Identifying and Understanding Multidrug Resistant Pathogens Isolated from Cystic Fibrosis Patients

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Introduction

Cystic Fibrosis (CF) is an autosomal recessive disease caused by a mutated Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene located on chromosome 7 [1]. The CFTR protein is a chloride ion channel. When the CFTR gene is mutated, it causes the protein to be absent or lose function, which leads to dehydration in the lung's airways and also traps mucus inside the lungs [2]. These conditions in the lung generate a perfect environment for bacterial colonization by multiple different species [3]. Chronic bacterial colonization and development of antibiotic resistance are serious concerns for CF patients. However, many recent studies looking at the complexity of these infections have suggested that many different types of bacteria persist in the CF lung and acquire antibiotic resistance through various mechanisms which significantly hinders treatment options and drastically impacts patient mortality [4]. The diverse bacterial populations are recovered from the sputa using biochemical selective media, catalogued, and cryogenically stored. The goal is to isolate, test against various antibiotics, and sequence general sputa from cystic fibrous patients in order to compare their sequences and resistance capabilities to wild type strains.

Methods

Forty-one clinical samples were struck onto *Mueller Hinton Agar (MHA)* plates and separated by colony morphology onto plates containing Gentamycin, Polymyxin B, Carbenicillin, and Ticarcillin. Colony types showing growth in the presence of three or more of the preliminary antibiotics were then tested for relative resistance using the Kirby Bauer Disk infusion test. In this test, resistant strains were struck in a lawn and had eight disks containing increasing concentrations of antibiotic placed on the agar surface. The zones of clearing around each disk after incubation at 37℃ for 24 hours were measured to determine relative resistance. Isolates displaying high levels of resistance to multiple antibiotics were frozen into stocks for further quantitative testing. Freezer stocks of eleven highly resistant colony types were then tested against the previously used antibiotics as well as Oxacillin, Ciprofloxacin, Erythromycin, Vancomycin, and Methicillin using the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration tests. From these tests, quantitative resistance data was deduced. These isolates had their DNA extracted, duplicated in Polymerase Chain reactions, and sequenced using the Sanger Sequencing method. These results were analyzed using NCBI Basic Local Assignment Search Tool (BLAST) to identify the unknown isolates to the genus level.

Results

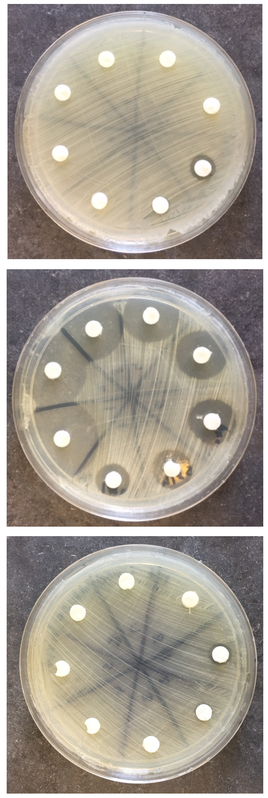

Figure 1. Kirby-Bauer Disk Infusion Photos

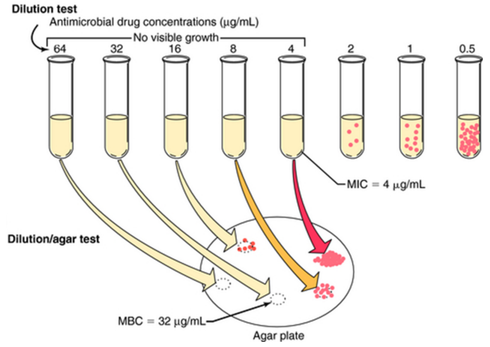

Figure 2: Differences in Methodology: MIC vs. MBC

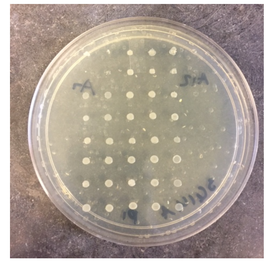

Figure 3. Visual MBC Result

Table 1: MIC Results* (Reported in mg/mL)

ID Number	PM	G	TC	CB	ER	OX	CIP	V	M
3614A	0.469	5.0	>5.0	1.875	>0.10	>2.5	1.75	<0.039	2.5
3614C #17	0.234	0.938	>5.0	>5.0	>0.10	>2.5	1.75	>5.0	1.875
3614C #21	>1.875	5.0	>5.0	>5.0	>0.10	>2.5	1.75	>5.0	2.5
5814F #1	0.469	1.875	1.25	1.25	>0.10	0.313	0.438	<0.039	1.25
5814F #5	0.351	0.938	1.25	2.5	>0.10	0.963	0.438	<0.039	>2.5
5814B	0.469	>5.0	>5.0	>5.0	>0.10	0.469	0.656	>5.0	>2.5
5813E	0.234	1.875	2.5	>5.0	>0.10	0.625	0.438	>5.0	>2.5
61214B	0.234	<0.04	1.875	0.938	>0.10	0.313	0.027	<0.039	>2.5
6514A	0.703	1.875	2.5	>5.0	>0.10	0.313	1.75	<0.039	1.25
7314A	0.176	<0.04	0.938	0.938	>0.10	0.234	0.656	0.098	0.328
8714C	0.938	0.234	0.313	1.25	>0.10	1.25	0.656	<0.039	<0.020

Table 2: Quantitative MBC Results* (Reported in mg/mL)

ID #	PM	G	TC	CB	ER	OX	CIP	V	M
3614A	0.469	>5.0	>5.0	>5.0	>0.10	>2.5	>1.75	<0.039	>2.5
3614C #17	0.117	0.938	>5.0	>5.0	>0.10	>2.5	>1.75	>5.0	>2.5
3614C #21	>1.875	5.0	>5.0	>5.0	>0.10	>2.5	1.75	>5.0	>2.5
5814F #1	0.938	1.875	3.125	1.25	>0.10	0.313	0.656	<0.039	1.875
5814F #5	0.586	1.25	1.875	2.5	>0.10	1.25	0.438	<0.039	>2.5
5814B	0.469	>5.0	>5.0	>5.0	>0.10	0.469	>1.75	>5.0	>2.5
5813E	0.234	5.0	>5.0	>5.0	>0.10	>2.5	>1.75	>5.0	>2.5
61214B	0.234	<0.04	3.75	0.938	>0.10	0.469	0.027	<0.039	>2.5
6514A	0.938	1.875	2.5	>5.0	>0.10	0.313	1.75	<0.039	1.25
7314A	0.469	<0.04	0.938	0.938	>0.10	0.234	0.656	0.098	0.328
8714C	0.938	0.234	0.313	1.25	>0.10	1.25	>1.75	<0.039	0.059

***PM = Polymyxin B, G = Gentamycin, TC = Ticarcillin, CB = Carbenicillin, ER = Erythromycin, OX = Oxacillin, CIP = Ciprofloxacin, V = Vancomycin, M = Methicillin**

ID Number	Genus ID
3614A	Enterococcus
3614C #17	Trichosporon
3614C #21	Trichosporon
5814F #1	Staphylococcus
5814F #5	Staphylococcus
5814B	Candida
5813E	Candida
61214B	Staphylococcus
6514A	Staphylococcus
7314A	Staphylococcus
8714C	Staphylococcus

Table 3: Isolate Genus Identification

Conclusions

- Multiple pathogens have the capability of resisting multiple different antibiotic therapies
- A greater number of CF isolates may have multidrug resistance than previous studies suggest
- CF isolates may have more resistance to a greater variety of antibiotics than originally predicted

Future Directions

- Continue sequencing and identifying prevalent pathogens in the CF lung
- Continue a random sampling technique to further identify highly resistant isolates
- Collect and compare genomic data to determine differences in wild type and clinical strains
- Collaborate in development of better clinical diagnostic testing to identify and treat mixed culture infections

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References

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