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Review

Complicated treatment of *Burkholderia cepacia* complex (Bcc) and *Pseudomonas aeruginosa* in cystic fibrosis (CF) patients: an overview

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ABSTRACT

Cystic fibrosis (CF) disorder is thought to be the most autosomal recessive condition with high morbidity and mortality rates in Caucasians. The primary cause of these individuals' early death is persistent airway bacterial infections. *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex (Bcc) are the major pathogens that colonize the airways and cause progressive respiratory failure with high mortality in CF patients. It is important to note that both of these pathogens have large genomes compared to other known prokaryotes and can induce infections displaying a high degree of innate and acquired resistance to antimicrobial agents in the unique environment of CF lungs. They are not only harmful to CF patients but also are significant pathogens in other vulnerable patients. Bcc has frequently been misidentified with other non-fermentative Gram-negative bacilli (NFGNB), particularly *Pseudomonas species*, in cases from nations with poor infrastructure. It must be accurately identified and distinguished since it has an inherently different susceptibility pattern from P. aeruginosa. This narrative review aims to highlight the most important information about these selected difficult-to-treat pathogens, whose management is particularly challenging, to understand the similarities and differences better and provide possible therapeutic approaches.

Keywords: Burkholderia cepacia complex; *Pseudomonas aeruginosa*; misidentification; antibiotics, treatment.

1- *Burkholderia cepacia* complex (Bcc)

Bacteria belonging to the genus *Burkholderia* were first identified in the 1950s. They are Gram-negative bacteria that can be found in nature, frequently in soil, the rhizosphere of plants, or water. Some species in this genus can infect people, plants, and animals, while other species have positive impacts that are significant for agriculture or industry [1]. Walter Burkholder identified it as a pathogenic bacterium in plants that caused onion rot in the mid-1940s. It was first known as *Pseudomonas cepacia*. The *Burkholderia* genus is a member of the beta-proteobacteria class with the Burkholderiales order and Burkholderiaceae family. According to a 1992 proposal, seven species were separated from *Pseudomonas* ribosomal RNA group II based on DNA–DNA homology, sequences of 16s rRNA, and composition of cell-membrane lipid [2]. There are currently about 100 species of *Burkholderia* [3]. Within the *Burkholderia* genus, Bcc is a subgroup [4]. It is an oxidase-positive, catalase-positive, aerobic, non-spore-forming, non-sugar-fermenting bacteria. It contains genetically different species but similar phenotypes [5,6]. Currently, Bcc has approximately 21 species known previously as genomovars (closely related species) [7]. These bacteria typically contain three chromosomes in addition to a large plasmid in their genomes, which range in size from 7 to more than 9 million base pairs (Mbps) [8]. Genomes of Bcc are assumed to be more flexible in losing and gaining genes due to their massive size. This extensive genetic capacity increases Bcc adaptability in infections and biological processes [6].

1.1- *Burkholderia cepacia* complex in cystic fibrosis (CF) patients

In Caucasians, CF is the most prevalent and fatal autosomal recessive condition, affecting nearly 70,000 people globally. The epithelia of the pancreas, airways, liver, small intestine, sweat glands, and reproductive tract are among the tissues with abnormal viscous secretions because of the CF condition [9]. The cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation results in the dysfunctional CFTR protein. Reduced exchange of chloride ions and subsequent dysregulation of epithelium lining fluid transport occur when the protein is not functioning properly [10]. Chronic bacterial infections in the CF patients` airways are a great concern. Despite the potentially harmful effects of *CFTR* mutations, chronic airway infections cause 80 to 90% of fatalities [9]. The decrease in secretions` water content and reduced periciliary fluid, which traps inhaled germs and slows clearance, is thought to be the cause of bacterial colonization in the respiratory tract. Patients with CF are more prone to recurrent and

chronic airway infections because of their thick mucus. An increasing amount of research also points to the possibility that CF neutrophils exhibit delayed apoptosis, which prolongs inflammation and causes the release of pro-inflammatory cytokines, which in turn causes damage to the airways [11]. Ninety-five percent of CF patients die from respiratory failure caused by the subsequent inflammatory reaction which in turn releases cytokines, damages lung parenchyma, and causes bronchiectasis in the tissue [12].

Patients of CF experience recurrent infections, and as they get older, different microorganisms have been found in their respiratory tracts. While species of the Bcc include *Pseudomonas aeruginosa, Stenotrophomonas maltophilia,* and *Achromobacter species.* Are more prominent in older children and adults; *Staphylococcus aureus* infection is typically seen in younger children [13]. Bacteria of Bcc is believed to be responsible for serious respiratory tract infections within CF populations. It became a significant CF pathogen in the 1980s, when specific infected individuals experienced a rapid clinical decline brought on by sepsis and necrotizing pneumonia that led to early death [8]. Isles *et al*. reported the first remarkable report of infection with Bcc in CF individuals in 1984 [14], and within a year, Tablan *et al*. confirmed the infection by a second report [15]. The infections observed in the early reports were extremely virulent, in contrast to infections with other infectious agents such as *P. aeruginosa*, and it resulted in an uncontrollably rapid clinical decline that is fatal in almost 10% of patients (a clinical manifestation which is currently referred to as cepacia syndrome) [16]. The symptoms of cepacia syndrome include high fever, decreased leukocyte and erythrocyte counts, rapidly deteriorating respiratory function, bacteremia, sepsis, and necrosis [2]. Even with vigorous antibiotic therapy, cepacia syndrome is an almost deadly consequence of Bcc infection [1]. Strains of *B. cenocepacia, B. multivorans, B. dolosa,* and *B. cepacia* have all been highly transmittable between CF patients when they come into contact [8]. The second main microbiological concern for CF patients is Bcc. Despite their lower prevalence (approximately 3%–4% of CF patients have Bcc infection), they are particularly feared because of the ease with which they can spread among the patients, the widespread use of antibiotics, and the possibility of cepacia syndrome [9]. The mean survival of CF patients with Bcc infections decreases by approximately 10 years in the population compared to those with *P. aeruginosa* infections. The serious consequence of Bcc infection, besides the disease severity, is that it drastically limits the

number of CF patients who are capable of receiving lung transplants due to the high risk of postoperative sepsis and death [17].

1.2- *Burkholderia cepacia* complex in non-cystic fibrosis patients

It is not just CF patients who are affected by Bcc pathogenicity. It can result in infections including respiratory tract infections, bacteremia, urinary tract infections, septic arthritis, and peritonitis in non-CF patients. It is becoming highly recognized as a serious pathogen in humans, especially in individuals with compromised immune systems and those receiving hospital care who can get the infection from contaminated objects or from other infected patients [18]. The increasing number of reports of hospital-acquired infections caused by Bcc led to its recognition as an emergent causative agent of nosocomial infections in patients who are not suffering from CF, particularly in cancer patients. There have been more incidences of Bcc-caused bacteremia among hospitalized non-CF patients [8]. In addition to being extremely virulent, five species of Bcc (*B. cepacia, B. cenocepacia, B. multivorans, B. dolosa,* and *B. contaminans*) have the ability to spread via aerosol droplets, which makes them capable of rapidly infecting hospitalized patients [19].

It also have the ability to infect chronic granulomatous disease (CGD) patients [8]. The nicotinamide adenine dinucleotide phosphate (NADPH) complex is where problems occur in CGD, which was first discovered in 1959. Eczematoid dermatitis, chronic suppurative lymphadenitis, and hepatosplenomegaly were the distinguishing characteristics of that illness. The first four children who were diagnosed with this deadly granulomatous disease of childhood all passed away before the age of 6 years old; therefore, it earned that proper name (deadly granulomatous disease) [20]. It is a heritable disorder brought on by a mutation in the NADPH oxidase gene, which is usually linked to the X chromosome. The patient is more vulnerable to bacterial and fungal infections as a result of these alterations, which decrease the phagocytes' capacity to create reactive oxygen species (ROS) [1]. Infections caused by Bcc are remarkably aggressive and have the greatest mortality rates despite being a rare cause of respiratory infections in CGD patients [21]. These high fatality rates are frequently because of cepacia syndrome. Although the precise causes of this syndrome are poorly understood, *in-vitro* research has demonstrated that neutrophils from CGD patients who lack ROS are unable to eradicate *B. cenocepacia* after phagocytic internalization. This particular form of necrotic neutrophil death permits toxic intracellular contents to diffuse into tissues, resulting in further tissue damage, the

persistence of the inflammatory response, and sepsis. This could assist to explain the increased mortality rate of Bcc infections in CGD patients as well as cepacia syndrome [2]. B. multivorans and B. cenocepacia are the most common species of Bcc causing infections in CF patients, but B. cepacia is the most common in non-CF patients [6].

Bacteria of Bcc has also been isolated from pharyngeal infections, pediatric neck infections, and otitis media infections in immunocompetent individuals [8].

1.3- Antimicrobial resistance and virulence factors of *Burkholderia cepacia* complex

There are only a few antibiotics, including meropenem, levofloxacin, ceftazidime, cotrimoxazole, chloramphenicol, and minocycline, available for the treatment of infected patients with Bcc because this pathogen has a broad range of intrinsic resistance (defined as innate or inherent (not acquired) resistance) to antimicrobial agents [22]. The Bcc has intrinsic resistance to aminoglycosides, other beta-lactams, cationic antimicrobial peptides, and polymyxins. It also displays many resistance pathways to other types of antibiotics, including chloramphenicol, quinolones, trimethoprim, and tetracyclines [2,23]. Multiple cellular resistance pathways are responsible for antibiotic resistance in Bcc species [24]. Since the majority of Bcc members contain chromosomally encoded modified beta-lactamases and penicillin-binding proteins, altered drug targets and enzymatic modifications are the main causes of beta-lactam resistance [25]. Some Bcc species can survive and grow by utilizing penicillin G as their sole carbon source [2]. Modified dihydrofolate reductase, the target enzyme of the trimethoprim antibiotic, is the cause of some Bcc strains' resistance to this antibiotic. Because of the unique structure of the lipopolysaccharide (LPS) and the decreased permeability of the outer membrane in comparison to many other bacteria, Bcc is resistant to many classes of antibiotics, including aminoglycosides, several beta-lactams, polymyxins, and cationic antimicrobial peptides [2,26]. *Burkholderia species* have altered LPS that prevents binding and decreases the anionic charge on the cell surface, which prevents the binding of polymyxins and cationic antimicrobial peptides and inhibits their expected bactericidal effects [27]. A major resistance mechanism in the Bcc species is the efflux pump-mediated antibiotic extrusion, which is believed to cause resistance to tetracyclines, trimethoprim, quinolone antibiotics and chloramphenicol [28]. The Bcc's unique LPS, which lacks binding sites, and the Efflux systems (AmrAB-OprA) account for its inherent resistance to aminoglycosides [26]. BpeEF-OprC, BpeAB-OprB, and AmrAB-OprA, can exclude tetracyclines. Tetracyclines are not the only antibiotics that BpeEF-OprC gives

resistance to; they also cause resistance to cotrimoxazole, fluoroquinolones, and chloramphenicol. AmrAB-OprA can effluxe macrolides [29]. Additionally, Bcc treatment can often be complicated by its ability to form biofilms, particularly in individuals with CF [26]. It is extremely difficult for clinicians to provide antimicrobial treatment for Bcc since acquired resistance features are common [30,31]. Some Bcc species also had extended-spectrum beta-lactamases, which have strong activity to penicillins and cephalosporins [30].

It exhibits a wide range of virulence features in addition to the multiple mechanisms of antibiotic resistance, including the formation of biofilms [32], an extracellular matrix that encases bacterial cells and adheres to surfaces. Polysaccharides, lipids, proteins, and extracellular DNA make up the majority of the matrix. Bacteria are able to cooperate because of the biofilm formation, which also shields them from antibiotics and neutrophil phagocytosis. Some bacteria may escape the biofilm and spread to infect further locations [33]. It is not a component of the human microbiome so it is clear that the environment is both a major source of infection and Bcc's natural habitat [34]. Invasion as a biofilm and paracytosis are examples of penetration mechanisms that Bcc uses successfully to invade the internal system of hosts. Extracellular lipase, serine proteases, metalloproteases, and various bacterial surface features like pili, flagella, and LPS can also be identified as virulence factors produced by Bcc species. Production of exopolysaccharide (EPS), specifically cepacian, is another essential virulence factor necessary for biofilm formation to shield Bcc from the host's defense mechanisms and the treatment with antimicrobials [9].

2- *Pseudomonas aeruginosa*

Pseudomonas aeruginosa has been known as a widespread facultative anaerobic Gram-negative environmental bacterium that has frequently been isolated from fruit, vegetation, soil, and water settings, including swimming pools, lakes, and rivers [35]. It belongs to Pseudomonadaceae family and can thrive in various conditions [36,37]. It is catalase-positive, oxidase-positive [38], a non-sugar fermenter [13], and cannot produce spores [36,37]. In comparison with other bacteria like *Escherichia coli* (4.6 Mbps), *Mycobacterium tuberculosis* [4.4 Mbps], and *Bacillus subtilis* [4.2 Mbps], the genome of *P. aeruginosa* is large (5.5-7 Mbps). It encodes regulatory enzymes in a high percentage, which are crucial for organic compounds extrusion, metabolism, and transportation [39].

2.1- *Pseudomonas aeruginosa* in cystic fibrosis patients

Pseudomonas aeruginosa is a common cause of chronic infections in patients with CF, accounting for 80% of CF adults with chronic infections [9]. It is clearly defined as a pathogen that CF patients acquire early. It is frequently acquired from natural sources. Once the bacteria reside in the CF airways, they adapt through modifications like flagellum expression downregulation, which reduces motility and decreases other virulence factors [13,40]. Additionally, it produces excessive exopolysaccharides like alginate, resulting in a mucoid status. Early mortality, along with rapid lung function deterioration, has been linked to chronic infection. Early and new-onset infections have to be aggressively treated to clear the organism from the airways of CF patients to avoid these negative effects. However, failure of eradication still affects this patient population. A potential risk factor for eradication failure is the chronic phenotype of the isolate, including the mucoid status [13].

2.2- *Pseudomonas aeruginosa* in non-cystic fibrosis patients

As an opportunistic pathogen, *P. aeruginosa* can infect various hosts, including people, plants, and animals. It is the most prevalent pathogen in humans and infects people who are most susceptible, such as those with obstructive respiratory diseases [41]. It often causes respiratory, blood, and urinary tract infections. It has been identified among the bacteria that causes hospital-acquired infections, including ventilator-associated pneumonia, sepsis, and meningoencephalitis. The bacterium most frequently colonizes medical devices such as nebulizers, catheters, and humidifiers. Additionally, it has been shown to infect patients who are ventilated or who have long-term bladder catheters [42]. It is also a significant pathogen in diabetic foot ulcers, burns, wounds, and infections that affect other healthy individuals, including keratitis and otitis media [43]. Treatment for infections brought on by this bacterium is difficult. Despite notable regional variations, chromosomal mutations that lead to resistance are more prevalent and have increased the probability of multidrug resistance through the past decades due to the ability to tolerate high antibiotic concentrations and the consequent need for long-term therapy. It is also one of the "ESKAPE" pathogens prioritized for developing novel antimicrobials and alternative therapies [12].

2.3- Antimicrobial resistance and virulence factors

Pseudomonas aeruginosa has resistance to a number of antibiotics, including several beta-lactams, aminoglycosides, and quinolones. Many defense mechanisms have been employed against antibiotics in *P. aeruginosa* which generally can be divided into acquired, adaptive, and intrinsic resistance. Examples of intrinsic resistance are efflux pumps that extrude drugs out of the cell, low outer membrane permeability, and the development of inactivating enzymes. For acquired resistance, either horizontal gene transfer or mutational alterations might result in resistance [36]. On gaining exogenous genes, *P. aeruginosa* strains become multidrug-resistant (MDR), which can resist fluoroquinolones, carbapenems, and aminoglycosides [44]. Acquiring genes for multiple types of beta-lactamases aids in developing carbapenem resistance [45]. Aminoglycoside resistance has also been linked to the uptake of exogenous genes that encode for various modifying enzymes, including aminoglycoside acetyltransferase (AAC) and 16S rRNA methylases [46]. On the other hand, it was discovered that fluoroquinolone resistance is caused by mutations in the target genes coding for topoisomerases IV (*parC* and *pare*) and DNA gyrases (*gyrA* or *gyrB*) [44]. In order to prevent the bacterial cells from being accessed by antibiotics, the adaptive resistance implies the production of biofilm in the lungs of infected individuals. It is also possible for the biofilm to develop multidrug-tolerant cells, which can resist antibiotic attacks and result in prolonged recurrent infections in CF patients [47].

It typically grows as an aerobe but may also endure in anaerobic environments. It can develop antimicrobial resistance either through horizontal transfer of genes or via chromosomal mutation, and it is inherently resistant to a number of beta-lactams. Moreover, it possesses a range of virulence characteristics that contribute to host infection [13]. An important step in infection is bacterial adherence to the epithelia of the respiratory tract, which is mediated via interactions between the host receptors and adhesins of bacteria. Bacteria of *P. aeruginosa* has single flagellum that is necessary for the formation of biofilm, motility, and adhesion to cells, as well as type IV pilli, which is also important for motility in addition to helping the bacterial cells to attach to respiratory epithelial cells and form biofilm. They are appendages made of pilin polymers. Both the single flagellum and the type IV pilli are the main adhesins for infection with *P. aeruginosa*. Gene expression in *P. aeruginosa* can be controlled by quorum sensing, a mode of bacterial communication, which enables bacteria to coordinate their attacks on the host when an infection is present [48].

3- The *Burkholderia cepacia* complex compared to *Pseudomonas aeruginosa*

Pseudomonas aeruginosa and Bcc have been isolated from various sources, including plants, industrial settings, soil, and water. They are both widely distributed in the environment and are both opportunistic pathogens. These organisms' remarkable metabolic flexibility, their ability to interact with both other bacteria and their eukaryotic hosts, and their capacity to modify their genetics to the requirements of their specific environment by acquisition of genetic material from other microorganisms are possible explanations for how they can exist in such a wide range of various habitats while also having the potential to infect humans [49] severely. The genomes of Bcc species typically exceed 7 Mbps in length, making them larger than *P. aeruginosa* strains. Given that the bacterium is constantly evolving within the host lung, it is believed that its enormous genome gives it a significant advantage over antimicrobial treatments and the host's immune response. A prolonged infection by *P. aeruginosa* and infections by Bcc bacteria typically develop following *P. aeruginosa* colonization, can flourish in the CF lung's impaired mucus clearance. Infections of Bcc can also spread quickly among people with CF due to both the vulnerability of the host and the bacterium's remarkable evolutionary progress [9].

4- Misidentification of *Burkholderia cepacia* complex

The Bcc was formerly only referred to as one species. Researchers discovered that *B. cepacia* actually consisted of several species in the mid-1990s. When *B. cepacia* was initially identified from patients with CF, it was identified to be *Pseudomonas cepacia* [7]. Studies revealed significant variation among Bcc bacteria, which led to the addition of new species to this complex, including *B. pyrrocinia* and *B. ambifaria*. However, due to their great degree of similarity, accurate identification of these bacteria can be extremely difficult [50]. Studies have shown that phenotypic identification tests are inappropriate to identify the species in this complex, and neither manual nor automated phenotypic identification techniques, such as VITEK 2, VITEK MS, and Phoenix can produce accurate findings. Molecular biology methods, including PCR and analysis of 16S rRNA, *hisA,* and *recA* sequences, are utilized to identify these pathogens [51]. It has frequently been identified as Gram-negative non-fermentative bacilli, particularly *Pseudomonas species*, in cases from nations with poor infrastructure [52–54]. This explains why there aren't many reports regarding *B. cepacia* infections in Egypt and other nations [5]. It can be challenging to distinguish between species within the Bcc as well as between these species and other closely related taxa, including *Cupriavidus, Ralstonia,*

Achromobacter, Pandoraea, Comamonas, Delftia, and *Brevundimonas* species [54]. Because Bcc has an inherently different susceptibility pattern from *P. aeruginosa*, it must be accurately identified and distinguished from that organism. This fact gives a high value of Bcc's accurate identification [5,18,55]. The comparison of several bacterial identification techniques is shown in various studies. In order to identify species that are Bcc-related, *recA* gene sequencing was found to be the most effective technique. Although the 16S and 23S rRNA gene sequences are commonly employed to identify bacteria but for this complex, it may be able to identify the *Burkholderia* genus without identifying the species [51].

5- Possible treatment perspectives

5.1- Antibiotic therapy

As a result of the increased use of broad-spectrum antibiotics, the prevalence of MDR *P. aeruginosa* and Bcc has increased, which has reduced the effectiveness of the treatment [56].

Levofloxacin, ceftazidime, meropenem, minocycline, cotrimoxazole, and chloramphenicol are among the few antibiotics that can be used to treat Bcc infections due to its broad range of inherent resistance to antibiotics [57]. It has intrinsic resistance to polymyxins, β-lactams, cationic antimicrobial peptides, and aminoglycosides in addition to having different resistance mechanisms to several other antibiotic classes, including quinolones, tetracyclines, chloramphenicol, and trimethoprim [2]. Its susceptibility pattern is intrinsically different from *P. aeruginosa* [5,18,55].

These antimicrobial agent groups were recommended by CLSI 2022 guidelines to be investigated against Bcc. Group A: contains antimicrobial agents regarded for inclusion in usual, primary testing and also for regular reporting of results: levofloxacin, meropenem, and cotrimoxazole. Group B: consists of antimicrobials that may require initial testing but could be reported under specific circumstances, for example, when the organism resists antimicrobials from the same class as those in group A: minocycline and ceftazidime. Group C: represents supplemental or alternative antimicrobial agents that may need to be tested in facilities where there are epidemic or endemic strains, for patients who are allergic to the primary medications, or to be indicated to the prevention of infections for epidemiological aid: chloramphenicol [57] but it has been partially abandoned in developed countries because its systemic administration is associated with fatal aplastic anemia [58] and later on it was excluded in the CLSI 2023 edition as the guidelines have been changed to only have one group of

antimicrobial agents appropriate for routine, primary testing and reporting that includes only meropenem, ceftazidime, levofloxacin, cotrimoxazole, and minocycline.

Treatment options are frequently considered based on case-by-case, taking account of prior clinical outcomes and information regarding *in-vitro* antibiotic susceptibility [26]. There is still no agreement or recommended treatment plan for CF patients infected with Bcc, despite the data presented by the distributed antibiograms and the results of *in-vitro* research [59].

In a review conducted by Bogaart and Manuel, it was reported that the main antibiotic options for Bcc infection are cotrimoxazole, ceftazidime, and levofloxacin while alternative treatment includes minocycline and meropenem. For MDR Bcc, ceftazidime/avibactam is the main antibiotic treatment, and cefiderocol is an alternative option For MDR Bcc resistant to ceftazidime/avibactam, imipenem/relabactam and piperacillin/tazobactam plus ceftazidime/avibactam are the main treatment options, while cefiderocol and temocillin are alternatives [60].

Van Dalem *et al*. reported that colistin, tobramycin, and amikacin were shown to have little to no activity based on *in-vitro* studies in addition to tigecycline and ciprofloxacin which had also little activity. Cotrimoxazole had a significantly high susceptibility *in-vitro* (82%) across Bcc isolates, while for beta-lactams, the MICs varied greatly [61]. This variance may be attributed to the existence of multiple resistance mechanisms in Bcc [62]. The beta-lactam antibiotic ceftazidime-avibactam exhibited high susceptibility *in-vitro*. Moreover, ceftazidime's susceptibility was raised by about 20% when avibactam was added. It is noted that avibactam's ability to increase ceftazidime activity is highly variable, indicating that beta-lactamase synthesis is not the only cause of this resistance. The susceptibility of Bcc isolates for piperacillin increased slightly (approximately 10%) by adding tazobactam (58% versus 47%). This slight increase in susceptibility could be the result of tazobactam`s inability to inhibit AmpC beta-lactamases in contrast to avibactam [61]. However, according to Lee *et al*., Bcc had a high (90.3%) susceptibility rate to piperacillin-tazobactam and a 72.3% susceptibility rate to meropenem. Consequently, they proposed that piperacillin-tazobactam could be an efficient substitute for cotrimoxazole in treating Bcc infection [63].

Of the studies that reported clinical outcomes, Horsley *et al*. reported that intravenous (IV) antibiotics were administered for a median of two weeks in all patients investigated. Typically, this included an intravenous combination of the medication meropenem and

tobramycin taken together with cotrimoxazole, ceftazidime, and chloramphenicol serving as a supplementary therapy. Nebulized antibiotics were also administered for a median of 12 weeks, with tobramycin or meropenem being the most commonly used. Oral antibiotics were also administered for a median of 7 weeks; cotrimoxazole and/or minocycline were the most often utilized antibiotics. Favorable outcomes were observed in 37% of studied cases [64].

In a study conducted by Gruzelle *et al.,* the antibiotics used in the treatment were administered orally, intravenously, and nebulized. Every antibiotic regimen consisted of two or more combined antibiotics, with an IV beta-lactam administered in 8 cases (72.7%) and an IV or inhaled aminoglycoside and/or IV ciprofloxacin. Four patients received oral therapies consisting of cotrimoxazole, levofloxacin, and/or ciprofloxacin. Aztreonam lysine was used for inhalation in three patients. All IV medications required a duration of 14 days, while oral treatments required 21 to 28 days [65].

The low in permeability of *P. aeruginosa*'s outer membrane*,* the expression of efflux pumps, and the synthesis of enzymes that inactivate antibiotics such as inducible cephalosporinases confer intrinsic resistance to multiple antibiotics. These antimicrobial agent groups were recommended by CLSI 2022 guidelines to be investigated against *P. aeruginosa*. Group A: contains antimicrobial agents regarded for inclusion in usual, primary testing and also for regular reporting of results: ceftazidime, gentamicin, tobramicin, and piperacillin-tazobactam. Group B: consists of antimicrobials that may require initial testing but could be reported under specific circumstances, for example when the organism has resistance to antimicrobials from the same class as those in group A: amikacin, aztreonam, cefepime, ceftazidime-avibactam, imipenem-relebactam, ceftolozane-tazobactam, ciprofloxacin, levofloxacin, doripenem, imipenem, meropenem, and cefiderocol [57].

Beta-lactam/beta-lactamase-inhibitor combinations such as ticarcillin-clavulanate and piperacillin-tazobactam, in addition to antipseudomonal active cephalosporins such as cefoperazone, ceftazidime, and cefepime, are the first-line beta-lactam antibiotics for *P. aeruginosa* infections. Carbapenems, such as meropenem, doripenem, and imipenem, can be considered as second-line treatments [66].

Additional medications involve aztreonam, a member of the monobactam class that provides an alternative for individuals allergic to penicillins. Although aminoglycosides such as tobramycin, amikacin, and gentamicin can be effective against *P. aeruginosa*, they are not

recommended for use as monotherapy due to their increased rates of mortality [67]. The increased resistance levels, which have been linked to up to 54% of nosocomial *P. aeruginosa* infections, have prompted the development of novel anti-pseudomonal medications, which include novel cephalosporins as cefiderocol or novel combinations of beta-lactam/beta-lactamase inhibitors as ceftazidime/avibactam, imipenem-cilastatin/relebactam, and ceftolozane-tazobactam [68]. Combination empirical therapy should be considered for investigation in light of the emergence of antimicrobial resistance and the potential risks of insufficient empirical antibiotic therapy [69,70].

Patients with CF with persistent P. aeruginosa infections have improved from inhaled tobramycin and oral azithromycin. The two antibiotics are commonly used together [71]. Furthermore, Ren *et al*. demonstrated that azithromycin and gentamicin combination therapy had a synergistic effect against *P. aeruginosa* in both *in-vivo* and *in-vitro* conditions. Azithromycin did not, however, exhibit any synergistic effects with other studied aminoglycoside antimicrobial agents, such as amikacin, neomycin, and tobramycin [72].

5.2- Role of repurposed drugs

Considering the scarcity of effective antimicrobial agents for diseases caused by pathogenic bacteria, the rise of resistance to several antimicrobial agents in these bacteria has emerged as an urgent threat to public health [73]. Although novel therapeutic approaches have been investigated, individuals with infections require efficient treatments until they can be applied in clinical settings [74]. We aimed to overview different treatment perspectives that could eradicate Bcc bacteria as well as *P. aeruginosa*.

The increasing concern over MDR bacteria has led to the study of the availability of adjuvant medications to increase antibiotic actions, which is considered crucial [75].

N-acetyl cysteine (NAC) has drawn interest as a potential medication for MDR infections since it is a thoroughly investigated medication with a high safety profile and pleiotropic properties [75]. It is a glutathione endogenous precursor that has been used for a long time as an antioxidant, mucolytic, and anti-inflammatory. It has been used as an inhaled mucolytic in clinical practice [76] for managing lower respiratory tract disorders marked by increased production of thick mucus, especially in individuals with CF or chronic obstructive pulmonary disease [77]. Because NAC is also proteolytic, it may prevent bacterial adhesion and development of EPS along with that it could even improve the effect of the co-administered

antibiotics. Growing evidence shows it exhibits antibacterial and antibiofilm properties against significant respiratory infections *in-vitro* [78]. Several mechanisms have been hypothesized for its antimicrobial activity [79], and the -SH group was believed to be primarily responsible for that effect. It contributes in intra- and intermolecular protein disulfide group degradation, can destroy their dimensional structure, and eventually render them inactive. Therefore, there are different ways that NAC can destroy EPS: either directly through the sulfhydryl group, destroying the disulfide bonds of the bacterial enzymes involved in EPS formation, or indirectly through the antioxidant action, interfering with cell metabolism and EPS formation [80]. For instance, a class of enzymes known as disulfide oxidoreductases, which includes Disulphide Bond Proteins as DsbA, DsbB, DsbC, and DsbD regulates the redox activity in the periplasm of Gram-negative bacteria. The bacterium's proteins can actually have a tertiary configuration because of the coordinated action of these enzymes, which create an oxidative state in the periplasm that permits the formation of disulfide bridges, resulting in the native conformation of the protein and enabling its particular functionality. On the other hand, a NAC thiol group may change the periplasmic redox state of bacteria, deactivating this finely tuned mechanism and leading to protein misfolding that results in cytoplasmic accumulation and exocytosis [81]. Paxman *et al*. demonstrated that a nonsense mutation in the DsbA protein of *Escherichia coli* might inactivate beta-lactamases and increase penicillin sensitivity [82]. NAC was previously shown to have antimicrobial activity against *P. aeruginosa* [77] and has recently been shown to have an effect against Bcc and *Stenotrophomonas maltophilia* [78].

Glutathione (GSH) is the main intracellular antioxidant and a thiol-based tripeptide produced by the body from glycine, L-glutamate, and cysteine. GSH level in healthy cells is within the range from 1 to 10 mM that is equivalent to 307.3 – 3073 μg/mL, however in CF patients, these are much lower due to reduced GSH transport to the lung fluid caused by CFTR mutations [83], leading to inflammation, oxidative stress, oxidation of the mucus cysteine thiols which cause increasing viscosity and difficult expectoration. Research conducted *in-vitro* demonstrates that the inherent acidity and thiol (-SH) component of GSH offer favorable mucolytic characteristics, bacteriostatic efficacy, and the ability to break biofilms [84].

Ascorbic acid, often known as vitamin C (VC), is a reducing agent necessary for intracellular enzymes in the respiratory system's linings and helps inhibit redox reactions. By

preventing quorum sensing, VC can also prevent the production of biofilms, which will alter bacterial chemotactic activity and augment the effects of antibiotics [85].

A recent study proposed that combining an antibiotic with NAC, GSH, or VC might improve the effectiveness of eradicating bacteria at different phases of biofilm formation. The three antioxidants were evaluated for their ability to disturb the biofilms of *B. cenocepacia* bacteria isolated from CF patients by screening them against three antibiotics: ceftazidime, tobramycin, and ciprofloxacin. The combination of ciprofloxacin and NAC resulted in a statistically significant disruption of the biofilm in all tested isolates [78]. A study by Aksoy *et al.* reported that the addition of NAC significantly enhanced the meropenem activity against clinical isolates tested, including *P. aeruginosa* [86].

Other examples of adjunctive treatments to improve antibiotic activities are amiloride and verapamil, which could cause sodium channel blockage, inhibit the activity of efflux pumps, and enhance the activity of different antibiotics.

Amiloride is most well-known for being a moderate diuretic that spares potassium and blocks Na+ channel. It works as a diuretic in the renal distal convoluted tubule, and inhibits Na+ channel activity on the surface of epithelial cells. It is believed that a malfunction in Cl-transport at the apical cell membrane causes excessive Na+ reabsorption in CF, which in turn causes viscous secretions in the airways that make them prone to obstruction of airflow and bacterial infections. In an attempt to stop this process in CF, amiloride has been researched to lower the viscosity and enhance the clearance of secretions from airways. Numerous trials have also shown that nebulized amiloride has a good safety profile [87].

For CF patients who are infected with Bcc or *P. aeruginos*a, amiloride has been demonstrated to be highly effective when combined with tobramycin *in-vivo*. It can also enhance the effects of aminoglycoside treatment [88].

The calcium channel blocker verapamil hydrochloride can potentially be used as an autophagy host-directed therapy for treating intracellular *Mycobacterium tuberculosis*. It is being researched as an inhalable treatment for tuberculosis infections, and it has been shown that the combination of verapamil with antibiotics has an improved antibacterial effect [89]. Investigations showed that it has an inhibitory effect on multi-drug ATP-dependent efflux pumps as well as other efflux pumps; these investigations have focused on mycobacterial efflux pumps [90].

In-vitro studies have indicated that cotrimoxazole combined with verapamil or amiloride works synergistically, and the majority of these combinations had positive clinical outcomes [59]. Both verapamil and amiloride enhanced the tobramycin's *in-vitro* activity against Bcc and could inhibit the bacterium efflux pump activity. Even so, in a group of CF patients, nebulized tobramycin combined with amiloride couldn't successfully treat a chronic lung infection with *B. dolosa* [26].

5.3- Prophylaxis

Administration of flucloxacillin as prophylaxis effectively reduces the incidence of infection with *Staphylococcus aureus* in CF patients [91], but a similar strategy hasn't proven successful with *P. aeruginosa*. However, early detection and elimination may effectively avoid or delay chronic infection. Nebulized or intravenous antimicrobial therapy, or both, could preserve lung function as well as decrease bacterial load in sputum following chronic infection [11]. Early intense therapy with suitable antibiotics for particular Bcc species could postpone or prevent long-term infection. It has been demonstrated that 94% of infections with *B. cenocepacia* and 50% of infections with *B. multivorans* progress to chronicity in spite of treatment [92].

The preferred alternative is to develop effective vaccines that can offer widespread protection. For many years, efforts have been made to develop vaccines against either *P. aeruginosa*, Bcc, or a combination of the two [93]. Understanding the virulence factors, pathogenesis, and interactions between host and pathogens, besides immune responses of hosts, has advanced significantly. However, no ideal vaccines have emerged, highlighting the difficulties in developing secure and efficient vaccinations that can produce persistent immunity against both acute and chronic bacterial infections caused by these two pathogens [94].

Vaccines against many identified antigens are presently undergoing preclinical or clinical trials; nevertheless, none are ready for clinical application. New tools for finding novel antigens that can be used to develop vaccines have lately been available to researchers due to recent advances in proteomics, genomics, and bioinformatics. Both *P. aeruginosa* and Bcc have an astounding amount of complete and publicly accessible genomes available; the *Burkholderia* Genome database had 287 complete genomes and 2979 draft genomes, and the *Pseudomonas* Genome database had 613 complete genomes and 9184 draft genomes. With the help of this

accessible data, there is a great chance of discovering new antigens and developing innovative immunotherapies to fight these infections [93].

6- Conclusion

Patients with CF condition might experience infection of the airways by a variety of pathogens, including *Staphylococcus aureus, Stenotrophomonas maltophilia, Haemophilus influenzae, Mycobacterium species* other than *Mycobacterium tuberculosis*, and *Aspergillus fumigatus*. Each of these microorganisms has the potential to decrease lung function. However, *P. aeruginosa* and Bcc represent the greatest risks to the survival of CF patients. The main risk of morbidity and early death is chronic infections caused by these bacteria, which have the ability to persist in the airways on colonization. Because of their multi-drug resistance, severity, and poor eradication efficiency with current treatments, human infections caused by these bacteria are particularly problematic. They are amongst the hardest to cure and eradicate. However, their pathogenicity is typically not limited to CF. Individuals who possess impaired immune systems can also develop an infection. Effective therapies are needed for infected patients until new therapeutic options for both infections can be converted into practical applications. The two bacteria can produce mixed biofilms in the lungs of people with CF and share the same environmental habitats. It's important to correctly identify and differentiate Bcc species from *P. aeruginosa* since they have an intrinsically different susceptibility pattern. Bcc species have an unremarkable phenotype and a wide variety of genotypes that make identifying this pathogen challenging.

- **Conflict of Interest**

  The authors declare no conflict of interest.

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