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Measuring antibiotics in exhaled air in critically ill, non-ventilated patients: A feasibility and proof of concept study

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ABSTRACT

Purpose: Measurement of antibiotic concentrations is increasingly used to optimize antibiotic therapy. Plasma samples are typically used for this, but other matrices such as exhaled air could be an alternative.
Materials and methods: We studied 11 spontaneously breathing intensive care unit patients receiving either piperacillin/tazobactam or meropenem. Patients exhaled in the ExaBreath® device, from which the antibiotic was extracted. The presence of antibiotics was also determined in the condensate found in the device and in the plasma.
Results: Piperacillin or meropenem could be detected in the filter in 9 patients and in the condensate in 10. Seven patients completed the procedure as prescribed. In these patients the median quantity of piperacillin in the filter was 3083 pg/filter (range 988–203,895 pg/filter), and 45 pg (range 6–126 pg) in the condensate; meropenem quantity was 21,168 pg/filter, but the quantity in the condensate was below the lower limit of quantification. There was no correlation between the concentrations in the plasma and quantities detected in the filter or condensate.
Conclusions: Piperacillin and meropenem can be detected and quantified in exhaled air of non-ventilated intensive care unit patients; these quantities did not correlate with plasma concentrations of these drugs.

1. Introduction

Antibiotic pharmacokinetics are fundamentally changed in critically ill patients, which result in highly variable antibiotic concentrations in plasma and in the tissues [1,2]. Several strategies to improve antibiotic treatment have been introduced and measuring actual drug concentrations to maintain the plasma concentration and ameliorate individual dosage regimens, or therapeutic drug monitoring (TDM) is increasingly popular to guide antibiotic therapy [2,3]. The majority of these strategies use plasma samples to measure the drug concentrations, but it is generally assumed that tissue concentrations at the site of infection will determine antibiotic efficacy [1].

For most infections, getting tissue concentrations is difficult or impossible. With the exception of abdominal fluid and cerebrospinal fluid, most tissues are not readily accessible for sampling. Both blood and infection site sampling are invasive techniques, and therefore other, non-invasive strategies for measuring antibiotic concentrations are highly desirable.

Exhaled breath analysis is commonly used for alcohol testing. Good correlations between arterial blood concentrations and breath concentrations have been demonstrated for several compounds [4]. It is well-known that exhaled air includes non-volatile substances which are carried in the bioaerosol particles [5-7]. Recent research has provided evidence of the presence of different drugs in exhaled breath of patients. Methadone, cocaine, opiates, cannabis, amphetamines, propofol, fentanyl, nicotine, and valproic acid can be detected in exhaled breath [8,9].

At the moment, there are no data in the literature on the presence of antibiotics in exhaled breath of patients. Therefore, we wanted to explore whether antibiotics can be detected in exhaled air of critically ill patients using the commercially available sampling ExaBreath®-device (SensAbues® AB, Sollentuna, Sweden), and whether these can be quantified and potentially correlated with plasma concentrations.

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2. Methods

2.1. Study subjects

Eleven patients admitted to the intensive care unit (ICU) and treated with intravenous piperacillin/tazobactam or meropenem were included in the study. Patients were included if all the following inclusion criteria were met: age ≥ 18 years, treated with piperacillin/tazobactam or meropenem for >24 h prior to inclusion, conscious and considered capable of exhaling in the device used for 3 min; minors, patients with anaemia (haemoglobin < 7 g/dL) or receiving aerosols were excluded.

2.2. Sampling

The commercially available ExaBreath®-device was used in this study. The device consists of three parts (Fig. 1 A): the mouthpiece contains two saliva barriers in order to separate larger saliva particles present in the breath from the micro particles, the second part contains an electret (electrostatic based) filter. This filter is used to capture and retain submicron bioaerosol particles which originate from the airway lining fluid and the third part is a control bag, in order to standardise the procedure.

The filter used in this device is an electret filter. This type of filter combines two mechanisms: the mechanical and electrostatic mechanism. The mechanical mechanism includes impaction, interception, and diffusion. The electrostatic mechanism includes Coulombic and dielectrophoretic attraction, predominantly to capture small particles (10–500 nm). The combination of both mechanisms has a significantly higher filtration performance than the merely mechanical ones [10]. The maximum diameter of the aerosol particles is 0.8 μm [11]. This diameter is lower when the patient is breathing with a lower tidal volume [12].

Patients were asked to exhale through the device until the control container was full or until the patient wanted to stop the procedure. The fully inflated container indicates that the minimum volume of exhaled air (30 L) has passed through the filter, which equates to 3 min of tidal breathing. The devices were sealed, labelled and immediately sent to the laboratory. Upon arrival, the devices were stored at −80 °C until further analysis. Two mL of blood was collected within 10 min before/after the exhaled breath collection. The plasma samples were stored together with the exhaled breath devices.

In all the patients, antibiotics were analysed in both the plasma and the exhaled breath. Only samples obtained from patients who were able to complete the full sampling protocol were compared to the plasma concentration.

2.3. Analysis of exhaled breath samples

Following storage, the ExaBreath®-devices were thawed under ambient laboratory circumstances. Test tubes were labelled and a 3D-printed (PLA black Ultimaker filament, Geldermalsen, The Netherlands) spacer was put into the test tube (Fig. 2). The spacers have a height of 32 mm. The upper surface has been developed as a grid with 1 mm square holes. The containers were opened, and the filters were placed into the test tubes. The extraction was achieved by the addition of 2 mL 70:30 4% NaCl in water: isopropanol (Fig. 2). The closed test tubes were placed on a roller mixer for 10 min at ambient temperature. Centrifugation was performed under standard conditions: 4750 rpm for 5 min at 4 °C in a Sigma 4 K15 centrifuge (Sigma, Osterode, Germany). Due to the instability of piperacillin and meropenem, evaporation of the solvent with nitrogen gas (while heating) was not possible. Thirty microliters (300 pg/filter) internal standard piperacillin/tazobactam-D_5 and meropenem-D_6 was added to 170 μL of the centrifuged solution. The solution was shaken at 3200 rpm using the vortex shaker for 15 s at ambient laboratory temperature.

The inner surface of the ExaBreath devices also contained exhaled breath condensate. After removing the filter, the devices were washed out with 1 mL 70:30 4% NaCl in water: isopropanol. The same quantity of internal standard was added to 170 μL of this solution. The linearity, LLOQ, within-run accuracy and precision were defined using blank SensABues® filters spiked with standards of meropenem and piperacillin which were purchased from Sigma–Aldrich (Bornem, Belgium). The within-run accuracy and precision were measured in a single analytical run of five replicates per QC level, using four QC levels, together with eight calibrators. The coefficients of variation ranged from 3.9% to 8.6%. Accuracy values ranged from 81 to 128%. The signal of the QC with lowest concentration that could be measured with acceptable accuracy and precision (LLOQ) was compared with the signal of blank sputum samples (n = 3). The analyte signal of the LLOQ should be at least 5 times the signal of a blank sample to comply with the EMA guideline [13]. The lower limit of quantification for both piperacillin and

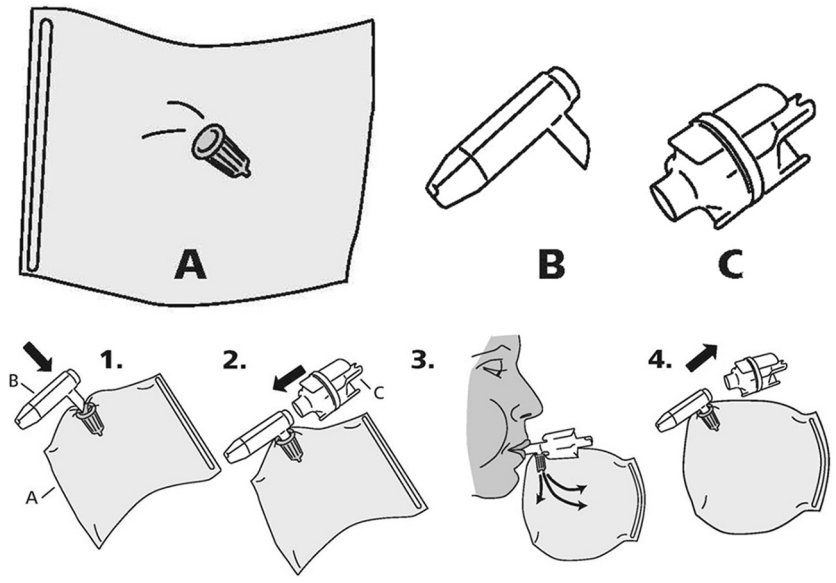

Fig. 1. A. Schematic representation of the SensAbues device. The device consists of three parts: the mouthpiece (B) contains two saliva barriers, the central part (C) contains the electret filter. The third part is a control bag (A). B. Procedure for the collection of exhaled air using the SensAbues device.

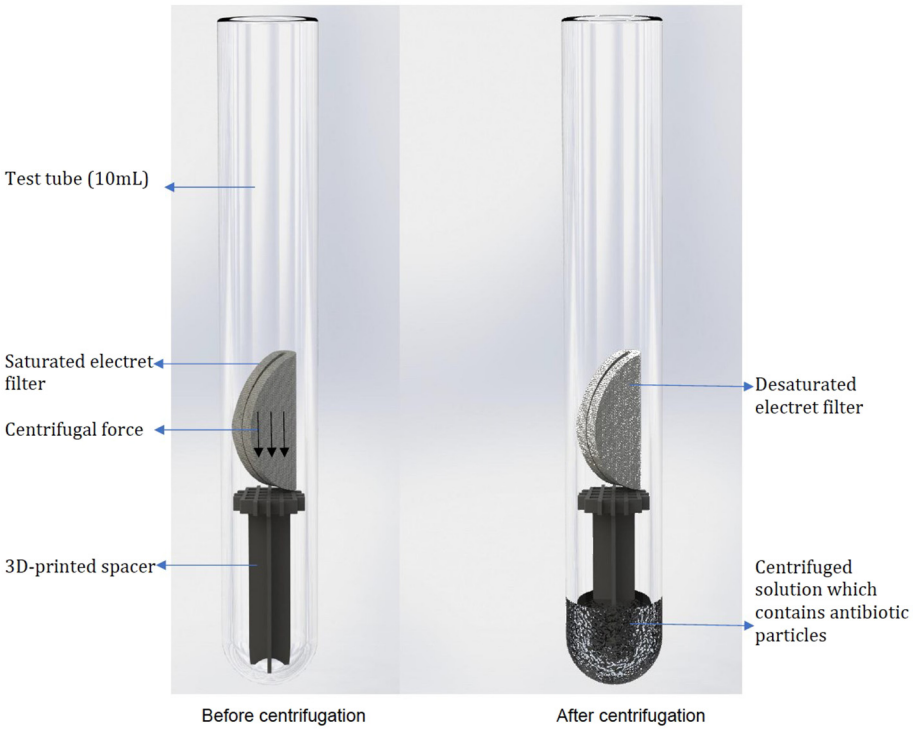

Fig. 2. Extraction method for antibiotics in electret filter which is used for exhaled breath sampling.

meropenem was 1000 pg/filter for filter samples and 5 pg/mL for condensate. The LOD was not determined.

2.4. Mass spectrometry analysis system

2.4.1. Instruments

The analyses were performed on an ultra high-pressure liquid chromatography high-resolution mass spectrometry (UHPLC-HR-MS) system: the Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermo Fisher Scientific, Waltham, USA). Separation was performed on the Accucore phenylhexyl-column (100 × 2.1 mm) with a 2.6 μm particle size.

2.4.2. Chromatographic conditions

The column and autosampler temperature were 40 °C and 10 °C, respectively. The flow rate was 0.4 mL/min. A volume of 10 μL was injected. The mobile phase A was composed of 2 mM ammonium formate (NH_4HCO_2), 0.1% formic acid (CH_2O_2) in water, mobile phase B consisted of 2 mM NH_4HCO_2, 0.1% CH_2O_2, 1% water in 50/50 (v/v) methanol/acetonitrile. The multi-step gradient ranged from 2% B to 90% B and 10% A, and the total analysis duration was 7.6 min.

2.4.3. Mass spectrometric conditions

We used positive electrospray ionization mode (ESI+) in targeted SIM mode with an isolation window of 30.0 *m/z* and an offset of 4 m/z around the masses of meropenem and piperacillin (in order to detect both the proton and the ammonium adduct of the analyte and the internal standard) with a resolution of 70,000, automatic gain control target of 2×10^5 and a maximum time of accumulating ions of 240 ms. The software used for processing the results was Tracefinder 3.3.

2.4.4. Plasma analysis

The total drug concentration in plasma was measured with a validated chromatographic method in combination with tandem mass spectrometric detection [14].

2.5. Statistics

Statistical analyses were performed with IBM SPSS 24 Statistics software. The Pearson correlation coefficient was calculated to assess the correlation between the amount of antibiotic found in the filter and the condensate, versus the plasma concentration.

2.5.1. Ethics

The ethics committee of the Ghent University Hospital approved the study (reference 2016/1298); written informed consent was obtained from all participants.

3. Results

3.1. Patient characteristics

Nine participants (82%) were male; the median age of the patients was 66 years (range 38–81), the mean weight 78 kg (range 57–93). The median acute physiology and chronic health evaluation II score at admission was 11 (range 2–21), the median sequential organ failure assessment score at the day of the of sampling was 2 (range 0–9).

Nine patients were treated with piperacillin/tazobactam, 2 with meropenem. The daily dose for piperacillin/tazobactam varied between 8/1 g and 16/2 g per day and was based on kidney function; meropenem was dosed at 3 g per day. All patients received piperacillin/tazobactam or meropenem as a continuous infusion using a syringe pump and a dedicated central line. The majority of the patients were treated for intra-abdominal infections (n = 5); other infections included infected vascular prosthesis (n = 4), catheter related blood stream infection (n = 1), and one patient was treated empirically.

3.2. Sampling

The sampling occurred 100 h (median, range 24–216) after the first antibiotic administration. Seven out of eleven patients were able to exhale in the device until the control container was completely filled

(equivalent to 30 L). The remaining four did not exhale correctly and also inhaled through the device (patient 3, 5, 6), were tired (patient 3), and/or were unable to fill the control container completely (patients 3, 4, 5, 6).

The median sampling duration was 120 s (range 100–270). Two patients had pre-existing lung disease: chronic obstructive pulmonary disease (patient 8) and asthma (patient 11).

None of the patients experienced any respiratory complication after performing the sampling procedure.

The amount of piperacillin and meropenem was high enough to produce an appropriate response using UHPLC-HRMS in the filter of nine out of eleven patients, two filter samples (patient 2, 6) did not contain the antibiotics at a concentration above the LLOQ and were excluded (Table 1). In the patients who correctly completed the sampling procedure and fulfilled the analytical conditions, the median quantity of piperacillin in the filter was 3083 pg/filter, range 988–203,895 pg/filter. In the condensate, a median quantity of 45 pg (range 6–126 pg) piperacillin was found. Meropenem quantity was 21,168 pg/filter. The meropenem quantity in the condensate was below the LLOQ.

No correlation was found between the quantity of piperacillin in the exhaled breath and the plasma concentration ($r = -0.319$, $p = .6$). Furthermore, no correlation between the quantity of piperacillin in exhaled breath and in the condensate was found ($r = 0.27$, $p = .66$).

4. Discussion

This study evaluating the use of the ExaBreath-device for the detection of antibiotics in exhaled breath found that both piperacillin and meropenem can be detected in the exhaled air from critically ill patients. Using UHPLC-HRMS, it is also possible to quantify the quantity of antibiotic present in the filter. Furthermore, quantifiable amounts of antibiotics were found in the condensate present on the inner parts of the device. In their analysis of cocaine, Ellefsen et al. suggested that the device reflects the drug in oral fluid as well as lung microparticles [15].

This is the first study to demonstrate that antibiotics are present in the exhaled air of patients receiving antibiotics and may offer the opportunity to non-invasively measure antibiotic concentrations in a yet unexplored matrix.

If this proof of concept study is confirmed in other types of patients, performing TDM of antibiotics in exhaled air may be within reach. Concentrations in the exhaled air could potentially reflect the concentrations in the epithelial lining fluid (ELF). ELF concentrations are difficult to obtain in critically ill patients; this can be done using a invasive bronchoalveolar lavage (BAL) but the technique is invasive and may not be tolerated in a patient with respiratory failure.

At this stage, we have not been able to correlate the quantity of antibiotics in the filter with plasma concentrations in this small sample with highly variable amounts of antibiotics detected. As such this is not surprising and future investigations should focus on how antibiotics in exhaled air relate to the tissue concentrations rather than investigating plasma concentrations alone. Also, normalising using an endogenous marker e.g. urea or PC16:16 could assist in improving the correlation.

Whereas antibiotics were found in all samples, the study also demonstrates practical difficulties in exhaling in a coordinated way for some patients, although the impact on the measured quantities is uncertain.

This alternative matrix could be developed into a new method for real-time monitoring, similar to what is available for the detection of other molecules in exhaled air such as alcohol. Regular or even continuous, non-invasive monitoring of antibiotic concentrations may provide a better insight in the tissue pharmacokinetics of antibiotics, which is considered to be the most important determinant in antibiotic efficacy in the treatment of infections [16]. For patients with respiratory infections, the alveolar space is the site of infections and the ELF are considered to be the relevant target for antibiotic therapy; future studies will

Table 1
Patient characteristics and measured antibiotic concentrations in the respective matrices.

Patient number	Age (y)	Gender	Weight (kg)	Length (cm)	Sampling method reliable	Antibiotic administered and dosing (g/24 h)	Interval between initiation of antibiotic therapy and sampling (hours)	Use of humidifcation	Duration of exhalation manoevre	Pre-existing lung disease	Antibiotic concentration in filter (pg/filter)	Antibiotic concentration in condensate (pg/device)	Antibiotic concentration in plasma (mg/L)
1	63	F	70	157	Yes	TZP, 16	121	Yes	4′30″	No	5786	45	174
2	77	M	82	177	Yes	TZP, 16	45	Yes	2′00″	No	<LLOQ	129	174
3	81	F	57	155	No	TZP, 16	164	No	5′43″	No	1576	51	152
4	64	M	87	178	No	TZP, 16	37	Yes	4′53″	No	5574	84	126
5	77	M	85	170	No	TZP, 16	216	Yes	3′07″	No	12042	85	86
6	66	M	78	172	No	MEM, 3	165	No	4′00″	No	<LLOQ	49	17
7	54	M	85	183	Yes	TZP, 16	115	Yes	3′00″	No	203895	78	36
8	68	M	70	180	Yes	TZP, 16	32	No	1′45″	COPD	1495	126	55
9	50	M	73	176	Yes	TZP, 8	100	No	2′30″	No	988	15	30
10	66	M	93	180	Yes	MEM, 3	24	Yes	1′40″	No	21168	<LLOQ	11
11	38	M	80	190	Yes	TZP, 16	86	No	1′46″	Asthma	3083	6	63

Abbreviations. TZP = piperacillin/tazobactam; MEM = meropenem.

need to investigate whether exhaled air concentrations are indeed a valid alternative for TDM in lung infections.

Further research into the feasibility of using the ExaBreath device in other types of critically ill patients is necessary before clinical use can be considered. Taking samples in mechanically ventilated patients could bypass the difficulties in exhaling in a non-standardized way, although this sampling technique would necessitate some adaptations of ventilation tubing to fit the device. The electret filter which is secured in a plastic frame could be inserted in a holder in the expiratory limb of a ventilator.

The non-volatile fraction can be trapped as exhaled breath condensate [6]. This method could also be standardized in ventilated patients.

This study has a number of limitations. We did not collect data on outcome, so linking concentrations in the exhaled breath to any clinical parameter was not possible. Furthermore, patients who suffered from pneumonia were not included in this study. Pneumonia results in a changed pulmonary capillary permeability, this could have an impact on the antibiotic concentrations in exhaled air.

Comparative research between different devices is necessary to define the most appropriate device to collect representative antibiotic concentrations. Despite the accurate analysing method, it was not always possible to reliably quantify the antibiotic concentration as they were below the LOQ.

In conclusion, piperacillin and meropenem can be detected and quantified in exhaled air of non-ventilated ICU patients. Although not all patients could complete the sampling manoeuvre and there appears to be no correlation with plasma concentration in the samples obtained, this could be a promising step in non-invasive monitoring of antibiotic concentrations that can be applied in many patients, depending on the further development of tools to obtain samples of exhaled air.

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