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Research Article

Comparison of the Effects of Antibiotic Sensitivity and Physical Parameters on the Growth of *Burkholderia cepacia* complex and *Burkholderia cenocepacia*

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Abstract

B*urkholderia cepacia* complex (Bcc) has been tied to various FDA drug recalls over the past years. It was found that these bacteria can survive on a broad range of molecules in oxygenic and anoxygenic environments as well as sterilized and non-sterilized environments. The main research question focused on how physical requirements and antibiotics can be used to control Bcc and *B. cenocepacia* growth. Four replicates of TSB tubes that had pH 4, 6, 7, and 8 were inoculated with Bcc and *B. cenocepacia* and incubated overnight at 4, 20, 25, 37, and 85 ℃. The transmission readings of the broth cultures were measured to estimate bacterial growth using a Genesys 2 spectrophotometer. The Kirby-Bauer test was performed using Polymyxin, Ticarcillin, Ticarcillin with Clavulanic acid, Penicillin, Ampicillin, Chloramphenicol, Tetracycline, Erythromycin, and Streptomycin. The E-test was performed using gradient strips of Cefiderocol (C) l (0.016-256 mg/L) and Imipenem-relebactam (IR) (0.002/4-32/4 mg/L). The antibiotic dilution test was performed for Chloramphenicol and Tetracycline after observing larger zones of inhibitions with the Kirby-Bauer test. There was no visible growth of Bcc and *B cenocepacia* at 4 ℃ and 85 ℃ at any pH and pH 4 across the temperatures. However, subcultures showed bacterial growth the following day. The growth rates increased significantly at 25 and 37 ℃ as well as pH 6 and 7. The average diameters of the zones of inhibitions of PXB, TCC, TIC, C30, and TE30 for Bcc were 1.3, 3.7, 3.1, 2.0, and 1.16 mm and for *B. cenocepacia* were 0, 1.2, 1.3, 1.1, and 1.6 mm, respectively. Both Bcc and *B. cenocepacia* were resistant to P10, AM10, E15, and S10. MIC for the E-test of Bcc and *B. cenocepacia* for IR and C were 0.67 and 10 and 0.88 and 0. 016. mg/L, respectively. MIC and MBC for the dilution test of the C30 and TE30 for Bcc were 1 and 8 and 64 and 128 and *B. cenocepacia* 8 and 128 and 16 and 128 μg/ml. These bacteria had faster growth rates with no significant difference in their growth under the various temperature and pH conditions used. The research concluded that both Bcc and *B. cenocepacia* can grow in typical storage conditions such as 4 ℃ and pH 4, without showing any visible signs of growth. This study showed that *B. cenocepacia* has significantly higher resistance to antibiotics than Bcc. These results are beneficial for developing strategies to prevent *Burkholderia* cross-contamination in clinical environments.

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Keywords

Burkholderia cepacia complex, *Burkholderia cenocepacia*, Physical Requirements, Antibiotic Sensitivity

1. Introduction

Burkholderia cepacia complex (Bcc) was linked to various FDA drug recalls such as nasal spray, curl styler, wipe, oral electrolyte, and anesthetic hydrogel in recent years [1]. Bcc contains 24 distinct opportunistic pathogen species that are phylogenetically close relatives including *B. cepacia*, B. *multivorans*, *B. cenocepacia*, *B. stabilis*, *B. vietnamiensis*, *B. dolosa*, *B. ambifaria*, *B. anthina*, and *B. pyrrocinia*. The members of Bcc including *B. cepacia*, *B. multivorans*, *B. pseudomallie*, *B. mallei*, and *B. cenocepacia* are being classified as human pathogens [2, 3].

Burkholderia spp. can thrive in a broad spectrum of molecules, including disinfectants, hospital equipment, medicinal drugs, biocides, petroleum products, antimicrobial solutions, sterile solutions, preserved pharmaceuticals, cosmetics, and toiletries [4]. Their ubiquity is associated with their capability to survive on minimal nutrient requirements, broad metabolic adaptabilities, and rapid mutations during infections [2].

This gram-negative aerobic rod shaped betaproteobacteria has three circular chromosomes weighted as 3.87 Mb, 3.22 Mb, and 0.88 Mb and one plasmid (0.09 Mb) [5, 6]. These opportunistic pathogens are positive for catalase and oxidase tests and negative for lactose fermentation. The bacteria cause nosocomial infections due to patients' prolonged hospitalization, extensive usage of broad-spectrum antibiotics and poor ventilation at hospitals [2]. The bacteria worsen lung infections in patients who have *cepacia* syndrome with necrotizing pneumonia and sepsis [7]. Bcc can also infect immunocompromised patients with cystic fibrosis and chronic granuloma diseases, a fatal pneumonia accompanied by septicemia [8, 9]. Among members of Bcc, *B. cenocepacia* has been identified as the most common clinically important bacteria isolated from North America and Europe [10]. Furthermore, *Burkholderia* can transfer from one cystic fibrosis patient to another in hospital environments [11, 12]. In addition, *cepacia* syndrome causes pulmonary decline in patients after lung transplant. It was shown that *B. cenocepacia* infected patients before lung transplant were six times higher to die within first year after the transplant [13].

A study showed that the bacteria that were isolated from clinical samples had similarities to the Bcc isolated from chlorhexidine mouthwash [14]. Clinical samples of Bcc have been isolated from the sputum, blood, and tracheal aspirates of the patients. Häfiger et al., 2020 [6] included 111 nosocomial Bcc outbreaks in their study including twenty outbreaks in Europe, 38 in North America, 29 in Asia., 10 in the Middle East, 11 in South America, 3 in Australia. They reported that 73.9% of the cases had been matched with their sources including 53.2% cases were tied to contaminated medical solutions and medications, and 12% of the cases tied to contaminated disinfectants [6].

Burkholderia are also commonly found in soil, water, and plants including flowers [16]. These bacteria are capable of being viable for months in moist environments even though dry conditions were not favorable for their growth [6]. William Burkholder was the first person to describe this bacterium in 1949 as a plant pathogen causing onion rot in New York and to name the species *cepacia* by using the Latin word for onion rot [17]. Initially the bacteria were known as *Pseudomonas cepacian*, but ribosomal RNA analysis showed that the *Pseudomonas cepacian* was distantly related to the new group *Burkholderia* [18].

Bcc has been identified for its potential use in agriculture as a biofertilizer due to their nitrogen-fixing ability [3]. Among nitrogen-fixing *Burkholderia* species, *B. vietnamiensis* was the first to be discovered in association with rice, maize, and coffee plants and *B. brusilensis* and *B. tropicalis* were found in association with banana and pineapple plants. In addition, the evidence showed that insect species harbor *Burkholderia* spp. in symbiotic relationships [25].

It was suggested that water could be potential contaminant for Bcc cross-contamination during the manufacturing processes of sterile and non-sterile products including inhalers, parenteral solutions, disinfectants, laxatives, and lubricants for urinary catheterization [19]. To overcome Bcc contaminations, the FDA enforced routine testing for both raw materials and finished products of manufacturing processes.

Burkholderia have the potential to be used as bioremediation, biocontrol, and biofertilizer, but the use of *Burkholderia* has moratorium due to pathogenicity in humans and plants [18-20].

Bcc uses various ways to invade host defensive mechanisms including flagella to help them with their movements and pili to attach the epithelial cells of the lungs, as the first step for infections [21, 22]. In addition, their resistance-nodulation-division (RND) genes, RND3, RND4, and RND9 control efflux pumps to help them with surviving in biocides. They use quorum sensing for cell signaling. In addition, lipase, metalloprotease, and siderophores increase their virulence factors. Moreover, there is an increased concern about their capability of making biofilms inside organisms [23]. Bcc is also capable of altering the targets of antibiotics and developing resistance to them [24]. The main research question focused on how physical requirements and antibiotics can be used to control Bcc and *B. cenocepacia* growth.

All the data have been statistically analyzed using ANOVA with a 95% significance.

2. Materials and Method

2.1. Bacterial Strain Collection

Lyophilized *B. cepacia* complex and *B. cenocepacia* (Carolina Biology Supply Company, NC) were rehydrated using Tryptic Soy broth, and the culture broth tubes were incubated at 37 ℃ overnight in the microbiology lab at Frostburg State University. The aliquots of the cultures were stored in the -80 ℃ freezer until further use.

2.2. Effect of Temperature on *Burkholderia* Growth

The bacteria cultures stored in the -80 ℃ were thawed and subcultured in autoclaved Tryptic Soy Broth (10 ml). Two replicates of Bcc and *B. cenocepacia* broth cultures were used to adjust pH at 4, 6, 7, and 8 and incubated the tubes overnight at 4, 15, 25, 37, and 85 ℃ to understand the effect of temperature and pH on *Burkholderia* growth and to understand their controlling measures.

2.3. Antibiotic Sensitivity Using the Kirby-Bauer Test

Four replicates of lawns of bacteria were used for the Kirby-Bauer test using cotton swabs and Muller-Hinton agar plates for both Bcc and *B. cenocepacia*. The impregnated antibiotics, such as Amoxicillin (10 mg), Erythromycin (15 mg), Penicillin (10 mg), Streptomycin (10 mg), Chloramphenicol (30 mg), Tetracycline (30 mg) and Polymyxin B (30 mg) from Fisher Scientific, and TCC (85 mg) and TIC (75 mg) from Germany, were placed on the Muller-Hinton plates around a negative control and the plates were incubated overnight at 37 ℃. The zones of inhibitions (Table 1) were measured in mm the following day to assess bacterial sensitivity for each antibiotic.

2.4. Antibiotic Sensitivity Using the E-test

Gradient strips of Imipenem-Relebactam and Cefiderocol were placed on four replicates of lawns of Bcc and *B. cenocepacia* plates prepared on Muller-Hinton and were incubated at 37 ℃.

The E-strips indicated the minimal inhibitory concentration (MIC) of the antibiotic required to inhibit the growth of Bcc and *B. cenocepacia*.

2.5. Dilution Test for Tetracycline (TE30) and Chloramphenicol (C30)

Serial dilutions of antibiotics at fifty percent concentrations were prepared using nine Mueller Hinton broth tubes (1 ml/tube) and dehydrated Tetracycline (TE30) and Chloramphenicol (C30) powder as shown in table 1. A sterile pipette was used to transfer 2 ml of the antibiotic (256 μg/ml) to tube 1. After mixing Tube 1, 1 ml of the broth was transferred to Tube 2 using a new pipette. The transferring of 1 ml of the broth in the tubes continued using new pipettes up to Tube 8 with 1 ml being removed from Tube 8. The concentration of antibiotics was reduced 50% every time the broth transferred from tube 1 to 8. The tube 9 served as the negative control and contained no antibiotics.

Bacteria inoculum for the test was prepared by transferring five colonies to 5 ml of Mueller Hinton broth and 100 μl of the bacterial suspension was transferred to 20 ml Mueller Hinton broth. All the broth culture tubes were incubated overnight at 37 ℃.

The least concentration of the antibiotic in the tube that did not have visible bacterial growth with a cloudy appearance was recorded as the MIC. Then the tubes with no visible growths were streaked on TSA plates and incubated overnight at 37 ℃. Minimum concentration of the antibiotic that did not have growth on the TSA was recorded as the MBC.

Table 1. *The 50% dilutions of the antibiotic.*

Tube no	Antibiotic concentration (μg/ml)
1	128
2	64
3	32
4	16
5	8
6	4
7	2
8	1
9	0

3. Results and Discussion

3.1. Temperature and pH Effect on Bacterial Growth

Four replicates of Bcc and *B. cenocepacia* were inoculated in TSB that had adjusted pH at 4, 6, 7, or 8 and incubated them at 4, 20, 25, 37, and 85 ℃. After overnight incubation, transmission readings were recorded as shown in the tables below (Tables 2-6) using the Genesys 2 spectrophotometer. Transmission is inversely proportional to the bacterial growth. Therefore, higher transmission corresponds to lower bacterial growth at each tested temperature and pH level.

Table 2. Mean absorption readings of the broths at 4 ℃.

Bacteria	pH 4	pH 6	pH 7	pH 8
B. *cepacia* complex	100%	100%	100%	99.4$
B. *cenocepacia*	100%	100%	99.8%	99.6%

Table 3. Mean absorption readings of the broths at 20 ℃.

Bacteria	pH 4	pH 6	pH 7	pH 8
B. *cepacia* complex	100%	91.9%	88.6%	93.9%
B. *cenocepacia*	99.9%	84.8%	88.4%	93.7%

Table 4. Mean absorption readings of the broths at 25 ℃.

Bacteria	pH 4	pH 6	pH 7	pH 8
B. *cepacia* complex	99%	79.6%	80.8%	79.5%
B. *cenocepacia*	99.6%	70.1%	77.7%	84%

Table 5. Mean absorption readings of the broths at 37 ℃.

Bacteria	pH 4	pH 6	pH 7	pH 8
B. *cepacia* complex	58.4%	56.9%	61.6%	70.5%
B. *cenocepacia*	55.2%	57.0%	64.5%	60.1%

Table 6. Mean absorption readings of the broths at 85 ℃.

Bacteria	pH 4	pH 6	pH 7	pH 8
B. *cepacia* complex	99%	100%	100%	100%
B. *cenocepacia*	100%	100%	100%	100%

*4 replicates

To confirm the absence of visible bacterial growth at pH 4, all broth cultures of Bcc and *B. cenocepacia* were spread plated on TSA, incubated overnight at 37 ℃, and observed the following day. (Figure 1 and Figure 2).

Figure 1. Spread plated Bcc at pH 4; from left at 4, 20, 25, 37, and 85 ℃.

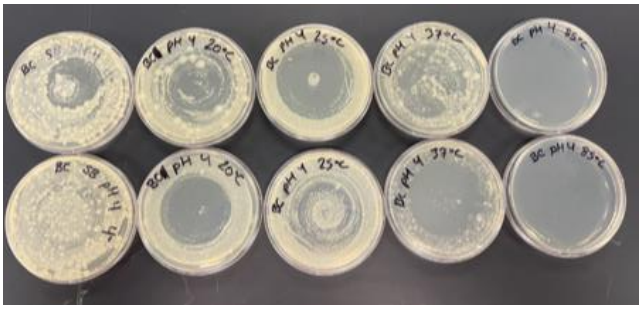

Figure 2. Spread plated B. cenocepacia at pH 4; from the left at 4, 20, 25, 37, and 85 ℃.

3.2. Antibiotic Sensitivity with the Kirby-Bauer Test

The Kirby-Bauer test was conducted to test the antibiotic sensitivity of the bacteria to Amoxicillin (10 mg), Erythromycin (15 mg), Penicillin (10 mg), Streptomycin (10 mg), Chloramphenicol (30 mg), Tetracycline (30 mg), Polymyxin B (30 mg), Ticarcillin (TIC 75), and Ticarcillin with Clavulanic acid (TCC 85). After overnight incubation, the zones of inhibitions were compared with the negative control at the center of the plate and the diameters were measured in millimeters (Table 7).

Table 7. Antibiotics sensitivity (diameter in mm).

Bacteria	AM10	E15	P10	S10	C30	TE30	PXB30	TCC85	TIC75
B. *cepacia* complex	0	0	0	0	2	1.16	1.3	3.7	3.1
B. *cenocepacia*	0	0	0	0	1.1	1.7	0	1.2	1.3

3.3. Antibiotic Sensitivity with the E-test

The E-test was performed using gradient strips of Cefiderocol (C) l (0.016-256 mg/L) and Imipenem-relebactam (IR) (0.002/4-32/4 mg/L). The tables below (Tables 8 and 9) show MIC values of four replicates.

Table 8. E-test results; MIC for Bcc.

Cefiderocol	6	4	6	4
Imipenem	0.064	0.094	0.047	0.064

Table 9. E-test results; MIC for B. cenocepacia.

Cefiderocol	0.016	0.016	0.016	0.016
Imipenem	0.75	1.5	0.5	0.75

* Four replicates

3.4. MIC and MBC of Antibiotics Using the Dilution Test

The MIC and MBC values were recorded for both of Tetracycline (TE30) and Chloramphenicol (C30) as shown in the tables below (Tables 10 & 11).

Table 10. Dilution test results: MIC and MBC of Bcc.

Antibiotics	MIC	MBC
Tetracycline	64 µg/ml	128 µg/ml
Chloramphenicol	1 µg/ml	8 µg/ml

Table 11. Dilution test results: MIC and MBC of B. cenocepacia.

Antibiotics	MIC	MBC
Tetracycline	16 µg/ml	128 µg/ml
Chloramphenicol	8 µg/ml	128 µg/ml

4. Results and Discussion

Four replicates of Bcc and *B. cenocepacia* were inoculated in TSB with pH levels of 4, 6, 7, and 8 and incubated at 4, 20, 25, 37, and 85 ℃. After overnight incubation, broth cultures were tested for transmission using a Genesys 2 Spectrophotometer. There were no visible growths observed for either Bcc or *B. cenocepacia* at 4 ℃, 85 ℃, or at pH 4 across all the temperatures (4, 20, 25, 35, and 85 ℃). However, transmission readings and spread plates with Bcc and *B. cenocepacia* indicated the presence of bacteria even at pH 4. These observations suggest that Bcc and *B. cenocepacia* can grow in liquid media without producing visible signs.

Kirby-Bauer, E-strip, and the dilution tests were used to assess the antibiotic sensitivity of both Bcc and *B. cenocepacia*. The average diameters of the inhibition zones of PXB, TCC, TIC, C30, and TE30 on Bcc were 1.3, 3.7, 3.1, 2.0, and 1.16 mm and that of *B. cenocepacia* were 0, 1.2, 1.3, 1.1, and 1.6 mm, respectively. The Bcc and *B. cenocepacia* were resistant to P10, AM10, E15, and S10 with no inhibition zones.

The E-strip test for Cefiderocol (C) l (0.016-256 mg/L) and Imipenem-relebactam (IR) (0.002/4-32/4 mg/L) showed average MIC of Cefiderocol for Bcc and *B. cenocepacia* were 5 and 0.016 mg/L, and that of Imipenem-relebactam were 0.67 and 0.88 mg/L, respectively.

The fifty precent serial dilution tests showed that the MIC and MBC of the C30 and TE30 for Bcc were 1 and 8, and 64 and 128 and that of *B. cenocepacia* were 8 and 128, and 16 and 128 µg/ml. Bcc had MBC at 128 µg/ml for Tetracycline and 8 µg/ml for Chloramphenicol while *B. cenocepacia* had MBC at 128 µg/ml, tube 2, for both Tetracycline and Chloramphenicol.

The overall antibiotic test results showed that the *B. cenocepacia* is more resistant to antibiotics compared to Bcc. According to our results, Bcc and *B. cenocepacia* are multidrug resistance bacteria. H äfiger et al., 2020 [6] also identified that medical preparations such as solutions, drugs and disinfectants were 66% of the source of the Bcc outbreaks with leading to 240 deaths. In addition, they showed 62.1% of the cases were due to medical devices testing including bronchoscopy and anesthesia equipment.

Statistical analysis using ANOVA revealed a 95% confidence level of significant differences between Bcc and *B. cenocepacia* in response to physical parameters and antibiotics. The results showed that *B. cenocepacia* exhibited higher growth rates than Bcc, as well as significantly greater antibiotic resistance.

5. Conclusion

Bcc and *B. cenocepacia* growths were tested at 4, 20, 25, 37, and 85 ℃ and pH 4, 6, 7, and 8 to explore potential control measures especially in clinical environments. Additionally, antibiotic sensitivity testing was conducted to examine their resistance for treatments.

TSB broth tubes were adjusted to pH 4, 6, 7, and 8 and after inoculation with bacteria, four tubes of each pH were incubated overnight at 4, 20, 25, 37, and 85 ℃. The effect of physiological parameters for the growth of Bcc and *Burkholderia cenocepacia* were tested using the transmission

values. Transmission value is inversely proportional to the bacterial growth. There were no visible growths of Bcc and *Burkholderia cenocepacia* at 4 and 85 ℃ at any pH, and none at pH 4 across temperatures even with more than 50% transmission at 37 ℃. However, bacterial growth rates significantly increased at 20, 25, and 37 ℃. At 85 ℃, TSB turned a darker color. These results suggest that the products such as medicinal drugs should be stored at a temperature less than 4 ℃ or adjusted to a pH less than 4 if applicable for a slower bacterial growth. However, there was still bacterial growth observed on spread plates with Bcc and *B. cepacia* at pH 4 after overnight incubation, suggesting that these bacteria can grow without producing visible signs in liquid media.

This study also confirmed that preventing Bcc and *B. cenocepacia* growth is challenging even if the source of the outbreak has been identified in controlling infections and agreed with the report of asymptomatic colonization in Cystic Fibrosis patients [6].

In the antibiotic sensitivity test using Kirby-Bauer test, the average diameter of the inhibition zones of PXB, TCC, and TIC on Bcc were 1.3, 3.7, and 3.1 mm, and that of *B. cenocepacia* were 0, 1.2, and 1.3 mm, respectively. Both Bcc and *Burkholderia cenocepacia* bacteria were resistant to P10, AM10, E15, and S10 and the average diameter of the inhibition zones for C30 and TE30 were 2 and 1.16 and 1.1 and 1.7 mm for Bcc and *B. cenocepacia*, respectively. E-test results showed that the average MIC of Imipenem-relebactam and Cefiderocol for Bcc and *B. cenocepacia* were 0.067 and 5 and 0.88 and 0.016 mg/L, respectively. The dilution test results for MIC and MBC showed for Bcc for C30 and TE30 were 1 and 8 and 64 and 128 μg/ml, and that of *B. cenocepacia* were 8 and 128 and 16 and 128 μg/ml.

Our findings agreed with Bcc and *B. cenocepacia* have inherent resistance to antibiotics, along with the ability to survive in nutrient-limited conditions [2]. Therefore, it is imperative to explore all possible ways of physiological control measures for Bcc and *B. cenocepacia*, while highlighting difficulty in control and prevention due to invisible growth of bacteria in low temperature and pH. Furthermore, our results suggest that medicinal products, such as drugs, should be stored at temperatures below 4 ℃ and/or adjusted to a pH below 4, where applicable, to slow bacterial growth. This research will continue to investigate the pathogenicity of *Burkholderia* spp. and explore the synergistic effects of antibiotics for potential treatments.

Abbreviations

Bcc	*Burkholderia cepacia* Complex
AM10	Amoxicillin (10 mg)
E15	Erythromycin (15 mg)
P10	Penicillin (10 mg)
S10	Streptomycin (10 mg)
C30	Chloramphenicol (30 mg)
TE30	Tetracycline (30 mg)
PXB30	Polymyxin B (30 mg)
TCC85	Ticarcillin with Clavulanic Acid (85 mg)
TIC75	Ticarcillin (75 mg)
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
C	Cefiderocol
IR	Imipenem-relabactam

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Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] FDA, https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-multistate-outbreak-burkholderia-*cepacia*-infections (17 October 2021).

[2] Tavares, M., Kozak, M., Balola, A., and Sá-Correia, I. *Burkholderia cepacia* Complex Bacteria: a Feared Contamination Risk in Water-Based Pharmaceutical Products. *Clin Microbiol Rev*. 2020, 33(3). https://doi.org/10.1128/CMR.00139-19

[3] De Soyza, A., Alba, S., Rosa, L., John, G., and Molinaro A. Chemical and biological features of Burkholderia *cepacia* complex lipopolysaccharide. *Innate immunity*. 2008, 14(3), 127-144. https://doi.org/10.1177/1753425908093984

[4] Jimenez, L. 2007. Microbial diversity in pharmaceutical product recalls and environments. PDA *J. Pharm. Sci. Technol*. 2007, 61, 383–399. https://doi.org/pubmed.ncbi.nlm.nih.gov/18047177/

[5] O'Grady, E. P. and Sokol, P. A. *Burkholderia cenocepacia* differential gene expression during host-pathogen interactions and adaptation to the host environment. *Frontiers in Cellular and Infection Microbiology*. 2011. https://doi.org/10.3389/fcimb.2011.00015

[6] Häfiger, E., Atkinson, A., and Marschal, l J. 2020. Systematic review of healthcare-associated *Burkholderia cepacia* complex outbreaks: presentation, causes and outbreak control. *Infection Prevention in Practice*. 2020, 2(3), 1-6. https://doi.org/10.1016/j.infpip.2020.100082

[7] Rushton, L., Sass, A., Baldwin, A., Dowson C. G., Donoghue, D., and Mahenthiralingam, E. Key role for efflux in the preservative susceptibility and adaptive resistance of complex bacteria. *Antimicrobial Agents Chemotherapy*. 2013, 57, 2972–80. https://doi.org/10.1128/AAC.00140-13

[8] Coutinho, C. P., Dos Santos, S. C., Madeira, A., Mira, N. P., Moreira, A. S., Sá-Correia, I. Long-term colonization of the cystic fibrosis lung by *Burkholderia cepacia* complex bacteria: epidemiology, clonal variation, and genome-wide expression alterations. *Front Cell Infect Microbiol*. 2011, 1: 12. https://doi.org/10.3389/fcimb.2011.00012

[9] Johnston, R. B, Clinical aspects of chronic granulomatous disease. *Curr Opin Hematol*. 2001. 8(1), 17–22. https://doi.org/10.1097/00062752-200101000-00004

[10] Slade, L A. and Miguel, V. A. A Decade of *Burkholderia cenocepacia* Virulence determinant research. Infect. Immun. 2010. 78(10), 4088-4100. https://doi.org/10.1128/IAI.00212-10

[11] Govan, J. R. W., Brown, P. H., Maddison, J., Doherty, C., Nelson, C. J., Dodd, M., Greening, A. P., and Webb, A. K. Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis patients. Lancet.1993. 342: 15-19. https://doi.org/10.1016/0140-6736(93)91881-l

[12] Bennett, J. E., Dolin, R., and Blaser, M. J. *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. (9th ed). 2020. Elsevier. ISBN 978-0-323-55027-7.

[13] Alexander, B. D., Petzoldb, E. W., Rellera, L. B., Palmerc, S. M., Davisd, R. D., Woodsa, C. W., and LiPuma, J. J. Survival After Lung Transplantation of Cystic Fibrosis Patients Infected with Burkholderia *cepacia* Complex. *American Journal of Transplantation*. 2008, 8, 1025–1030. https://doi.org/10.1111/j.1600-6143.2008.02186.x

[14] Leong, E. X., Lex, L. D., Carter, P. G., Wang, P., Smith, K., Stinear, P., Tim, S. D., Sintchenko, V., Wesselingh, L, Stevan, B. I., and Rogers, B. G. Impact of long-term erythromycin therapy on the oropharyngeal microbiome and resistance gene reservoir in non-cystic fibrosis bronchiectasis. 2018. *Emerg. Infect Dis*. 2018, 24 (11), 2109-2111. https://doi.org/10.1128/msphere.00103-18

[15] Leong, E. X. L., Lagana D., Carter, G. P., Wang, Q. Smith, K., Stinear, T. P. Shaw, D., Sintchenko, V., Wesselingh, S. L., Bastian, I., Rogers, G. B. *Burkholderia lata* infections from intrinsically contaminated chlorhexidine mouthwash, Australia, 2016. *Emerg Infect Dis*. 2016. 24(11), 2109-2111. https://doi.org/10.3201/eid2411.171929

[16] Vial, L., Annelise, C., Groleau, M., and Deziel, E. The various lifestyles of the *Burkholderia cepacia* complex species: a tribute to adaptation. *Environmental Microbiology*. 2011. 13(1): 1-12. https://doi.org/10.1111/j.1462-2920.2010.02343.x

[17] Burkholder, W. H. Sour skin, a bacterial rot of onion bulbs. *Phytopathology*. 1950, 40, 115–117. https://www.cabidigitallibrary.org/doi/full/10.5555/19501101355

[18] Yabuuchi, E., Kosako, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y.1992. Proposal of Burkholderia Gen. Nov. And Transfer of Seven Species of the Genus Pseudomonas Homology Group II to the New Genus, With the Type Species Burkholderia *Cepacia*. *Microbiol. Immunol.* 1992, 36 (12): 1251–1275. https://doi.org/10.1111/j.1348-0421.1992.tb02129.x

[19] Sommerstein, R., Führer, U., Lo Priore, E., Casanova, C., Meinel, D. M., Seth-Smith, H. M., Kronenberg, A., Koch, D., Senn, L., Widmer, A. F., Egli, A., Marschall, J., Anresis, S., A. *Burkholderia stabilis* outbreak associated with contaminated commercially available washing gloves, Switzerland, May 2015 to August 2016. *Euro Surveill*. 2017, 22(49): 17. https://doi.org/10.2807/1560-7917.ES.2017.22.49.17-00213

[20] Baldwin, A., Mahenthiralingam, E., Drevinek, P., Vandamme, P., Govan, J. R., Waine, D. J., Environmental *Burkholderia cepacia* complex isolates from human infections. *Emerg. Infect. Dis*. 2007, 13(3), 458–461. https://doi.org/10.3201/eid1303.060403

[21] McClean, S., Callaghan, M. *Burkholderia cepacia* complex: epithelial cell-pathogen confrontations and potential for therapeutic intervention. *J. Med. Microbiol.* 2009, 58, 1–12. https://doi.org/10.1099/jmm.0.47788-0

[22] Schwager, S., Agnoli K., Kothe M., Feldmann, F., Givskov, M., Carlier A., et al. (2013). Identification of *Burkholderia cenocepacia* strain H111 virulence factors using nonmammalian infection hosts. *Infect. Immun.* 81 143–153. https://doi.org/10.1128/IAI.00768-12

[23] Coenye, T., Van Acker, H., Peeters, E., Sass, A., Buroni, S., Riccardi, G. 2011. Molecular mechanisms of chlorhexidine tolerance in Burkholderia *cenocepacia* biofilms. *Antimicrob Agents Chemother*. 55(5): 1912–1919. https://doi.org/10.1128/AAC.01571-10

[24] Foweraker, J. 2009. Recent advances in the microbiology of respiratory tract infection in cystic fibrosis. *Br Med Bull*. 2009 89(1): 93-110. https://doi.org/10.1093/bmb/ldn050

[25] Eberl, L. and Peter, V. Members of the genus Burkholderia: good and bad guys. *Food research*. 2016, 5. https://doi.org/10.12688/f1000research.8221.1