Evaluation of Bacterial Isolates From Cystic Fibrosis Patients

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ABSTRACT

Gram positive, *Staphylococcus aureus*, and gram negative, *Pseudomonas aeruginosa*, and *Burkholderia cepacia* are bacteria that can cause infections in cystic fibrosis (CF) patients. The purpose of this study was to assess colonial and cellular morphology of clinical isolates that were collected from northeastern Oklahoma CF patients. Colonial and cellular morphology were assessed by conventional methods. The *P. aeruginosa* isolates were similar with colorless, mucoid colonies while one *S. aureus* isolate was slightly mucoid and the other was butyrous. The *B. cepacia* isolates were the most diverse with 53% of the isolates being butyrous while 47% were mucoid. Additionally, 75% of the isolates were colorless and 25% of the isolates were yellow in color. These data support the conclusion that the *B. cepacia* isolates had the most diverse colonial morphology while *P. aeruginosa* and *S. aureus* isolates had similar morphology among their species.

Table 1. Colonial and Cellular Morphology on Isolates

Organism	Size (mm)	Form	Margin	Elevation	Consistency	Color	Opacity	Size	Shape	Group	Gram Result
Staphylococcus aureus											
SFL 42	0.56	C	E	CX	SM	CL	O	M	C	G	+
SFL 47	0.25	C	E	CX	B	CL	O	M	C	G	+
Pseudomonas aeruginosa											
SFL 37	0.57	C	E	CX	M	CL	O	M	R	I	-
SFL 40	0.27	C	E	CX	M	CL	O	M	R	I	-
SFL 49	0.49	C	E	CX	M	CL	O	M	R	I	-
Burkholderia cepacia											
SFL 31	0.70	C	E	CX	B	Y	O	M	R	I	-
SFL 32	0.23	C	E	CX	B	CL	O	M	R	I	-
SFL 33	0.80	C	E	CX	M	CL	O	M	R	I	-
SFL 35	0.51	C	E	CX	M	CL	O	M	R	I	-
SFL 36	0.28	C	E	CX	M	CL	O	M	R	I	-
SFL 38	0.53	C	E	CX	B	Y	O	M	R	I	-
SFL 39	0.13	C	E	CX	B	CL	O	M	R	I	-
SFL 41	0.14	C	E	CX	B	CL	O	M	R	I	-
SFL 43	0.12	C	E	CX	B	Y	O	M	R	I	-
SFL 44	0.35	C	E	CX	M	CL	O	M	R	I	-
SFL 45	0.53	C	E	CX	B	CL	O	M	R	I	-
SFL 48	0.20	C	E	CX	B	CL	O	M	R	I	-

C: Circular, **E:** Entire, **CX:** Convex, **SM:** Slightly Mucoid, **B:** Butyrous, **M:** Mucoid, **CL:** Colorless, **Y:** Yellow, **O:** Opaque, **M:** Medium, **R:** Rod, **G:** Cluster, **I:** Individual, **+:** Gram Positive, **-:** Gram Negative

INTRODUCTION

CF is a hereditary disease that affects various organs of the body, most commonly the respiratory and digestive systems (1). This genetic disease causes non-functioning CF transmembrane conductance regulator (CFTR) protein. The CFTR protein is located on cell membranes and acts as a passage for the production of liquids such as sweat, mucus, and other digestive enzymes. In CF patients theses mediums do not remain viable for enough time to perform chloride transport, causing there to be an influx of chloride in their perspiration (2). The mutation also affects the epithelial cells, causing the mucus in patients to become more sticky and thick. This leads to inundation bacteria becoming encompassed into the mucus. The first occurring bacteria in colonizing in CF patient's lungs is *S. aureus*. Then *P. aeruginosa* will begin to colonize in the mucus, overtaking the *S. aureus* as the predominant organism. *B. cepacia* can then infect the patients, co-existing with *P. aeruginosa*. The effects of *B. cepacia* varies inside patient's lungs, initially as a simple infection, however, can lead to necrotizing pneumonia and death. Studies show that colonies producing extra polysaccharides have the ability to colonies CF lungs by producing biofilms (3,4). Organisms that produce and live in biofilms are more difficult to kill, which causes the patient to have chronic lung infections. The purpose of this study was to assess colonial and cellular morphology of CF clinical isolates in order to better understand the organisms that are infecting northeastern Oklahoma CF patients.

METHODS

Bacterial Isolates and Maintenance Conditions

Clinical isolates were contributed by a local hospital in northeast Oklahoma, U.S.A, between October 2013 and February 2015. All cultures were maintained under cryoprotective conditions at -80℃ (3, 5).

Inocula Preparation

Inocula was obtained by streaking Luria Bertani agar (LBA; Difco Laboratories) with cells from cryopreserved stock cultures, incubating at 37℃ for 24 h, and storing at 4℃ for no longer than 7-10 days to provide working cultures (3, 5).

Colonial Morphology

LBA plates were streaked for isolation and incubated for 37˚C for 24 h. Colonial morphology was

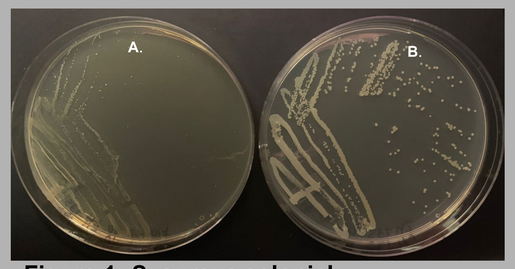

Figure 1. *S. aureus* colonial morphology
Isolates were cultivated on LBA. SFL 42 (A) and SFL 47(B).

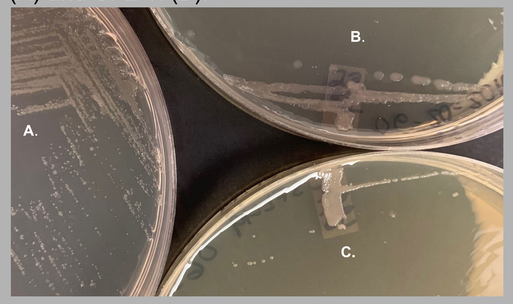

Figure 3. *P. aeruginosa* colonial morphology
Isolates were cultivated on LBA. SFL 37 (A), SFL 40 (B), SFL 49 (C).

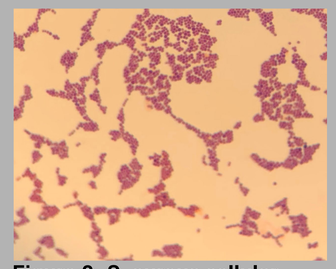

Figure 2. *S. aureus* cellular morphology
SFL 47 with an appearance of gram positive cocci clusters.

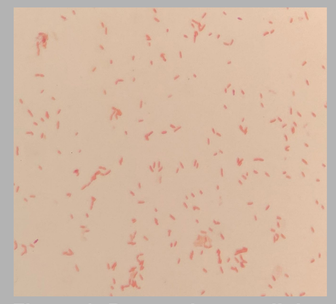

Figure 4. *P. aeruginosa* cellular morphology
P. aeruginosa appears as gram negative, individual, bacilli.

RESULTS

A. *S. aureus* isolates had similar colonial and cellular morphology except for consistency, where one was butyrous and the other was slightly mucoid.

B. *P. aeruginos*a isolates were similar regarding colonial and cellular morphology.,

C. *B. cepacia* isolates were similar regarding cellular morphology.

D. *B. cepacia* isolates had diverse consistency with 53% of the isolates being butyrous and 47% being mucoid.

E. *B. cepacia* isolates had diverse colonial color with 75% being colorless and 25% being pigmented yellow.

CONCLUSION

These data support the conclusion that the *B. cepacia* isolates had the most diverse colonial morphology while *P. aeruginosa* and *S. aureus* isolates had similar morphology among their species. Future studies should include assessment of capsule production and biofilm formation.

REFERENCES

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3. Ruskoski SA and Champlin FA. Cell surface physiology and outer cell envelope impermeability for hydrophobic substances in *Burkholderia multivorans*. J Med Microbiol. 2017;66:965-971.