistant (100%), moderate (50% each) to cotrimoxazole, ciprofloxacin, clindamycin and tetracycline, respectively. Maximum susceptibility was observed to gentamicin, tigecycline, levofloxacin, benzylpenicillin, linezolid and vancomycin (100) (Figure 13). Lastly, *Enterococcus faecalis* was very resistant to levofloxacin (67%), ciprofloxacin (66.67%), benzylpenicillin (66.67%), and tetracycline (66.67%). Vancomycin and teicoplanin (33.33 and 33%), and linezolid (100%) had moderate resistance and complete susceptibility respectively (Figure 14).

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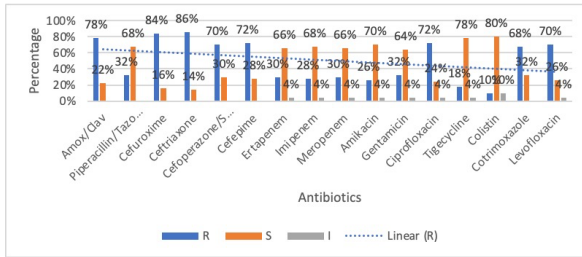


Figure 7: Antibiotic susceptibility pattern observed among *E. coli* isolates

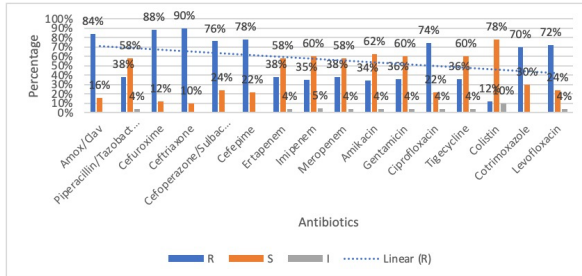


Figure 8: Susceptibility pattern of *K. pneumoniae* against tested antibiotics

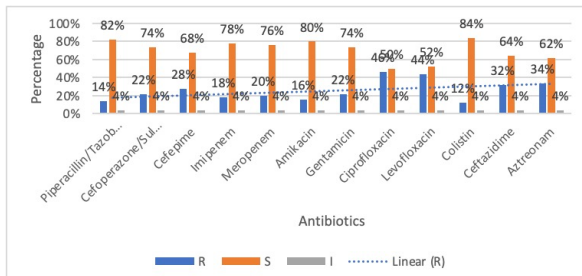


Figure 9: Antimicrobial susceptibility profile of *P. aeruginosa* isolates

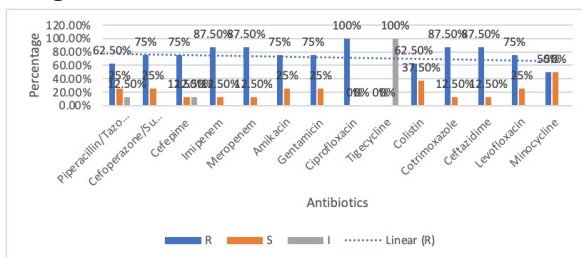


Figure 10: Antimicrobial resistance profile of *A. baumannii* isolates

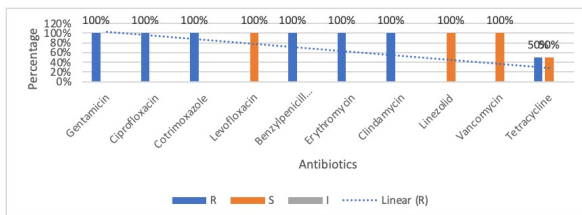


Figure 11: Antibiotic sensitivity profile of *S. pneumoniae*

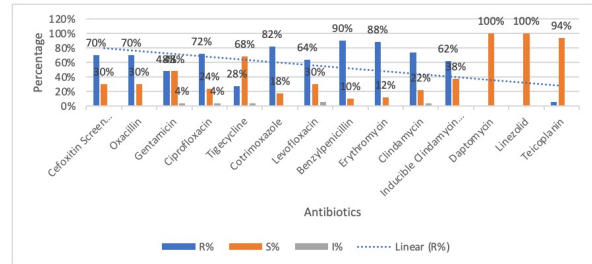


Figure 12: Distribution of antibiotic susceptibility among *S. aureus* isolates

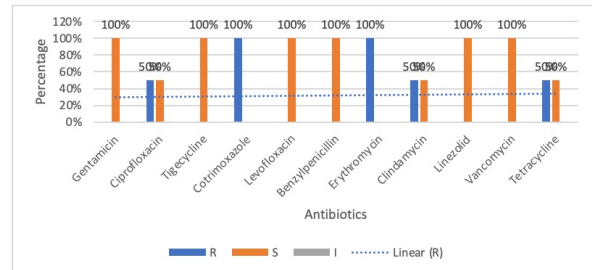


Figure 13: Distribution of antibiotic susceptibility among *S. pyogenes* isolates

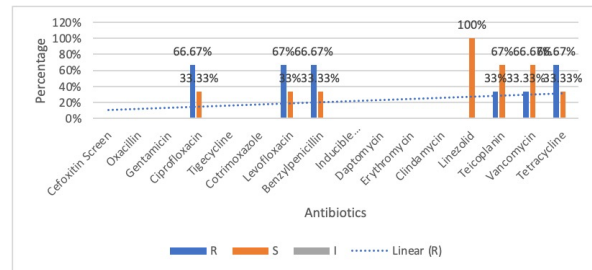


Figure 14: Distribution of antibiotic susceptibility among *E. faecalis* isolates

Discussion

The data was analyzed on 460 respiratory samples of 41.52% bacteria growth where *E. coli*, *S. aureus*, *P. aeruginosa*, *A.baumannii*, *K. pneumoniae*, *S. pneumoniae*, *S. pyogenes* and *E. faecalis* were prevalent in a tertiary care hospital in Delhi NCR. The rate of *S. aureus* which was methicillin resistant (MRSA) was high (65.7%). *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* were the most commonly recovered respiratory-collected bacterial species, which were detected in our investigation. Similar results have been indicated in the past studies. Indicatively, the research conducted on ICU-acquired pneumonia has reported the presence of *Klebsiella spp.*, *Staphylococcus aureus*, *Pseudomonas spp.*, or *Acinetobacter spp.* as the major organisms obtained in the tracheal or endotracheal samples of patients in intensive care units (Pasquale et al., 2014; Esperatti et al., 2010; Rello et al., 2002). Similarly, a study by Rose et al. (2016) in patients with ventilator-associated

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pneumonia (VAP) ascertained the prevalence of the most commonly identified pathogen in respiratory samples, which was *Klebsiella* spp., which is also aligned with the findings of the present study. Conversely, microbial distributions have been reported to be different in other studies. Indicatively, Kabak et al. (2019) found that the Gram-positive cocci *Staphylococcus aureus* was the most common pathogen in endotracheal samples among mechanically ventilated ICU patients.

Gram-negative bacteria represented the most significant number of isolates in the present research (78.01%), with Gram-positive organisms (21.99%). This trend aligns with the results of Mahendra et al. (2018) who also indicated that Gram-negative pathogens were predominant among the patients hospitalized in a respiratory ICU. Epidemiological research by Paterson et al. (2006) and Sydnor et al. (2011) indicates that the augmented frequency of Gram-negative bacterial infections in the ICU setting might be attributed to their capacity to inhabit the medical catheters such as urinary catheters, hospital equipments, and the contaminated fluids. These organisms can survive on nonliving surfaces over long durations of time, usually longer than Gram-positive bacteria. Moreover, *Pseudomonas* spp. are also known to thrive in damp environments such as aerators, sinks, respiratory devices as well as other sources involving water in hospital environments (Paterson et al., 2006). These pathogens along with the *Acinetobacter* species may be transmitted either via direct person-to-person contact or through the contaminated surface of the environment (Jakribettu et al., 2012).

In the current research, *Escherichia coli* and *Klebsiella pneumoniae* exhibited a high level of resistance to cephalosporins, amoxicillin/clavulanic acid, and fluoroquinolones alongside a higher susceptibility to colistin, tigecycline, aminoglycosides, and carbapenems. *Pseudomonas aeruginosa* showed comparatively reduced resistance to the use of piperacillin/tazobactam, aminoglycosides and carbapenems with the maximum susceptibility to colistin. The *Acinetobacter baumannii* was highly resistant to most antibiotics, including carbapenem and fluoroquinolone with a limited susceptibility to minocycline. *Staphylococcus aureus* was the most resistant to various drugs and 65.7% of its isolates were found to be MRSA with all being sensitive to linezolid and daptomycin. *Streptococcus pneumoniae* was totally resistant to various antibiotics but fully sensitive to levofloxacin, linezolid and vancomycin. *Streptococcus pyogenes* and *Enterococcus faecalis*

were also variable resistant but linezolid was extremely effective. On the whole, the research has indicated a high prevalence of antimicrobial resistance, which has the significance of constant monitoring and responsible use of antibiotics.

Such results can be compared to the ones provided by Behera et al. (2020), who examined the patterns of antimicrobial susceptibility in ICU patients with lower respiratory tract infections. Various aspects lead to the development and transmission of very resistant Gram-negative bacilli in intensive care unit. These are longer stay in the intensive care units, hospital stay longer than 48 hours over the last three months, old age (more than 65 years old), use of invasive or mechanical ventilation, and inappropriate or overuse of broad-spectrum antibiotics like fluoroquinolones, carbapenems, and third-generation cephalosporins. Moreover, recent similar antimicrobial therapy in the last 3 months and high level of antibiotic resistance in the community contribute to the risk of resistant infections further (Khilnani et al., 2019). The species of *Klebsiella* are some of the Enterobacteriaceae family and they are common in nature. They may be commensal in a variety of habitats such as soil, water, plants, insects, birds and animals. They are typically colonizers of the oral cavity, skin, respiratory tract, urogenital tract as well as the intestinal tract in both humans and animals (Podschun et al., 1998). The formation of biofilms is one of the significant virulence phenotypes of the *Klebsiella* spp. The formation of biofilm also forms a defense that minimizes the effects of antimicrobial agents, thus decreasing resistance to antibiotics, including gentamicin, ampicillin, and ciprofloxacin (Desai et al., 2019).

In the current experiment, multidrug resistant (MDR) was found in around 82.35 percent of *Klebsiella* isolates. In a study of Asri et al., the predominant nosocomial MDR *Klebsiella* spp. was 32.8% in that study, which is generally in line with our results. In the world, the prevalence of MDR *Klebsiella* is widely spread, with the lowest rates of 12.9% in North America and the highest 82% in Bangladesh (Mohd et al., 2021; Aminul et al., 2021). There are various mechanisms involving antibiotic resistance in *Klebsiella* species, such as enzyme degradation or modification of antibiotics, the changes in antibiotic targets, mutations, resulting in the loss of porin channels, overexpression of efflux pumps, and biofilm formation (Mulani et al., 2021).

In this research *Pseudomonas aeruginosa* was found to be most prevalent at 21.6% and this is in line with the

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range of the previous researchers. Indicatively, a study by Vincent et al. (2020) on intensive care unit-acquired infections showed that up to 23 percent of patients admitted to an intensive care unit may be infected with *Pseudomonas* spp. The ability to resist various antimicrobial agents, such as ampicillin, amoxicillin-clavulanate, ceftriaxone, cefotaxime, tetracyclines, nitrofurantoin, ertapenem, cotrimoxazole, and chloramphenicol, is one of the essential traits of *Pseudomonas* spp. The combination of multidrug efflux pump systems, the presence of type-1 AmpC β -lactamase enzymes, and decreased permeability of bacterial outer membrane are the primary factors of this inherent resistance (Behera et al., 2020). A study performed in Spain showed that a range of 26% of *Pseudomonas* isolates was multidrug resistant (MDR), which is more than the rate in our study, where MDR *Pseudomonas* spp. represented 13.33% of isolates (Del Barrio-Tofiño et al., 2019). Nonetheless, other researchers have demonstrated that the incidence of resistant *Pseudomonas aeruginosa* in ICUs may be up to 48.7% (Ribeiro et al., 2019).

Staphylococcus aureus exists in the natural environment and is a normal constituent of human microbiota, mostly found on the skin and mucus surfaces, and mostly the nasal cavity of healthy humans (Lowy, 1998). One of the reasons why it has been known to be very pathogenic is because it is capable of attaching to medical equipment and synthetic materials. Following the insertion of the device into the host, *S. aureus* may adhere to host matrix molecules that cover the surface of devices and then form biofilms, thereby improving the survival and persistence of bacteria on the host (Otto, 2018). In the present research, *Staphylococcus aureus* isolates were the most susceptible to linezolid & daptomycin (100%), then teicoplanin (94%). Also, the percentage of the *Staphylococcus aureus* that were methicillin-resistant was about 65.7% of the *S. aureus* isolates. The MRSA infections can be said to be highly morbid, highly morbid, and highly hospitalized as opposed to the infections caused by the methicillin-sensitive *Staphylococcus aureus* (MSSA) (Ippolito et al., 2010). Our MRSA prevalence (65.7) value is higher than the value discussed by Patil et al. (2022), who in a meta-analysis of MRSA prevalence in India indicated a range of 25% to 55%. Although such findings are made, the current research has some weaknesses. The samples of data were gathered in one tertiary care hospital, which might not be applicable to other healthcare facilities or areas. Also, variables related to patients, including age, comorbidity, and the history of

antibiotic usage, were not taken into account, yet they could also have a strong impact on the patterns of antimicrobial resistance. Moreover, the researchers failed to provide comparative resistance results of alternative tertiary care hospitals in the Delhi NCR area. Bacterial identification and antimicrobial susceptibility testing may also be inaccurate depending on the diagnostic methods that the diagnostic uses.

Conclusion

The present study suggests the high prevalence of antibiotic resistance in respiratory isolates of a tertiary care hospital in Delhi NCR. The outbreak of multidrug-resistant gram negative and MRSA is a major concern to patient care and the hospital resources. The results of this research point at the urgency of continuous antimicrobial resistance monitoring, the reasonable use of antibiotics, and the introduction of hospital antibiotic stewardship programs.

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