Detection and Treatment of Antibiotic-Resistant Pseudomonas Aeruginosa and Burkholderia Cepacia in Healthcare Settings: A Review

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Abstract

This literature review focuses on the issue of antibiotic resistance in healthcare settings, primarily on the detection and treatment of two highly resistant pathogens, *Pseudomonas aeruginosa* and *Burkholderia cepacia*. This review discusses detection methods such as PCR and ELISA, as well as emphasizing the use of 16s- rDNA based PCR assays. This review also explores different current and future treatment strategies for dealing with the two bacteria, such as the use of quorum sensing inhibitors, nanoantibiotics, and phage therapies. This review also discusses a current lack of available research on B. cepacia's treatment methods, concluding by underscoring the urgent need to combat antibiotic resistance in healthcare environments.

Keywords

Antibiotic resistance, Healthcare, Detection, Treatment, Phage Therapy

Introduction

Antibiotic resistance is a highly prevalent issue in healthcare settings. This fact is especially true, where there is increased use of antibiotics to treat patients already suffering from bacterial illnesses. Increased broad spectrum antibiotic use has been shown to cause further antibiotic resistance. Ciprofloxacin, a broad-spectrum antibiotic commonly used to treat various types of infections, has correlated to a higher number of ciprofloxacin-resistant strains (1). Another reason for the high prevalence of antibiotic resistance in healthcare facilities is the prolonged stays of vulnerable patients inside hospitals, as the longer that immunocompromised individuals stay in hospitals, the more likely they are to contract further infections from prolonged exposure to antibiotic-resistant bacteria (2).

Due to the danger posed by different species of bacteria, the WHO has identified a list of twelve “priority pathogens” that pose the highest threat which require new antibiotics (3). This list includes a variety of different bacteria, such as carbapenem-resistant, vancomycin-resistant, and fluoroquinolone-resistant bacteria, among bacteria with resistance to other broad-spectrum antibiotics (3). Under the “critical priority” section of the list is carbapenem-resistant *Pseudomonas aeruginosa*, as well as carbapenem-resistant *Acinetobacter baumannii* and *Enterobacteriaceae*. Although the WHO’s list is an important guide in identifying dangerous bacteria in need of new antibiotics, it is not a comprehensive list of all bacteria that pose a threat (4) (5) (6). Other harmful bacteria insensitive to many antibiotics include *Burkholderia cepacia, Candida auris,* and *Clostridium difficile (*4).

Due to their presence in healthcare environments and difficulty in treatment the two organisms this review will bring attention to are *Pseudomonas aeruginosa* and *Burkholderia cepacia*. As mentioned earlier, *Pseudomonas aeruginosa* is a carbapenem-resistant bacterium in the family Pseudomonadaceae. It is an opportunistic pathogen that is able to survive in many environments, making it an especially dangerous pathogen (7). *Pseudomonas aeruginosa* is known to infect people suffering from cystic fibrosis (CF), as well as patients with nosocomial, or hospital acquired, infections such as ventilator-associated pneumonia (7). In 2017, the bacterium infected over 32,000 hospitalized patients, and resulted in an estimated 2,700 deaths (4). The second bacterium this review will highlight is *Burkholderia cepacia*, a member of the *Burkholderia cepacia* complex that is resistant to aminoglycosides and cephalosporins (8). *B. cepacia* is also an opportunistic pathogen that is known to be found in soil and water (8). Like *P. aeruginosa*, *B. cepacia* is known to cause respiratory infections in people suffering from CF, as well as chronic granulomatous disease. If left untreated, the bacterium can cause quick deterioration of the patient, resulting in fatal necrotizing pneumonia (6).

Detection

Currently, there are multiple methods of detecting bacteria within healthcare facilities, such as by culturing, polymerase chain reaction (PCR), and/or enzyme-linked immunosorbent assay (ELISA) (9). Culturing bacteria involves gathering samples of bacteria either from various surfaces or from patients themselves, then allowing them to grow in a controlled medium, before identifying bacteria present under a microscope. Although culture-based testing is most conventional for diagnostics, it is not often successful due to unsuitable culturing conditions and difficulties in identifying bacteria under microscope (10). Due to this downfall, methods such as PCR and ELISA have become more favored in detecting different bacteria. PCR is a laboratory technique that involves amplifying, then analyzing bacterial genes to identify their presence from

an environment. Unlike PCR, ELISA is a chromogenic technique that identifies antibodies from bodily fluids such as blood, urine, or saliva to detect the presence of bacteria infecting a patient (11). These three techniques are used on both *P. aeruginosa* and *B. cepacia* in healthcare settings to detect, identify, and develop treatment plans against these bacteria.

Detection: *Pseudomonas Aeruginosa*

Detecting *P. aeruginosa* in hospitals and other healthcare facilities is often carried out through PCR techniques, such as 16S- rDNA based PCR assays, which is a type of PCR using 16S ribosomal DNA that provides species-specific sequences, allowing identification of bacteria. This technique was used to identify 104 *P. aeruginosa* isolates found in an Iranian hospital in 2010 (12). Their study used a 16S- rDNA based PCR assay to confirm the presence of *P. aeruginosa* in the samples, and observed that the bacteria were highly resistant to the broad-spectrum cephalosporin antibiotic Ceftazidimine (12). Another method of testing for the presence of *P. aaeruginosa* is by testing for carbapenemase, an enzyme released by *P. aeruginosa* to break down to carbapenems (13). One specific test is the Carba NP test, which detects the hydrolysis of carbapenem by bacteria such as *P. Aeruginosa* (13). It was determined that in a test group of 260 carbapenemase-producing *P. Aeruginosa* strains, the test resulted in a pooled value of 92% correct detections (13). They concluded that although there is no ideal phenotypic test for *P. aeruginosa*, the Carba NP test is a reliable and effective method of testing for the bacterium (13).

Detection: *Burkholderia cepacia*

The process of detecting *B. cepacia* involves techniques such as PCR and western blots. Similar to *P. aeruginosa*, the use of 16S- rDNA based PCR assays, as well as recombinase-aided amplification (RAA) assay, has shown promise in detecting and identifying *B. cepacia* strains (14). A study determined that RAA is a superior test in detecting *Burkholderia cepacia* complex (BCC) bacteria due to lower cost, faster speed, and ease of use (14). They found that RAA was applicable for clinical detection of BCC bacteria in a timely manner (14). Another study sought to develop a lipopolysaccharide-specific monoclonal antibody that would react with *B. cepacia* and other members of the BCC (15). The project resulted in the creation of the first BCC-specific monoclonal antibody, which could be helpful in diagnosing infections caused by *Burkholderia* species in CF patients (15).

Treatment

There are many drugs and procedures currently used to treat infections caused by antibiotic-resistant bacteria, but as bacteria continue to evolve, these methods will become obsolete, and new treatment plans will need to be created. The two main methods for combating antibiotic-resistant bacteria are pathogen and host-directed strategies (16). Pathogen-directed strategies include immunotherapy using monoclonal antibodies to eliminate the pathogenic effects of bacteria, blockage of biofilm formation through drug usage, and strategies to neutralize bacterial toxins by modifying how pathogens bind to host cell receptors (16). Alternatively, host-directed strategies combat antibiotic-resistant bacteria by modifying immune and host cell functions instead of affecting the pathogens themselves (16). While these

strategies are effective in the present, constant reinvention is due, as highly-resistant bacteria will continue to evolve against these strategies faster than medicine can keep up with.

Future Treatment Methods

New treatment methods are constantly being developed to aid the fight against antibiotic-resistant bacteria, some of which include quorum-sensing inhibitors, nanoantibiotics, and phage therapies (17). Quorum sensing inhibitors are a type of medicine that would impede the ability of bacteria to communicate through quorum sensing, a type of cell-cell communication. While still in the early stages of development, many classes of compounds have been reported with the potential to inhibit quorum sensing (17). Another type of medicine that could be used to treat resistant bacterial infections are nanoantibiotics (17). Nanoparticles, especially metal and metal oxide-based ones, have been considered auspicious candidates for eliminating bacteria (17). Due to their high surface area to volume ratio, as well as their increased solubility and ease of delivery, nanoparticles are efficient drug carriers which show promise with antibiotics (18). Nanoparticles have also shown their own antibacterial properties such as the disruption of bacterial cell walls and biofilms (18). Although studies have shown that nanoantibiotics have antibacterial properties, further research needs to be done to ensure their safety and efficacy in patient treatment (17). A third future method to fight antibiotic resistant bacteria is phage therapy (17). Phage therapy entails using bacteriophage, or viruses that kill bacteria, to treat patients when antibiotics have not been effective. Phage therapy has gained popularity in recent years due to the ubiquity of bacteriophages and their harmlessness to humans, as well as their ability to be administered orally, topically, or intravenously (17). Phage therapy does show challenges though, as phages are species-specific, so there would need to be a complete library of phages for every possible bacterial infection (17).

Treatment: *Pseudomonas aeruginosa*

*P. Aeruginos*a has been particularly difficult to treat with antibiotics due to many aspects of its biology, such as a highly impermeable outer membrane, efflux pumps, and antibiotic-inactivating enzymes (7). Efflux pumps are responsible for removing compounds such as antibiotics and quorum sensing signal molecules from bacterial cells (19). *P. aeruginosa's* efflux pumps belong to the resistance-nodulation-division family, which play a crucial role in its antibiotic resistance (7). Particularly, the overexpression of the efflux pumps has contributed to the multidrug-resistant character of *P. aeruginosa* (7).

Although future methods are in the works, current procedures for treating *P. aeruginosa* rely on antipseudomonal antibiotics, including doripenem and plazomicin (7). Doripenem, a carbapenem antibiotic, has shown greater potency against *P. Aeruginosa* isolated from CF patients (7). A dose of 0.5 g and 1.0 g of doripenem three times a day showed 100 percent negativity in *P. Aeruginosa* blood cultures, and 60 percent negativity at 1.5 g three times a day (20). In another study, despite high cure rates, the use of doripenem for *P. Aeruginosa* associated pneumonia has caused headaches, nausea, and phlebitis (7). Another drug that has worked against *P. Aeruginosa* infections is plazomicin, an injectible aminoglycoside (7). When combined with other drugs such as cefepine, doripenem, and imipenem, plazomicin produced synergistic activity with no antagonism, pointing to multidrug uses of plazomicin as treatment for *P. Aeruginosa* (7).

Some of the future methods of treatment mentioned above show promise in treating *P. Aeruginosa*, such as quorum sensing inhibition and nanoparticles. Quorum sensing inhibition

has been shown as a promising treatment for *P. Aeruginosa*, due to its ability to prevent biofilm formation, decrease virulence, and its low risk of development of resistance (7). One example of a quorum sensing inhibitor is zeaxanthin, a carotenoid found in plants, that has reduced *P. Aeruginosa* biofilms (7). Another example is a derivative of halogenated furanone that repressed quorum sensing gene expression in *P. Aeruginosa* (21). Thus, quorum sensing inhibitors seem to be a good approach to treating *P. Aeruginosa* infections, which becomes an even more powerful strategy when combined with phage therapy (7). Another future method of treating *P. Aeruginosa* infections is through the use of nanoparticles, due to their high penetrability into cell membranes and their ability to carry antibiotics (7) As mentioned earlier, metal nanoparticles such as silver nanoparticles are especially effective against *P. Aeruginosa* because they produce silver ions which inhibit bacterial systems (7) (18). Other nanoparticles have been created to carry antibiotics into bacterial cells, such as porous silicon nanoparticles which improved the survival rate of mice with a *P. Aeruginosa* lung infection (7).

Treatment: *Burkholderia cepacia*

There is a lack of accessible research on the treatment of *B. cepacia* infections. This may be due to difficulties encountered while working with this bacterium as well as a lack of research funding or awareness of these infections. This is not to say that there are no current treatment methods though, simply ones without extensive accessible research.

Future methods for combatting *B. cepacia* infections include phage therapy and use of combination antibiotics. Phage therapy for *Burkholderia* infections has been in the works for over 20 years, and recent efforts are continuing to be made (22). The use of phage therapy has been advised for treatment of *Burkholderia* infections due to the fact that most cases of *B. cepacia* infections are in CF patients who have been treated with antibiotics their whole lives, and further use could cause persistent infections (22). Phage therapy for *B. cepacia* infections is limited, though, by the small number of phages known to target BCC strains (22). Some of the phages which can target *B. cepacia* are Myroviridae NS1, NS2, DK2, DK3, JB1, JB5, and RL1c (22). Although there is not much research on BCC phages in human and mammalian immune systems, phage therapy has shown great success in other animal models, giving hope that phage therapy will be an effective treatment method for *B. cepacia* infections (22). Another treatment method in testing for BCC infections is combination antibiotics, of which moxifloxacin-ceftazidime showed the most success (23). Moxifloxacin alone is typically used to treat pneumonia, tuberculosis, and sinusitis; and Ceftazidime is a cephalosporin antibiotic used to treat meningitis, pneumonia, and urinary tract infections. When combined, these antibiotics prevented the overgrowth of resistant cells and the ceftazidime continued its killing effect for 24 hours (23). Combining these antibiotics to fight BCC infections appears suitable for future applications, and seems worth researching further.

Conclusion

The escalating concern regarding antibiotic resistance in healthcare settings highlights the urgent need to tackle this global health threat. The overuse of antibiotics has resulted in the creation of highly-resistant bacteria, such as *Pseudomonas aeruginosa* and *Burkholderia cepacia*, resulting in the need for new detection and treatment strategies. These two bacteria

have been spotlighted due to their prevalence in healthcare environments, especially among patients with respiratory conditions, and the challenges associated with treatment.

With new detection methods constantly evolving, hospitals are no longer held back by conventional culturing. From today's standard of PCR and ELISA tests, to new tests such as Carba NP test and RAA, there is constant progress being made towards better detection of these bacteria. Similar ventures are being made in treatment strategies, with new methods like quorum sensing inhibitors, and further research in phage therapy showing great promise for the future of tackling antibiotic resistance. Ultimately, although there are great strides being made in research against antibiotic resistance, the movement towards tackling resistance remains a constant struggle.

References

1. Pachori P, Gothalwal R, Gandhi P. Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Vol. 6, Genes and Diseases. Chongqing University; 2019. p. 109–19.

2. Peters L, Olson L, Khu DTK, Linnros S, Le NK, Hanberger H, et al. Multiple antibiotic resistance as a risk factor for mortality and prolonged hospital stay: A cohort study among neonatal intensive care patients with hospital-acquired infections caused by gram-negative bacteria in Vietnam. PLoS One. 2019 May 1;14(5).

3. Tacconelli E, Carrara E, Savoldi A, Kattula D, Burkert F. GLOBAL PRIORITY LIST OF ANTIBIOTIC-RESISTANT BACTERIA TO GUIDE RESEARCH, DISCOVERY, AND DEVELOPMENT OF NEW ANTIBIOTICS [Internet]. 2017 Feb [cited 2023 Jul 25]. Available from: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

4. CDC. Antibiotic resistance threats in the United States, 2019 [Internet]. Atlanta, Georgia; 2019 Nov. Available from: https://stacks.cdc.gov/view/cdc/82532

5. Huemer M, Mairpady Shambat S, Brugger SD, Zinkernagel AS. Antibiotic resistance and persistence—Implications for human health and treatment perspectives. EMBO Rep. 2020 Dec 3;21(12). 6. Tavares M, Kozak M, Balola A, Sá-Correia I. Burkholderia cepacia Complex Bacteria: a Feared Contamination Risk in Water-Based Pharmaceutical Products. 2020; Available from: https://doi.org/10.1128/CMR

7. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Vol. 37, Biotechnology Advances. Elsevier Inc.; 2019. p. 177–92.

8. Denis O, Rodriguez-Villalobos H, Struelens MJ. The problem of resistance. Antibiotic and Chemotherapy: Expert Consult. 2010 Jan 1;24–48.

9. Peri AM, Stewart A, Hume A, Irwin A, Harris PNA. New Microbiological Techniques for the Diagnosis of Bacterial Infections and Sepsis in ICU Including Point of Care. 2021; Available from: https://doi.org/10.1007/s11908-021-00755-0

10. Järvinen AK, Laakso S, Piiparinen P, Aittakorpi A, Lindfors M, Huopaniemi L, et al. Rapid identification of bacterial pathogens using a PCR- and microarray-based assay. BMC Microbiol. 2009;9. 11. Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. Peptides (NY). 2015 Oct 1;72:4–15.

12. Fazeli H, Akbari R, Moghim S, Narimani T, Arabestani MR, Ghoddousi AR. Pseudomonas aeruginosa infections in patients, hospital means, and personnel's specimens. Journal of Research in Medical Sciences. 2012.

13. Bouslah Z. Carba NP test for the detection of carbapenemase-producing Pseudomonas aeruginosa. Vol. 50, Medecine et Maladies Infectieuses. Elsevier Masson s.r.l.; 2020. p. 466–79.

14. Fu H, Gan L, Tian Z, Han J, Du B, Xue G, et al. Rapid detection of Burkholderia cepacia complex carrying the 16S rRNA gene in clinical specimens by recombinase-aided amplification. Front Cell Infect Microbiol. 2022 Sep 5;12.

15. AuCoin DP, Crump RB, Thorkildson P, Nuti DE, LiPuma JJ, Kozel TR. Identification of Burkholderia cepacia complex bacteria with a lipopolysaccharide-specific monoclonal antibody. J Med Microbiol. 2010 Jan;59(1):41–7.

16. Parmanik A, Das S, Kar B, Bose A, Dwivedi GR, Pandey MM. Current Treatment Strategies Against Multidrug-Resistant Bacteria: A Review. Vol. 79, Current Microbiology. Springer; 2022.

17. Kumar M, Sarma DK, Shubham S, Kumawat M, Verma V, Nina PB, et al. Futuristic Non-antibiotic Therapies to Combat Antibiotic Resistance: A Review. Vol. 12, Frontiers in Microbiology. Frontiers Media S.A.; 2021.

18. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. Vol. 12, International Journal of Nanomedicine. Dove Medical Press Ltd.; 2017. p. 1227–49.

19. Soto SM. Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. Virulence. 2013;4(3):223–9.

20. Bretonniére C, Jacqueline C, Caillon J, Guitton C, Le Mabecque V, Miégeville AF, et al. Efficacy of doripenem in the treatment of Pseudomonas aeruginosa experimental pneumonia versus imipenem and meropenem. Journal of Antimicrobial Chemotherapy. 2010 Sep 21;65(11):2423–7.

21. Hentzer M, Riedel K, Rasmussen TB, Heydorn A, Andersen JB, Parsek MR, et al. Inhibition of quorum sensing in Pseudomonas aeruginosa biofilm bacteria by a halogenated furanone compound. Vol. 148, Microbiology. 2002.

22. Lauman P, Dennis JJ. Advances in phage therapy: Targeting the Burkholderia cepacia complex. Vol. 13, Viruses. MDPI AG; 2021.

23. El-Halfawy OM, Naguib MM, Valvano MA. Novel antibiotic combinations proposed for treatment of Burkholderia cepacia complex infections. Antimicrob Resist Infect Control. 2017 Nov 25;6(1).