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ASSESSMENT OF RECENT NEBULIZER DELIVERY SYSTEMS USING URINARY PHARMACOKINETICS METHOD AND AERODYNAMIC CHARACTERISTICS OF TOBI[®] NEBULIZED DOSE FOLLOWING INHALATION

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Abstract

Background: Chronic infections with *Pseudomonas aeruginosa* are a leading cause of morbidity in patients with cystic fibrosis (CF). Tobramycin nebulizer solution (TNS) is indicated for maintenance therapy in CF patients. TOBI[®] is a tobramycin nebulizer solution (TNS) approved by FDA for maintenance therapy for patient with CF. Adherence to recommended therapy in CF has always been a challenge and new generation nebulizers are increasingly used “off label” to reduce the time required for inhalation, potentially improving patient compliance.

Objectives: to assess the performance of selected recent nebulizer delivery systems for determination the optimum combinations to deliver TOBI[®]. Using the relative lung bioavailability of TOBI[®] to the lungs in healthy volunteers, following inhalation from selected nebulizer delivery systems, using a urinary pharmacokinetics method. *In vitro* aerodynamic characteristics of the nebulized dose were also determined.

Methods: Serial urine samples were collected from 12 healthy volunteers up to 24 hrs post-inhalation of TOBI[®] inhaled solution following delivery by Pari LC Plus[®], Sidestream[®], NE-U22-E Omron[®] and Aeroneb[®] Go nebulizers. *In vitro* aerodynamic characteristics of the nebulized dose were also determined according to the CEN (Committee European de Normalization) method.

Results: The mean (SD) relative lung bioavailability from Pari LC Plus[®], Sidestream[®], Omron[®], and Aeroneb[®] Go nebulizers was 4.9 (0.5), 3.9 (0.5), 7.1 (1.3), and 7.7 (0.7) %, respectively. The mean (SD) mass median aerodynamic diameter (MMAD) of the drug particles from the same systems was 2 (0.2), 2 (0.2), 1.2 (0.03) and 2.0 (0.1) μm , and the corresponding fine particle doses (FPD) were 2.2 (0.23), 1.5 (0.2), 3.44 (0.3) and 2.8 (0.3) mg.

Conclusion: The data obtained from *in-vitro* and *in-vivo* studies reflect poor relative lung bioavailability of tobramycin following jet nebulization.

Keywords

Lung Bioavailability, Urinary Pharmacokinetics, CEN (Committee European de Normalization) method, Tobramycin, Inhalation, Nebulizer.

Introduction

Cystic fibrosis (CF) is an autosomal recessive disease that affects approximately 60,000 people worldwide [1]. Chronic pulmonary *Pseudomonas aeruginosa* infection is the most common cause of morbidity and mortality in patients with CF and bronchiectasis [2].

Aminoglycosides are the antibiotics most effective against *P. aeruginosa* infections [3]. Parenteral administration of aminoglycoside antibiotics is limited by poor penetration into bronchial secretions and impairment of their biological activity by purulent secretions, resulting in peak sputum concentrations equivalent to only 12-20% of serum concentrations [4]. However, because components of CF sputum bind aminoglycosides, sputum concentrations must exceed 10 to 25 fold above the minimum inhibitory concentration (MIC), to overcome the effect of sputum binding on aminoglycoside availability to bacterial targets. Thus, to attain efficacious drug concentrations at the site of infection, large parenteral doses of aminoglycoside must be administered, placing the patient at risk to aminoglycoside-associated ototoxicity and nephrotoxicity [5].

Delivery of aminoglycosides by the aerosolised route to the lower respiratory tract is an attractive alternative method of antibiotic delivery that allows direct application of high concentrations of antibiotic to the site of the infection, with minimal systemic absorption and associated toxicity [6]. Aerosolised tobramycin is often prescribed as part of the treatment regimen for pulmonary *P. aeruginosa* infection in CF patients. There are many advantages for its use that make it the antibiotic of choice in the treatment of pulmonary infections in CF. It is more effective than other antibiotics against *P. aeruginosa* strains that are found in the sputum of pulmonary CF and bronchiectasis patients [7-12]. It is also well-tolerated and the least toxic drug of the aminoglycosides [13]. TOBI® (tobramycin nebulized solution 300mg/5ml) is licensed as maintenance therapy for chronic pulmonary *P. aeruginosa* infections in CF patients. The manufacturer of TOBI® recommends nebulization of the solution with the Pari LC Plus® jet nebuliser in combination with the DeVilbiss Pulmo-Aide® compressor. However, poor lung deposition and high drug wastage, due to a high residual volume, are the main disadvantages of this combination. Therefore, new

nebuliser systems have been investigated to improve tobramycin aerosol delivery to the peripheral airways and minimize residual drug wastage. Many in-vitro and in-vivo studies have been investigated to achieve these purposes [14-27]

In-vivo studies the pharmacokinetic (PK) methods can provide accurate and reproducible quantification of drug delivery by measuring plasma levels to reflect lung deposition and bioavailability [28,29]. PK technique has been difficult to apply to most inhaled drugs, as the doses delivered are generally too small (microgram quantities), and the resulting plasma levels are low, which may be below the accurate detection limits of standard assays. Recently, more sensitive assay methods have been developed to overcome this problem. The other problem of plasma PK technique is the drug plasma level involves pulmonary absorption resulting from lung deposition, and gastro-intestinal absorption of the swallowed fraction resulting from oropharyngeal deposition. Where gastro-intestinal absorption is excluded, systemic levels will reflect total lung deposition. This is achieved by blocking all gastrointestinal absorption with oral charcoal co-administration, or used sampling during the lag time of the absorption phase.

Borgström et al. [30] used charcoal-block technique to quantify the percentage of inhaled dose deposited in the lungs, and to determine absolute pulmonary bioavailability for formoterol, ipratropium bromide, budesonide, salbutamol, and terbutaline sulphate.

Many studies have used PK technique to compare different inhaler devices, such as for dry powder, pressurised aerosol and nebulized formulations, and to evaluate the bioequivalence of different drug formulations. Newnham et al. [31] have determined salbutamol plasma concentrations (C_{max}) to compare standard MDIs and modified actuator devices. Newnham & Lipworth [32] used the same methodology to compare performance of two nebulizer delivery systems, Ventstream[®] and Hudson Updraft II[®]. Inhaled doses usually are small, and only 10% of dose deposits into the lungs [33], so plasma concentration of drug is generally very low, especially if the volume distribution is large, and concentration is therefore difficult to measure.

The serum (t_{max}) may vary for different drugs due to differences in the rate of absorption across to lung epithelium, or to prolonged binding to lung receptors [34] or airway mucus [35], so the measurement of urinary excretion has been developed as an alternative indirect method for evaluating pharmacokinetics of inhaled drugs. A urinary PK method is ideal for polar and basic drugs such as β -agonists and

aminoglycosides, which have a high renal clearance rate and are highly excreted in urine. Thus the concentration of the drug in urine is often high in comparison to that of plasma, because of the comparatively small urine volume. Therefore, in these cases, urine provides useful PK data following inhalation [36,37]. It has been successfully employed in the determination of the relative lung bioavailability of many inhaled drugs, such as salbutamol [38], sodium cromoglycate [39], nedocromil sodium [40], inhaled gentamicin [41, 42] and inhaled tobramycin [43].

In a comparative study of plasma and urinary PK methods, Clark et al. [44] reported no significant differences apparent between salbutamol urinary excretion and salbutamol plasma level.

Another indirect urinary PK method has been introduced. This method is based on the measurement of urinary excretion in the first 30 min after inhalation to differentiate between the components of lungs and gastrointestinal absorption of inhaled drug. Hindle & Chrystyn [45] used the advantage of the biphasic renal excretion (over 24 hours) to determine and compare the relative bioavailability of salbutamol post-oral and inhaled dose. The initial elimination (i.e. 30 min) represents the fraction of dose administered to the lungs, rapidly absorbed via the alveoli, then excreted unchanged by the kidneys. The second phase represents the dose swallowed after impaction in the oropharynx. They showed that little drug is excreted in urine 30 min after oral ingestion because of the lag time between administration and start of absorption. The urine drug recovery 30 minutes after inhalation was significantly greater.

Hindle et al. [46] have measured the relative bioavailability to the lungs using 30-minute urine recovery of salbutamol following a variety of inhalation techniques in 10 healthy volunteers. They observed significantly greater elimination in the first 30 min when drug was inhaled slowly (10 L/min) versus quickly (50 L/min), and with breath-holding (10s) versus without breath-holding.

Recently, the 30-min urinary salbutamol method has been validated, and showed a linear and reproducible relationship with inhaled dose [47]. Tomlinson et al. [48] have assessed different methods of inhalation from salbutamol MDIs by the 30-min urinary excretion method.

The 30-min urinary salbutamol method could not distinguish between the three products. Total systemic delivery can be assessed by using urinary excretion, especially if the molecules are polar or basic [49].

Borgström et al. [50] compared gamma scintigraphy and PK methods for determination of pulmonary deposition of inhaled terbutaline. The mean (S.D) total terbutaline lung deposition identified by gamma scintigraphy was 26.9 (3.8) % of nominal dose, whilst 21.1 (3.2) % was collected by urinary excretion post-terbutaline inhalation with charcoal co-administration. In another study, Derendorf et al. [51] obtained similar data from PK and gamma scintigraphy methods in comparing between oral (with charcoal co-administration) and inhaled glucocorticoid.

Gamma scintigraphy identified total lung deposition of a drug that is cleared from the lungs either by absorption into the systemic circulation or by mucociliary clearance, whereas the PK methods identify total lung deposition (not regional), and cannot differentiate between deposition into different zones of the lungs or the amount removed by mucociliary clearance [52].

In this study, we have investigated the urinary pharmacokinetics of tobramycin post-nebulisation, and the sampling periods have been designed to establish whether t_{max} is prolonged. In addition, we have determined the aerodynamic characteristics of the emitted dose to determine if these could influence lung deposition.

Methods

Ethical approval was obtained from the University of Bradford, and all volunteers gave signed informed consent. All subjects were healthy adult and non-smoking volunteers. The demographic details of volunteers who completed the study are shown in [Table 1](#).

Table 1 Volunteer demographics.

No.	Volunteer	Gender	Age (years)	Height (cm)	Weight (kg)
1	A	Male	36	175	80
2	B	Male	33	175	73
3	C	Male	43	180	85
4	D	Male	27	173	72
5	E	Male	32	170	80
6	G	Female	24	172	62
7	H	Male	34	180	74
8	J	Male	25	168	68
9	K	Male	40	170	84
10	L	Female	26	175	85
11	N	Male	39	170	90
12	W	Female	31	175	60.33
	Mean	-	32.5	173.583	76.110
	SD	-	6.230	3.848	9.479

Different nebulizer delivery systems were used to assess their ability to deliver inhaled tobramycin to lungs: two different designs of jet nebulizers and two new nebulizers based on mesh technology. The jet nebulizers were the breath-enhanced Pari LC Plus[®] attached to PariBoyN[®] compressor (Pari GmbH; Starnberg, Germany), and the constant output Sidestream[®] (Medic-Aid Ltd; West Sussex, UK) attached to Porta-Neb[®] compressor (Profiles, UK). The new nebulizers were NE-U22-E Omron[®] (Omron, Japan) static mesh nebulizer (moderate frequency) and Aeronex[®] Go (Aerogen, Inc., USA) vibrating mesh nebulizer.

The PARI LC Plus nebulizer is the “gold standard” for aerosolized medication delivery. It is a breath-enhanced open-vent jet nebulizer that uses two valves to maximise aerosol delivery and minimise medication wastage. The inspiratory valve is opened during inspiration to allow extra air to be sucked through the nebulizer chamber and closed during exhalation to decrease air flow through the chamber. It is designed for children as well as adults and works with any compressor. It is a reusable nebulizer that is recommended for six months use and it can be cleaned in the dishwasher, boiled or autoclaved.

The Sidestream[®] attached to Porta-Neb[®] compressor is fast, accurate drug delivery every time. It is a constant-output open-vent jet nebulizer. The open vent on the top of nebulizer chamber improves aerosol output by allowing the negative pressure to suck extra air into the chamber. This markedly increases the air flow out of the nebulizer and pushes out more small droplets to be inspired. The aerosol is produced or released constantly during inspiration and expiration. It is a reusable nebulizer and can be cleaned in the dishwasher, boiled or autoclaved.

The MicroAir[®] NE-U22 nebulizer uses an ultrasonic transducer to generate vibration (180 kHz) of the drug solution and push the droplets through the static mesh which can then be inhaled directly by the patient. Unlike jet and ultrasonic nebulizers, the aerosol is not recycled in the mesh nebuliser. The mesh cannot be disinfected by an autoclave process and should be submerged in a 0.1% benzalkonium solution.

The Aeronex[®] Go nebulizer has a novel design and generates aerosol using the OnQ[™] electronic micropump. It consists of a domed aperture plate with precision-formed holes (1000 holes) and a vibrational element which vibrates at 100 kHz. This creates a micro-pumping action producing a fine particle, low velocity aerosol using

no propellants or compressors. It cannot be disinfected by an autoclave process and there are no specific recommendations for disinfecting.

The nebulizer fill volumes were 5 ml and 2.5ml for jet and new nebulizers, respectively. Twelve volunteers (3 females) received inhaled tobramycin (TOBI[®]; Chiron Corporation, Emeryville, USA): 300mg/5ml dose from Pari LC Plus[®] and Sidestream[®], and 150mg/5ml from NE-U22-E Omron[®] and Aeroneb[®] Go, on separate randomised study days, each separated by seven days apart.

All subjects were trained on how to use normal tidal breathing through their mouth during nebulization, and how to use the nebulizer device according to the manufacturer's patient information sheet. A low resistance electrostatic filter pad, which represents the exhalation filter, was connected to the nebuliser system by T-piece to captured waste aerosol released during expiration, a schematic diagram as shown in **Figure1**.

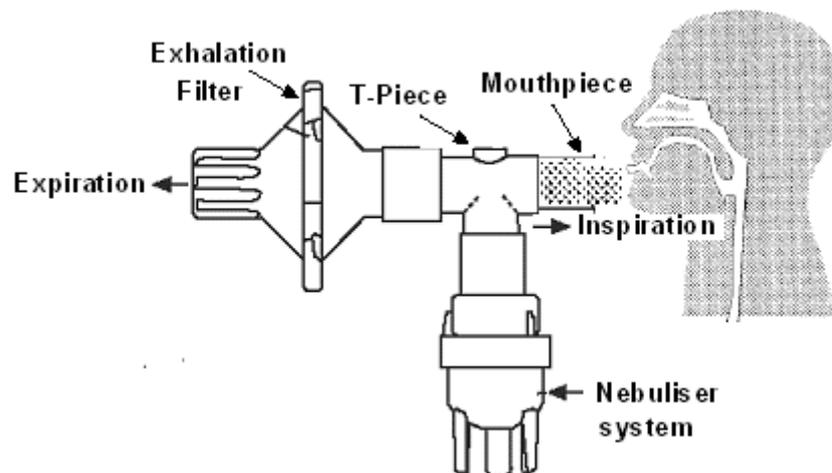


Figure 1 Schematic diagram of the general nebulization method was used for pharmacokinetic study.

All subjects voided their bladder 15 min pre-dosing. They swallowed 10g activated charcoal (Cabomix[®], Penn Pharmaceuticals, Tredegar, Gwent, UK) in 50ml of water immediately before, and another 10g charcoal in 50ml of water after each nebulized dose. Time zero was started on the first inhalation, and nebulization of each dose was continued until dryness of inhaled solution, and the time was recorded. After nebulization, the residual amount of tobramycin which remained in chamber, mouthpiece and on exhalation filter was determined by HPLC.

Urine samples were collected at 0.5, 1, 2, 3 4, 6, 9, 12 and 24 hr post-inhalation of each study dose. Urine was pooled during each collection time; aliquots were transferred to plastic bottles, and stored at 4° C. All samples were frozen at -20° C prior to analysis by HPLC.

The amount of tobramycin excreted in the urine during each collection period was determined analytically by HPLC assay, and then the cumulative amount excreted over the 24hr (Ae_{24}) post-nebulised dose was calculated. By using one-way ANOVA, all tested nebulizer systems were compared to Pari LC Plus® which was the reference nebuliser system. Comparisons between the tested nebulizers and the reference were made for the Ae_{24} , and for the amount of tobramycin excreted in the first 30 min. From the profiles of urinary tobramycin excretion against the midpoint of the collection period, the t_{max} was obtained. Comparison of t_{max} between the reference and tested nebulizers was also made. For each nebulizer system, comparisons of the relative bioavailability of tobramycin to the lung were made to the total dose available for inhalation, fine particle dose, and the mass median aerodynamic diameter (MMAD).

The *in-vitro* aerodynamic particle size characteristics of TOBI® emitted from each nebulizer systems (n=6) was determined according to the CEN (Committee European de Normalization) method [52].

2.6 Tobramycin Assay

The tobramycin concentration was determined by using validated HPLC method following pre-column derivatization with fluorescein isothiocyanate (FITC) [53]. The Guideline on Bioanalytical Method Validation. EMEA/CHMP/EWP/192217/ 2009, 21 July 2011 [54] was used as a guide to validated the analytical method.

Results and discussion

Twelve (3 females) healthy adult subjects completed the pharmacokinetic study. Their mean (SD) age, weight, and height were 32.5 (6.2) years, 76.11 (9.5) kg, and 173.58 (3.8) cm, respectively. From their urinary excretion profiles after receiving inhaled tobramycin 300 mg/5ml, using Pari LC Plus® and Sidestream® jet nebulizers, and 150 mg/2.5 ml, using electronic mesh nebulizers (Omron® and Aeronex® Go), the mean (SD) amounts of tobramycin excreted in the first 30 min were 0.5 (0.2), 0.4 (0.2), 0.4 (0.25), and 0.426 (0.12) mg, respectively. The cumulative urinary excretion profiles

over 24 hr are shown in Figures 2 (a) and 2 (b). The median (range) t_{max} values were 3.5 (2.5 – 7.5) hr for jet nebulizers, and 2.5 (1.5 – 5) hr for vibrating mesh nebulisers. The NE-U22-E Omron[®] showed less drug wastage, while Aeroneb Go[®] showed shortest nebulization time. The *in vivo* study data are summarised in Tables 2 (a - b) and 3 (a - b). As shown in Table 4, the tested nebulizers were compared to Pari LC Plus[®], which is recommended by the manufacturer of TOBI[®], combined with PariBoyN[®] compressor, by using one-way ANOVA test.

The mean (SD) MMAD of the drug particles from Pari LC Plus[®], Sidestream[®], NE-U22-E Omron[®] and Aeroneb[®] Go nebulizer systems was 2 (0.2), 2 (0.2), 1.2 (0.03) and 2.0 (0.1) μm , and the corresponding fine particle doses (FPD) were 2.2 (0.2), 1.5 (0.2), 3.4 (0.3) and 2.81(0.3) mg. All nebulized aerosol droplets were polydispersed, with span values ranging between 2.93-5.37 μm for jet nebulizers, and 1.76-2.21 μm for vibrating mesh nebulizers.

Nebulizer aerosol delivery is largely dependent on the nebulizer design and brand [55, 56]. In this study, the effect of nebulizer design on improving tobramycin aerosol delivery has been studied by using different nebulizer delivery systems. Two different designs of jet nebulizers and new nebulizers based on vibrating mesh technology have been used, the constant output Sidestream[®] and breath-enhanced Pari LC Plus[®] jet nebulizers. As shown in Table 4, the cumulative amount of tobramycin over 24 hr (Ae_{24}) and the relative lung bioavailability of tobramycin were significantly increased ($p < 0.05$) in breath-enhanced Pari LC Plus[®] when compared to the constant output Sidestream[®] jet nebulizer. Furthermore, the amount of tobramycin left in chamber was highly significantly decreased ($p < 0.001$) in the breath-enhanced Pari LC Plus[®] nebuliser. However, the relative lung bioavailability of drug was highly significantly increased ($p < 0.05$) using vibrating mesh nebulizers, which reflects their efficiency to nebulize inhaled tobramycin. No significant difference was found between the amount of tobramycin excreted in the first 30 min in all tested nebulizers and the reference. As shown in Figure 6, the residual volume percentage was highly significantly decreased in vibrating mesh nebulisers compared to jet nebulizers.

Decreasing the nebulization time also was highly significant in Aeroneb[®] Go nebulizer compared to all tested nebulizers.

In conclusion, , the lung bioavailability of tobramycin was poor following nebulization with jet nebulizers, while it was greater using electronic mesh nebulizer. The urinary pharmacokinetic method can be successfully employed in the determination of the relative lung bioavailability of tobramycin. The amount of tobramycin excreted in the urine in the first 30 minutes post-inhalation indicates the relative deposition of inhaled tobramycin in the lungs. The cumulative amounts of tobramycin excreted in the urine over the 24 hrs reflect the lung bioavailability. This index has been used to compare the performance of different nebuliser systems.

When the *in-vitro* and *in-vivo* study data are combined, the new technology nebulizers which depend on vibrating mesh are more efficient to nebulize TOBI[®] solution than jet nebulizers. Furthermore, the least drug wastage which was observed in vibrating mesh nebulizers helps the CF patients to continue the routine therapy of TOBI[®] by reducing medication cost and have shorter nebulisation times that should improve a patient's quality of life and compliance.

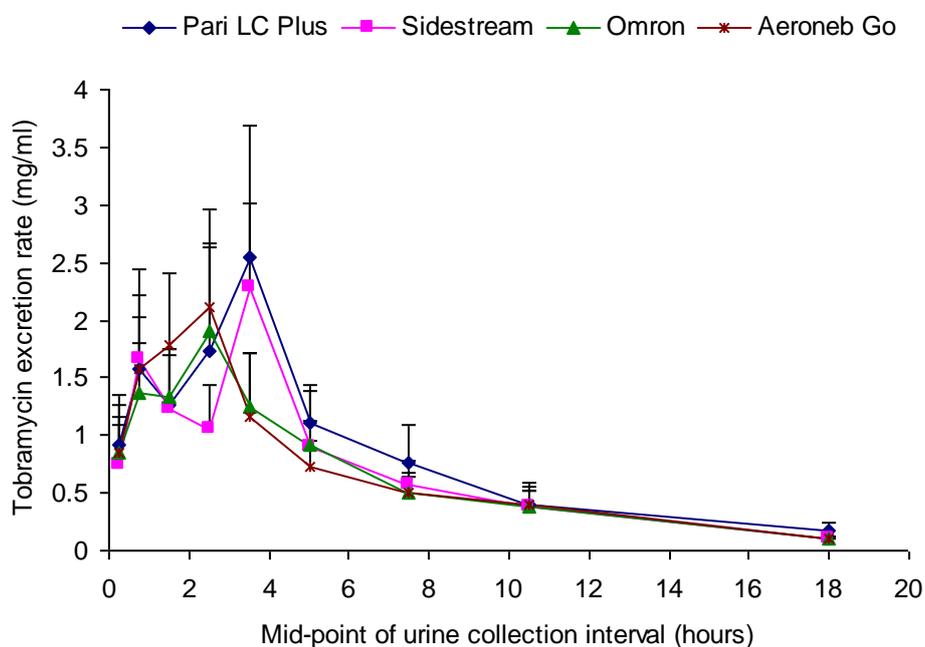


Figure 2 (a) The mean (SD) urinary excretion rate time profile for tobramycin obtained from 12 subjects following nebulized dosing of TOBI[®], using the different nebulizer systems.

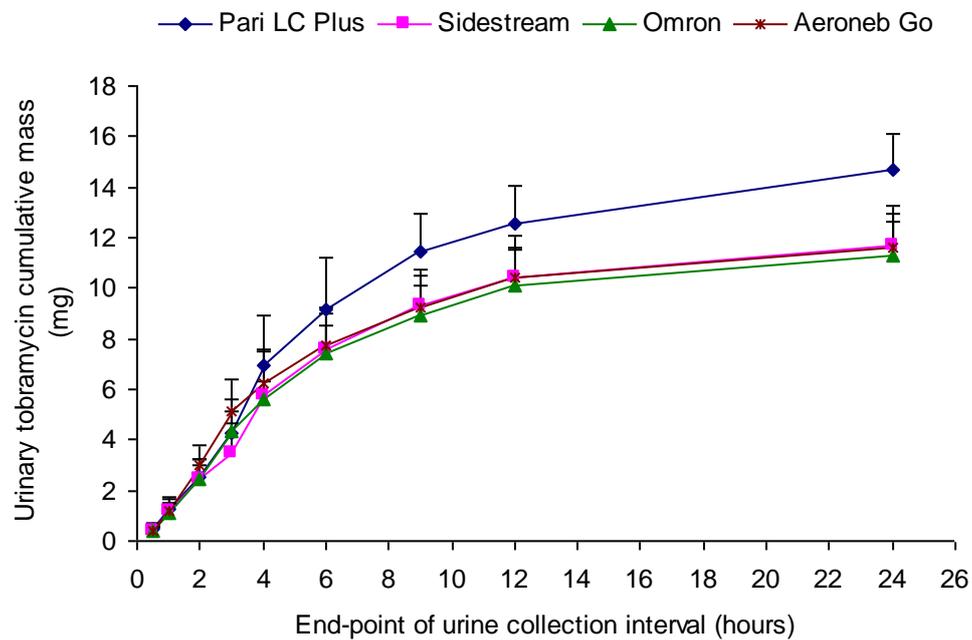


Figure 2 (b) The mean (SD) cumulative amounts (mg) of tobramycin excreted in the urine following administration of nebulized dose of TOBI[®], using the different nebulizer systems

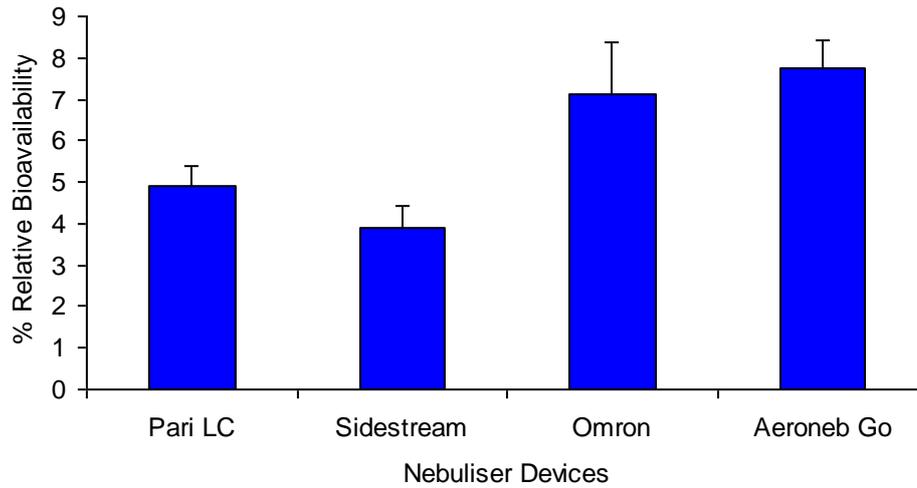


Figure 3 Mean (SD) lung bioavailability of nebulized doses obtained by selected nebulizer systems.

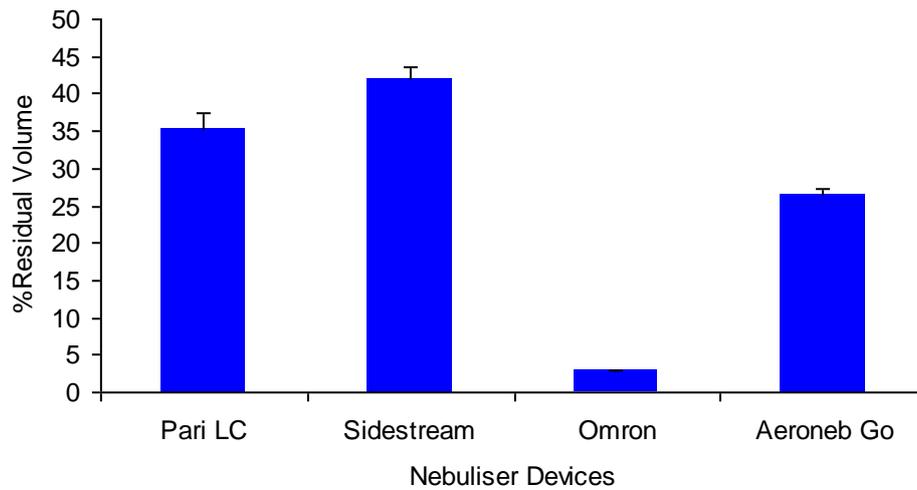


Figure 4 Mean (SD) residual volume percentage of nebulized doses obtained by selected nebulizer systems.

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