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Characterization of a Slit-Jet Nebulizer for use in Bacterial Inhalation Studies

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Abstract

Vegetative bacteria are susceptible to oxidative stress, desiccation, and sheer force stress when aerosolized with commercially available nebulizers which may result in decreased viability. We designed and developed a Slit-Jet nebulizer, based on the Babington principle [1], to operate at low pressure and deliver large liquid droplets to protect bacterial cells during aerosolization. We determined the volumetric flow rate, aerosol output, liquid use rate, and liquid droplet diameter of the Slit-Jet nebulizer using distilled water and Synfluid PAO 2cSt (Chevron Phillips, The Woodlands, TX). Volumetric flow rate, aerosol output, and liquid use rate were similar to published data for the Collison nebulizer [2] (BGI, Incorporated, Waltham, MA). However, the droplet diameter of the Slit-Jet nebulizer was significantly larger than the droplet diameter of the Collison nebulizer. 58% of the droplets produced by the Slit-Jet nebulizer were greater than or equal to nine microns and 51% were greater than or equal to 14 microns; whereas, 68% of the droplets generated by the Collison nebulizer are less than or equal to 2.5 microns [2]. We determined the viability, concentration, droplet size distribution, and particle size distribution of aerosolized *Yesinia pestis* KIM 6+ and KIM 10+ (YpK6 and YpK10) with the Slit-Jet and Collison nebulizers operated at nine and 30 psig, respectively. In comparison, there was no difference in viability for YpK10 between nebulizers. Conversely, we observed a 36% decrease in YpK6 viability for the Collison nebulizer. Despite having a lower flow rate, Slit-Jet YpK6 and YpK10 aerosol concentrations were 33% and 35% higher than respective Collison concentrations in our test system. The particle size distribution (MMAD, GSD) of YpK6 and YpK10 aerosols was 1.4 microns, 1.5 and 1.3 microns, 1.5, respectively, for the Slit-Jet nebulizer and 1.0 microns, 1.6 and 1.0 microns, 1.5 for the Collison nebulizer. In conclusion, the Slit-Jet nebulizer is an option for use in bioaerosol studies.

Design of the Slit-Jet nebulizer

A cross sectional diagram of the Slit-Jet nebulizer is shown in Figure 1. The reservoir had a capacity of approximately 70 mL. The compressed air slit jet was positioned directly opposite of the liquid inlet as shown in Figure 2. Liquid exited the reservoir through an outlet and was re-circulated to an inlet in the nebulizer body using a peristaltic pump as shown in Figure 3. Alignment of the jet was variable between 0° and 180°. At a jet orientation of 0°, a large droplet size fraction was able to exit the nebulizer while at jet orientations of 90° and 180°, the larger droplet size fraction was unable to exit the nebulizer.

Figure 1. Slit-Jet nebulizer cross sectional diagram.

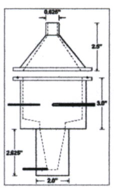


Figure 2. Top view of Slit-Jet nebulizer.

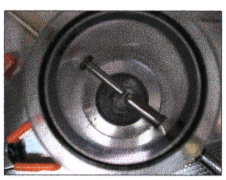
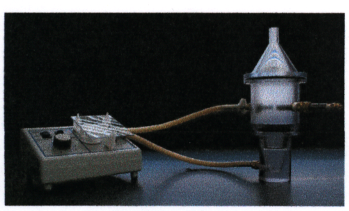


Figure 3. Slit-Jet nebulizer and peristaltic pump.



Methods

Use rate and aerosol output:
Slit-Jet nebulizer use rate and aerosol output were determined at room temperature using 0.9% NaCl solution, nebulizer operating pressures of 5, 10, 15, 20, and 30 psig, and jet orientations of 0°, 90°, and 180°.

Slit-Jet nebulizer droplet size distribution:
Slit-Jet nebulizer droplet diameter was determined using Synfluid PAO 2 CST. Nebulizer operating pressure was 10 psig and jet orientations were 0°, 90°, and 180°. Aerosol particles were collected with multi-jet cascade impactors and analyzed.

Slit-Jet nebulizer particle size distribution:
Slit-Jet nebulizer and Collison 3-Jet nebulizer particle size distributions were compared using *Yersinia pestis* KIM 6+ (YpK6) and *Yersinia pestis* KIM 10+ (YpK10) nebulizer stock suspensions. Aerosolized bacteria were gently dried, mixed with filtered dilution air, and directed into a sampling plenum. Slit-Jet nebulizer operating pressure was 10 psig and jet orientation was 0°. Collison nebulizer operating pressure was 30 psig. Sampling plenum and dilution flow rates were constant for all tests.

Slit-Jet nebulizer viability and aerosol concentration:
The viability of YpK6 and YpK10 bacterial aerosols generated with the Slit-Jet nebulizer were compared to a Collison 3-Jet nebulizer. Operating pressure for the Slit-Jet nebulizer was 10 psig and was 30 psig for the Collison 3-Jet nebulizer. The Slit-Jet nebulizer was operated in a 0° jet orientation. Aerosols were dried and mixed with dilution air and directed into a 24-port plenum.

Results

Use rate and aerosol output

- The liquid use rate and aerosol output were determined for the Slit-Jet nebulizer over an operating pressure range of 5 to 30 psig and 0°, 90°, and 180° jet orientations. Results of liquid use rate and aerosol output tests are presented in Figure 4.

- Liquid use rate was 0.19 mL/min to 0.48 mL/min for a 0° jet orientation over all operating pressure ranges while liquid use rates for 90° and 180° jet orientations were approximately 0.1 mL/min to 0.26 mL/min over all operating pressure ranges.

- Aerosol output was always highest with a 0° jet orientation. Aerosol output peaked at 44.5 µL/L with a pressure of 10 psig and decreased rapidly with increasing operating pressure. At 90° and 180° jet orientations, aerosol output was similar.

Figure 4A. Slit-Jet use rate.

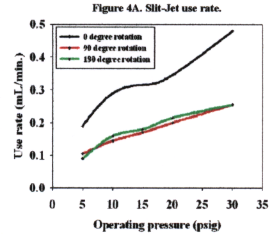
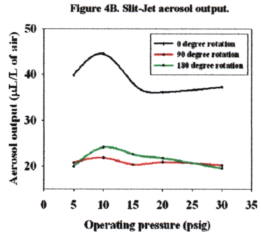


Figure 4B. Slit-Jet aerosol output.



Slit-Jet nebulizer droplet size distribution

- The droplet size distribution (MMAD, GSD) of the Slit-Jet nebulizer operated with a jet orientation of 0° was bimodal with modes at 4.6 µm, 3.2 and 16.6 µm, 1.1 (Figure 5A). Fifty-one percent of total droplet mass was greater than 14 µm. Droplet size distributions for 90° and 180° jet orientations were unimodal: 1.8 µm, 2.6, and 2.6 µm, 2.8, respectively (Figures 5B and 5C).

Figure 5A. 0° Distribution.

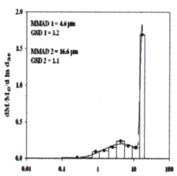


Figure 5B. 90° Distribution.

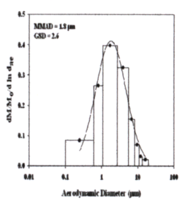
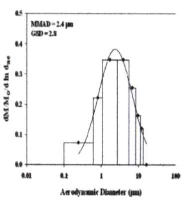


Figure 5C. 180° Distribution.



Slit-Jet nebulizer particle size distribution

- YpK6 and YpK10 mean particle size distributions (MMAD, GSD) for the Slit-Jet nebulizer were 1.4 ± 0.1 µm, 1.5 ± 0.0 (n = 3) and 1.3 ± 0.1 µm, 1.5 ± 0.0 (n = 3), respectively. YpK6 and YpK10 mean particle size distributions (MMAD, GSD) for the Collison 3-Jet nebulizer were 1.0 ± 0.0 µm, 1.6 ± 0.0 (n = 2) and 1.0 ± 0.0 µm, 1.5 ± 0.0 (n = 2), respectively. Particle size distributions were determined by analysis of Aerodynamic Particle Sizer samples and are presented in Table 1. The aerosol test system is presented in Figure 6.

Table 1. Particle size comparisons.

YpK6

Nebulizer Type	MMAD (µm)	GSD
Slit-Jet	1.3	1.5
Slit-Jet	1.4	1.5
Slit-Jet	1.4	1.5
MEAN	1.4	1.5
STDEV	0.1	0.0
% CV	4.1	0.0

YpK6

Nebulizer Type	MMAD (µm)	GSD
Collison	1.0	1.6
Collison	1.0	1.6
MEAN	1.0	1.6
STDEV	0.0	0.0
% CV	0.0	0.0

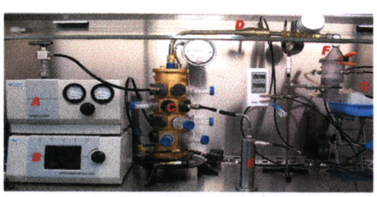
YpK10

Nebulizer Type	MMAD (µm)	GSD
Slit-Jet	1.4	1.5
Slit-Jet	1.2	1.5
Slit-Jet	1.4	1.5
MEAN	1.3	1.5
STDEV	0.1	0.0
% CV	7.7	0.0

YpK10

Nebulizer Type	MMAD (µm)	GSD
Collison	1.0	1.5
Collison	1.0	1.5
MEAN	1.0	1.5
STDEV	0.0	0.0
% CV	0.0	0.0

Figure 6. Aerosol test system. A. Aerodynamic Particle Sizer Diluter, B. Aerodynamic Particle Sizer, C. 24-port plenum, D. Mixer, E. Stainless steel impinger, F. Slit-Jet nebulizer, and G. Peristaltic pump.



Slit-Jet nebulizer viability and aerosol concentration

- Bacterial viability was defined as the ratio of post-spray (final) to pre-spray (initial) nebulizer concentrations. The Slit-Jet nebulizer did not affect YpK6 bacterial viability; however, the Collison nebulizer caused a 35.7 % decrease in viability. There was no difference in viability observed for YpK10 suspensions between nebulizers.

- Aerosol concentration was determined from samples collected with low-flow rate stainless steel impingers. The Slit-Jet nebulizer produced 33.7% and 35.4% higher aerosol concentrations than the Collison nebulizer for YpK6 and YpK10 suspensions, respectively.

- Viability and aerosol concentration data is presented in Table 2.

Table 2. Viability and aerosol concentration.

YpK6

Nebulizer Type	Pre-Spray (CFU/mL)	Post-Spray (CFU/mL)	Viability (%)	Aerosol Concentration (CFU/L)
Slit-Jet	2.8E+07	3.0E+07	107.1	2.6E+04
Slit-Jet	2.8E+07	2.8E+07	98.2	2.1E+04
Slit-Jet	2.8E+07	2.7E+07	95.4	3.2E+04
	MEAN	2.8E+07	100.6	2.7E+04
	STDEV	1.6E+06	5.7	5.5E+03
	%CV	5.7	5.7	19.9
Collison	2.8E+07	1.5E+07	51.8	6.5E+03
Collison	2.8E+07	2.2E+07	76.8	1.2E+04
	MEAN	1.8E+07	64.3	9.1E+03
	STDEV	4.9E+06	17.7	3.6E+03
	%CV	27.5	27.5	39.4

YpK10

Nebulizer Type	Pre-Spray (CFU/mL)	Post-Spray (CFU/mL)	Viability (%)	Aerosol Concentration (CFU/L)
Slit-Jet	2.1E+07	2.2E+07	105.0	1.9E+04
Slit-Jet	2.1E+07	2.2E+07	102.6	1.8E+04
Slit-Jet	2.1E+07	2.6E+07	124.1	3.0E+04
	MEAN	2.3E+07	110.6	2.2E+04
	STDEV	2.5E+06	11.8	6.9E+03
	%CV	10.6	10.6	31.1
Collison	2.1E+07	2.0E+07	96.5	8.0E+03
Collison	2.1E+07	2.0E+07	93.1	7.7E+03
	MEAN	2.0E+07	94.3	7.8E+03
	STDEV	3.5E+05	1.7	2.4E+02
	%CV	1.8	1.8	3.0

Conclusions

- The Slit-Jet nebulizer produces a large diameter droplet that provides protection from oxygen toxicity, desiccation, and nebulizer shear forces.

- The Slit-Jet nebulizer produces a variety of droplet distributions.

- The Slit-Jet nebulizer did not affect cell viability for YpK6 and YpK10 suspensions.

- The Slit-Jet nebulizer produced higher aerosol concentrations of vegetative bacteria than the Collison nebulizer.

References

1. The Babington nebulizer: a new principle for generation of therapeutic aerosols; American review of Respiratory Disease. Litt M. and Swift D.E. v105, 1972.

2. BGI Incorporated: Generalized Output Data For BGI Collison Nebulizers http://www.bgiusa.com/agc/Collison%20Data.xls.

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