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Basic nutritional investigation

The potential for isothermal microcalorimetry to detect venous catheter infection isolates and establish antibiograms

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ABSTRACT

Objectives: Because bloodstream infection and venous catheter (or cannula) bloodstream infection are associated with high morbidity and cost, early identification and treatment are important. Isothermal microcalorimetry can detect microbial growth using thermal power (heat flow), essentially in real time. The aim of this study was to examine the potential of this technique in clinical practice.

Methods: Thermal power of wild-type bacteria (*Escherichia coli, Staphylococcus epidermidis, Klebsiella pneumoniae,* and *Enterococcus faecium*) isolated from blood cultures of adult inpatients receiving parenteral nutrition in routine clinical practice was measured at 37°C every 10s using a Thermometric 2277 instrument. Temporal patterns of heat flow were used to detect the presence of bacteria, differentiate between them, and test their antibiotic sensitivity. Within and between batch reproducibility (% coefficient of variation [%CV]) was also established.

Results: Isothermal microcalorimetry always correctly detected the absence or presence of wild-type bacteria. Thermograms differed distinctly between species. Key thermographic features, such as peak heights, timing of peak heights, and interval between peak heights, were highly reproducible within each species (within-batch %CV usually about $\leq$1%, although between-batch %CV was usually higher). The antibiotic sensitivities (tested only for *S. epidermidis* and *K. pneumoniae*) confirmed the results obtained from the hospital laboratory.

Conclusions: Isothermal microcalorimetry is a promising and highly reproducible real-time measurement technique with potential application to the investigation, species identification, and targeted antibiotic treatment of bloodstream infection and venous catheter (or cannula) bloodstream infection.

Introduction

Bloodstream infection (BI) and venous catheter (or cannula) bloodstream infection (CBI) are associated with high morbidity and cost. BI/CBI is widespread, and occurs frequently in certain populations, such as immunosuppressed patients and those prescribed parenteral (intravenous) nutrition (PN). For example, a national report of UK clinical practice indicated that CBI was suspected in almost 10% of intravascular catheters used for inpatient PN [1]. In the United States, one-fourth of 68 984 adult inpatients administered PN in 196 hospitals had a BI [2]. More recently, CBI has been reported to vary from 0.27 to 11.5 occurrences per 1000 catheter-days [3,4] against a benchmark of <1 per 1000 catheter-days [5].

Blood culture from the venous catheter or as a peripheral sample is usually considered essential in the investigation of suspected CBI. The absence of growth could limit unnecessary empirical antimicrobial treatment, whereas rapid microbe identification with an antibiogram can lead to a targeted antimicrobial prescription. Because the patient's condition may deteriorate during investigations for suspected CBI, novel ways to rapidly establish microbial growth and antibiograms could prove useful.

Isothermal microcalorimetry can detect microbial growth as well as bacterial decline after growth using thermal power (heat flow) in real time. It is an essentially instantaneous technique that could rapidly identify microbial growth in blood during episodes

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of suspected CBI. It has already been demonstrated that it can detect pathogens more rapidly than conventional methods for urinary infection [6] and infection related to fracture [7] and in synovial fluid [8]. It also has potential for species identification from the pattern of heat flow associated with different microorganisms [9], and for establishing the susceptibility of microbes to antibiotics [6,10-13].

To facilitate these developments, it is necessary to address at least three issues. First, it is necessary to extend the sparse data on wild-type strains isolated from blood culture [12] reported without heat flow curves [12] or information on the nature of the source of infection [13]. There is also a need to address the surprising lack of microcalorimetry data on *Staphylococcus epidermidis*, one of the commonest organisms causing CBI (e.g., PN CBI), and other microbes causing CBI, such as *Klebsiella pneumoniae,* and *Enterococcus faecium*, although one paper [6] briefly reported on the presence of *E. faecium* in urosepsis without heat flow curves. Second, there is limited information on the precision or reproducibility of the technique when using the same and different batches of growth medium. Third, although isothermal microcalorimetry has been used to establish bacterial sensitivities to antibiotics [6,10–13], only a few [6] have been benchmarked against a standard clinical laboratory reference.

This study aimed to explore the potential use of isothermal microcalorimetry in the management of patients with suspected CBI. Specifically, it aimed to examine whether isothermal microcalorimetry can accurately and reliably determine the presence of wild-type bacteria in blood samples from patients on PN suspected of having CBI, and whether it can differentiate between species and their susceptibility to antibiotics, using hospital-reported antibiograms as benchmarks.

Materials and methods

Isothermal microcalorimetry directly measures heat flow into and out of a system at a constant temperature, reflecting physical and/or chemical processes. In this study, each test sample comprised of liquid growth medium inoculated with bacteria within a sealed ampoule. Control samples without bacteria were also used. Isothermal microcalorimetry is distinguished from indirect calorimetry, which measures the gaseous exchange of oxygen and carbon dioxide to estimate energy expenditure (heat production).

All microbiologic work involved standard routine methods under an aseptic technique, and all components were within their expiry date and, as necessary, sterilized before use.

Ethical considerations

This study was approved by the Proportionate Review Subcommittee of the South Central Oxford B Research Ethics Committee. The source patients were adults (≥18 y), who provided written informed consent to participate in this study, including the use of any isolates cultured from their blood. Each participant was free to withdraw consent at any time without a reason.

Source of microbial isolates

The wild-type (clinical) microbial strains were obtained from blood cultures taken for suspected CBI in patients receiving PN at a single large teaching hospital (Oxford, UK) between October 11, 2017 and November 16, 2018. Of the 103 consented patients who all received PN, 51 had suspected CBIs, and of those, 18 had at least one positive blood culture. For the 51 patients, 274 blood cultures were taken, including 47 that were positive (125 blood cultures were taken, including 47 that were positive for the 18 patients). According to routine clinical practice in the hospital, the blood cultures were tested using a BACTEC FX blood culture system (Becton, Dickinson, Wokingham, UK) and isolate species identified using matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (Bruker, Coventry, UK). Five isolates were used in this study: *S. epidermidis*, *E. faecium*, *K. pneumoniae* (two strains), and *Escherichia coli*. This is not an extensive range of bacteria and does not include fungi such as Candida or protozoa. However, these bacteria are used to establish initial insights into the potential use of isothermal microcalorimetry in clinical practice. None of the negative blood culture samples were analyzed using isothermal microcalorimetry in this study.

Preparation of microbial isolates

Each isolate was taken from an agar slope and cultured on tryptic soy agar (Sigma-Aldrich Company Ltd., Gillingham, UK) before being cultured in tryptic soy broth (Sigma-Aldrich Company Ltd.) to an exponential growth phase based on OD600. The microbial suspension was then centrifuged at 10,000rpm for 10 min under refrigeration at 4°C before the supernatant was discarded, and the remaining cells were resuspended in phosphate-buffered saline (PBS) (Sigma-Aldrich Company Ltd.). The resultant suspension was centrifuged under the same conditions as before, the supernatant discarded, and again the cells resuspended in PBS. This step was repeated one further time before the cells were resuspended in 1/4 Ringer's solution (ThermoFisher Scientific, Geel, Belgium) with 10% w/v sucrose (Sigma-Aldrich Company Ltd.) as a cryoprotectant. 1.5 mL aliquots were transferred to 1.8-mL cryovials (ThermoFisher Scientific) and frozen for storage at a nominal temperature of −80°C. Immediately before use, cryovials were dipped in a water bath at a nominal temperature of 40°C for 60s to thaw the microbial suspension. On the first thawing, each isolate was cultured on tryptic soy agar to confirm a single cell culture, and the OD600 was established (to account for potential variations in dilution during the preparation process). Immediately before use, all thawed isolates were diluted to an OD600 of 0.1 using PBS (Sigma-Aldrich Company Ltd.). Once thawed, cryovials and their content were disposed of after use (i.e., isolates from a single cryovial were not refrozen and reused).

Preparation of antibiotics

Antibiotics were prepared in tryptic soy broth (Sigma-Aldrich Company Ltd.) for each use by a single operator experienced in aseptic technique and intravenous medicine preparation. All dilutions were undertaken within a UniMAT-BS class II microbiologic safety cabinet to yield relevant concentrations based on the isolate sensitivities reported by the hospital (Table 1).

Isothermal microcalorimetry

Nominal 20-mL glass vials (22.2 mL total volume) were filled with 5 mL of either tryptic soy broth (as a control) or antibiotic in tryptic soy broth before 5 μL was replaced with 5 μL freshly thawed microbial suspension at an OD600 of 0.1 and a cap crimped onto the vial. Vials were then vortexed for 10s and inserted into

Table 1
Isolate sensitivities reported by the hospital and indicated by EUCAST, and the antibiotic concentrations tested against the isolates using isothermal microcalorimetry

		Hospital report*		EUCAST†		Test‡
Species	Antibiotic§	Sensitivity	MIC (mg/L)	Sensitive MIC (mg/L)	Resistant MIC (mg/L)	Antibiotic concentration (mg/L)
Staphylococcus epidermidis	Erythromycin	Resistant	>4	≤1	>2	1, 2, and 4
	Gentamicin	Sensitive	<1	≤1	>1	0.5 and 1
Klebsiella pneumoniae	Levofloxacin	Resistant	>2	≤0.5	>1	1 and 4
	Ertapenem	Sensitive	≤0.25	≤0.5	>1	0.1 and 1

EUCAST, European Committee for Antimicrobial Susceptibility Testing; MIC, breakpoint minimum inhibitory concentration
*Established during routine clinical practice.
†Different clinical breakpoint tables were used according to the date the isolates were isolated from blood culture (version 7.1 for the *S. epidermidis* and version 8.1 for the *K. pneumoniae*).
‡Test concentrations using isothermal microcalorimetry.
§Erythromycin from Acros Organics (part of ThermoFisher Scientific), Geel, Belgium; gentamicin ('Cidomycin') from Sanofi, Surrey, UK; levofloxacin from Cambridge Bioscience, Cambridge, UK; and ertapenem from ThermoFisher Scientific, Geel, Belgium.

the equilibration position of the calorimeter (Thermometric 2277 Thermal Activity Monitor; TA Instruments, New Castle, Delaware, USA) for a fixed period of 30 min from inoculation into the vial to allow the vial to reach temperature (37°C) before being lowered into the main instrument chamber where measurements of power were recorded every 10s at a fixed temperature of 37°C. To facilitate clear comparisons between organisms, data were taken from the curve baseline (the start of the exponential phase). Each time an antibiotic was tested against an isolate, a control sample was also tested.

Statistical analysis

Data were recorded by the isothermal microcalorimeter as power (mW) against time (s). The end of the lag phase was established to be the point at which the curve first rose and remained above zero power. Cumulative heat (area under the curve, mJ) was obtained by integration using the software OriginPro 2020 (OriginLab Corporation, Northampton, Massachusetts, USA). Within- and between-batch reproducibility (%CV) were calculated [14] using SPSS version 26 (IBM, Armonk, NY, USA).

Results

Data were obtained for all wild-type bacterial isolates tested (i.e., all thawed isolates were viable and single strain).

Detection of wild-type bacteria

Figure 1 shows the power-time data obtained when a control (Fig. 1A) and four wild-type viable bacteria (Fig. 1B, C) were grown in the isothermal microcalorimeter, clearly illustrating the ability of the technique to differentiate between the absence and presence of viable bacteria. The lag phases from inoculation varied from 0.8 h (*K. pneumoniae*) to 1 h (*E. coli*) to 1.4 h (*E. faecium*) to 1.5 h (*S. epidermidis*). The consistency of the duplicates of both gram-positive bacteria throughout the duration of testing is visually striking: The duplicate curves are so close to each other that, for the most part, they appear as a single curve (Fig. 1).

Reproducibility of heat flow curves

Figure 2 shows wild-type *E. coli* tested in triplicate using each of three separate batches of growth medium. The lag phase from inoculation was 1.3 h for batches A and C and 2.2 h for batch B. Figure 2A shows all nine curves superimposed. From these, the precisions and coefficients of variation are calculated. Figure 2B, C show both the within and between batch %CVs at individual time points, calculated every 10s throughout the 36 h (1296 separate time points), against power (mW) and area under the curve, respectively. The points are so close together that they appear to be joined up as a continuous curve. Similarly, %CVs for the area under the increasingly longer time intervals between 0 and 36 h (1296 10s intervals) also appear as continuous curves and are treated as such. The within-batch %CV was found to be particularly high near the start of microcalorimetry when the microbial signals over baseline were particularly small. Still, they were lower to about 2.6 h for the between-batch %CV, calculated from three mean values obtained from each of the three batches. They were found to be high when the change in microbial signal was high. For example, Figure 3 shows that the maximum %CV occurs when the slope of the curve is maximal (between 7.47 and 7.54 h) where small shifts of the "curve" to the left or right on the time axis correspond to large changes in heat flow. The pooled within-batch %CV corresponding to these time points is represented as a sharp peak in Figure 2B.

Table 2 shows the reproducibility of several features of the heat flow curves (thermograms), such as the time of appearance of peaks, time interval between peaks, power (heat flow) at peaks, and the ratio of power between peaks. The within-batch %CVs were typically $\leq$1% (range 0.2−2.4%), whereas the between-batch precision was typically $\geq$1% (range 0.4−9.4%; Table 2). When reproducibility involved differences between time points of peaks, or ratio of power (heat flow) at these peaks, the %CV was found to be better using ratios than differences (in Table 2, compare item 2A with 2B, and 4A with 4B). This was associated with a tendency for peaks to maintain their rank of heights within and between batches (Fig. 4) and for the time of appearance of these peaks to maintain their rank within and between batches. The findings of within-batch %CV for the *E. coli* (Table 2) are within the range of the four microorganisms in Figure 1 (time to highest peak 0.03−2.7%, heat flow at highest peak 0.3−2.9% and area under the heat flow curve from 0−10 h 0.09−2.4%).

Antimicrobial sensitivity

Figure 5 shows the isothermal microcalorimetry growth curves of a gram-positive bacterium (*S. epidermidis*; Fig. 5A, B), and one gram-negative bacterium (*K. pneumoniae*; a different wild-type strain to the *K. pneumoniae* in Fig. 1; Fig. 5C, D), each tested against two antibiotics, one of which the organism was resistant to (Fig. 5 left) and the other which the organism was sensitive to (Fig. 5 right; see Table 1 for antibiotic senitivities according to the hospital report and EUCAST). The lag phases from inoculation were 2.1 h (erythromycin) and 1.5 h (gentamicin) for *S. epidermidis* and 2.5 h

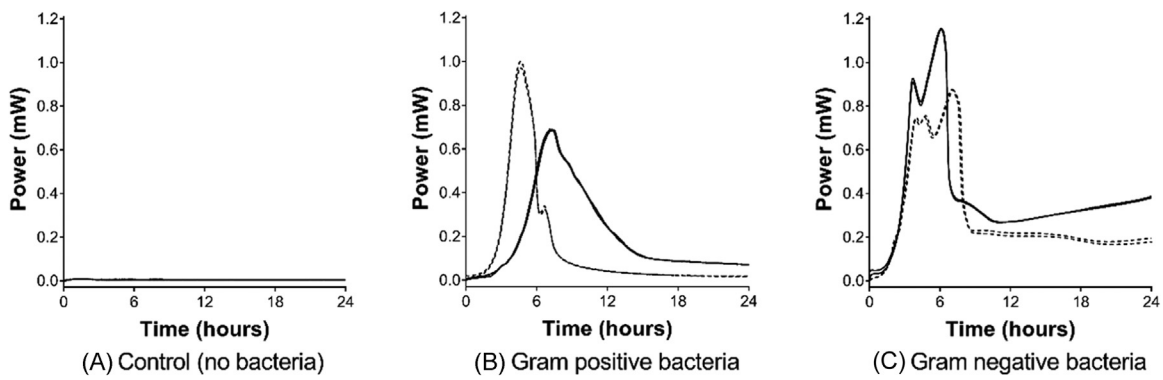

Fig. 1. Heat flow patterns observed during isothermal microcalorimetry in samples of tryptic soy broth in the absence of bacteria (control) and in the presence of different wild-type bacteria isolated from blood culture in routine clinical practice: (**A**) Tryptic soy broth without any microbe; (**B**) *Staphylococcus epidermidis* (solid lines) and *Enterococcus faecium* (dashed lines); (**C**) *Klebsiella pneumoniae* (solid lines) and *Escherichia coli* (dashed lines). All samples are shown in duplicate, and all bacteria were cultured in tryptic soy broth. All curves are shown from curve baseline at time = 0 h.

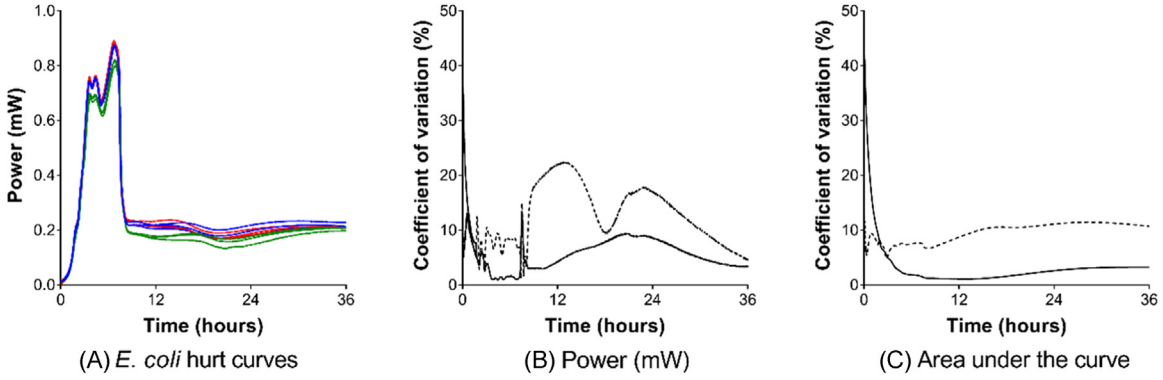

Fig. 2. Variability of heat flow curves obtained using isothermal microcalorimetry for a wild-type *Escherichia coli* tested in triplicate in each of three batches of growth media (tryptic soy broth): (**A**) Original heat curves with each batch shown in a different color (red, blue or green), each in triplicate at single time points every 10s between 0 and 36 h; (**B**) %CV within batches (solid line) and between batches (dashed line) at single time points every 10s between 0 and 36 h; (**C**) %CV within (solid line) and between (dashed line) batches, based on increasingly larger area under the curve measured contiguously every 10s between 0 and 36 h. The between-batch %CV are for single sample measurements within each batch (for replicates it becomes smaller by a factor of $\sqrt{n}$ (or 1.732 for three replicates)). All curves are shown from curve baseline at time = 0 h. %CV, percent coefficients of variation.

(levofloxacin) and 7.1 h (ertapenem) for *K. pneumoniae*. The heat flow curves obtained from bacteria exposed to antibiotics they were resistant to showed little attenuation and only a small shift to the right (delay in growth). For *S. epidermidis*, the delay was 0.5, 1, and <2 h at erythromycin concentrations of 1, 2, and 4 mg/L, respectively, compared with the control (not exposed to erythromycin). For *K. pneumoniae*, the delay was 0.5 and 2.5 h at concentrations of levofloxacin of 1 and 4 mg/L, respectively. In contrast, the heat flow curves obtained from bacteria exposed to antibiotics they were sensitive to showed much greater attenuation and were associated with greater delay in growth. For *S. epidermidis*, peak height was reduced by 35% and 43% at gentamicin concentrations of 0.5 and 1 mg/L, respectively; and for *K. pneumoniae* by 19% and 100% (no growth) at ertapenem concentrations of 0.1 and 1 mg/L respectively. The delay in growth for *S. epidermidis* was 13 and 22 h at gentamicin concentrations of 0.5 and 1 mg/L, respectively, and for *K. pneumoniae* by 8.5 and ≥36 h (no growth) at ertapenem concentrations of 0.1 and 1 mg/L, respectively.

Discussion

This study examined the potential use of isothermal microcalorimetry in the management of CBI, using wild-type bacteria obtained in routine clinical practice (*S. epidermidis, E. faecium, K. pneumoniae,* and *E. coli*) and antibiotic sensitivity using hospital reports and EUCAST as references.

CBI detrimentally affects clinical and economic outcomes of PN administration. It increases morbidity, disseminates infection, and adversely affects organ or tissue function and well-being. It may be necessary to remove the catheter and provide antibiotic treatment for up to ≥2 wk. The absence of a catheter (before replacement) may prevent administration of essential medicines, fluids, and nutrients that would help maintain or improve clinical status. CBI prolongs hospital length of stay (or increases risk for admission for those who develop CBI at home) and increases treatment needs at an estimated cost of $12,809 [15] for a single episode. By facilitating more rapid diagnosis and treatment of CBI, the above adverse consequences could be reduced or avoided in patients administered PN.

The clinical challenge is to fulfill three criteria as quickly as possible: detect the presence of microorganisms in blood, identify the specific causative microorganism (species identification), and provide antibiotic sensitivities. Each of these criteria is considered, followed by a discussion on reproducibility (precision).

Detection of microorganisms

In all cases, microcalorimetry correctly identified the growth of wild-type microorganisms from CBIs and no growth in control samples without microorganisms. Although this study did not specifically evaluate the time taken from blood sampling, an earlier study reported that it could be done in 6 h using neonatal blood

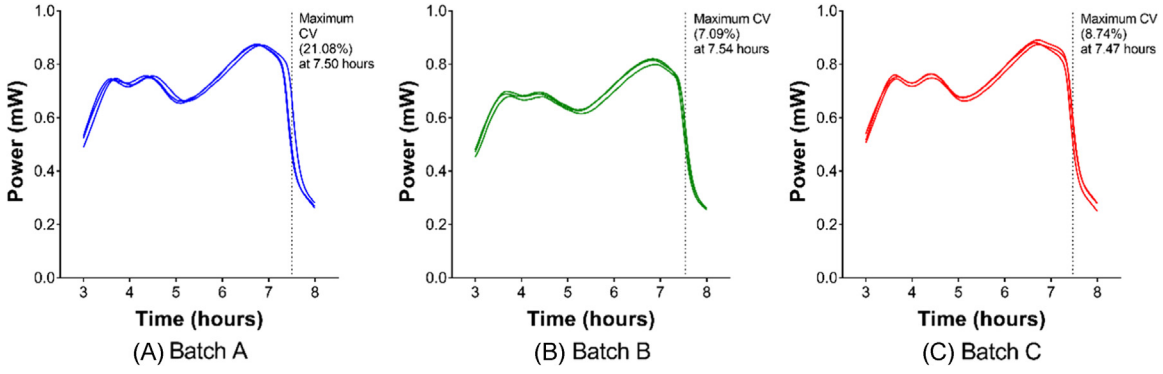

Fig. 3. Heat flow curves obtained between 3 and 8 h using isothermal microcalorimetry for a wild-type *Escherichia coli* tested in triplicate within each of three batches (**A**, **B**, and **C**) of growth medium (tryptic soy broth). All curves are shown from curve baseline at time = 0 h.

Table 2
Within- and between-batch variability of various aspects of heat flow curves for wild-type *Escherichia coli* tested in the same growth medium (tryptic soy broth)

			Within-batch*		Between-batch*,†	
Factor	Curve location	Mean	Precision	%CV	Precision	%CV
Time to peak (h)	Peak 1	3.665	0.050	1.4	0.058	1.6
	Peak 2	4.402	0.053	1.2	0.016	0.4
	Peak 3	6.779	0.048	0.7	0.120	1.8
Time interval between peaks (h)	Peaks 1 & 2	0.737	0.024	3.3	0.069	9.3
	Peaks 1 & 3	3.114	0.039	1.2	0.101	3.3
	Peaks 2 & 3	2.377	0.034	1.4	0.136	5.7
Ratio of time taken to reach peaks	Peak 1/peak 2	0.833	0.0051	0.6	0.0152	1.8
	Peak1/peak 3	0.541	0.0055	1	0.0088	1.6
	Peak 2/peak 3	0.649	0.0052	0.8	0.0139	2.1
Heat flow at peak (mW)	Peak 1	0.729	0.0072	1	0.060	8.2
	Peak 2	0.734	0.0074	1	0.069	9.3
	Peak 3	0.856	0.0082	1	0.066	7.8
Difference in heat flow between peaks (mW)	Peaks 1 & 2	0.0050	0.0024	‡	0.0097	‡
	Peaks 1 & 3	0.127	0.0055	4.3	0.0065	5.1
	Peaks 2 & 3	0.122	0.0056	4.6	0.0043	3.5
Ratio of heat flow at peaks	Peak 1/peak 2	0.993	0.0032	0.3	0.0129	1.3
	Peak 1/peak 3	0.851	0.0060	0.7	0.0042	0.5
	Peak 2/peak 3	0.857	0.0060	0.7	0.0146	1.7
Area under the heat flow curve (mJ§)	0–5 h	6633.1	140.6	2.1	284.9	4.3
	0–10 h	15652.8	178.9	1.1	682.9	4.4
	0–20 h	22698.2	437.4	1.9	1378	6.1
	0–30 h	29506.4	934.9	3.2	1939.5	6.6

%CV, percent coefficient of variation; mW, milliwatts.
*The within-batch precision is the pooled SD (pooling of three SD) within each of the three batches, which ignores the mean differences between batches. With the between-batch precision, the differences between batches are considered (precision = SD).
†The displayed values for the between-batch precision and the associated between-batch %CV are for single measurements of each sample in each of the three batches. For n replicate measurements of each sample within each batch, the between-batch precision and %CV become smaller by a factor of $\sqrt{n}$ (or 1.732 for three replicates).
‡%CV has little meaning when the mean value (the denominator) is close to zero.
§Calculated by integration of power in mW vs time in seconds.

containing 50 cfu/mL [16]. Other studies reported that appropriate antimicrobial therapy for urosepsis could be established in 7 h [6] and the time to positivity of synovial fluid was 9 h compared with 3 d for traditional culture [8]. The present work demonstrated rapid onset of growth after a lag phase of 0.8 to 7.1 h (0.8–2.5 h excluding *K. pneumoniae* with ertapenem). The delay depends on at least the type of microorganism, dose of inoculum [9,17,18], and presence of antibiotics, all of which need to be comprehensively assessed from a clinical perspective. Furthermore, formal evaluation of whether the technique reduces the time in routine clinical practice remains to be established.

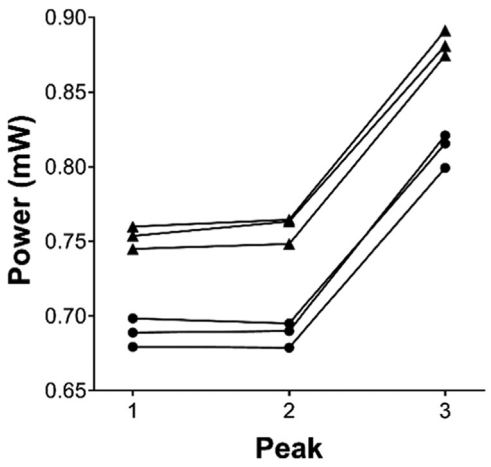

Fig. 4. Power (heat flow) at peaks 1, 2, and 3 obtained by analysis of triplicate samples. Each curve indicates the results from one sample. The upper three curves are the replicates from batch C and the lower three from batch B. Those from batch A are not shown for clarity because the curves overlap with those in the upper set. The curves retain their rank within and between batches, with the exception of the single and small crossover between peaks 2 and 3 at a power of ~0.75 mW, in the same batch (lower set of curves).

Species differentiation and identification

The microcalorimetry signals produced by different microorganisms appear to be distinct and reproducible, which is encouraging from the point of view of species identification. Figure 1, for example, shows that the heat flow curve produced by *S. epidermidis*, one of the commonest bacteria causing CBI, is reproducible and distinct from other gram-positive and gram-negative organisms causing CBI. Although this study adds to the literature, which generally lacks information on heat flow signals produced by *S. epidermidis, K. pneumonia*, and *E. faecium,* it also prevents comparisons with other studies employing different growth media and instruments. The heat flow signals from *E. coli*, which has been much more extensively studied [6,11,17,19–23], appear to show some similarities [17,20,21] with those from the present study. However, closer inspection reveals multiple differences [17,19–23]: in the reported number of peaks (three in this study compared with one [6], two [20,22], three [23], or four [11] in others); timing of peaks (which may be much earlier [22] or later [19] than those from the present study); ratio of peak heights or ratio of time of appearance of peaks; and the extent to which the curve returns toward baseline (well above baseline in this study even up to 36 h — corresponding to ~29% of the height of highest peak—compared with much lower values in other studies <0.1% to 5% by 27 to 33 h [19,20,23] or as early as 10 h [21].

These differences can be explained by the metabolism of *E. coli,* which uses substrates in the presence and absence of oxygen (facultative anaerobe) and which can use amino acids, fatty acids, and

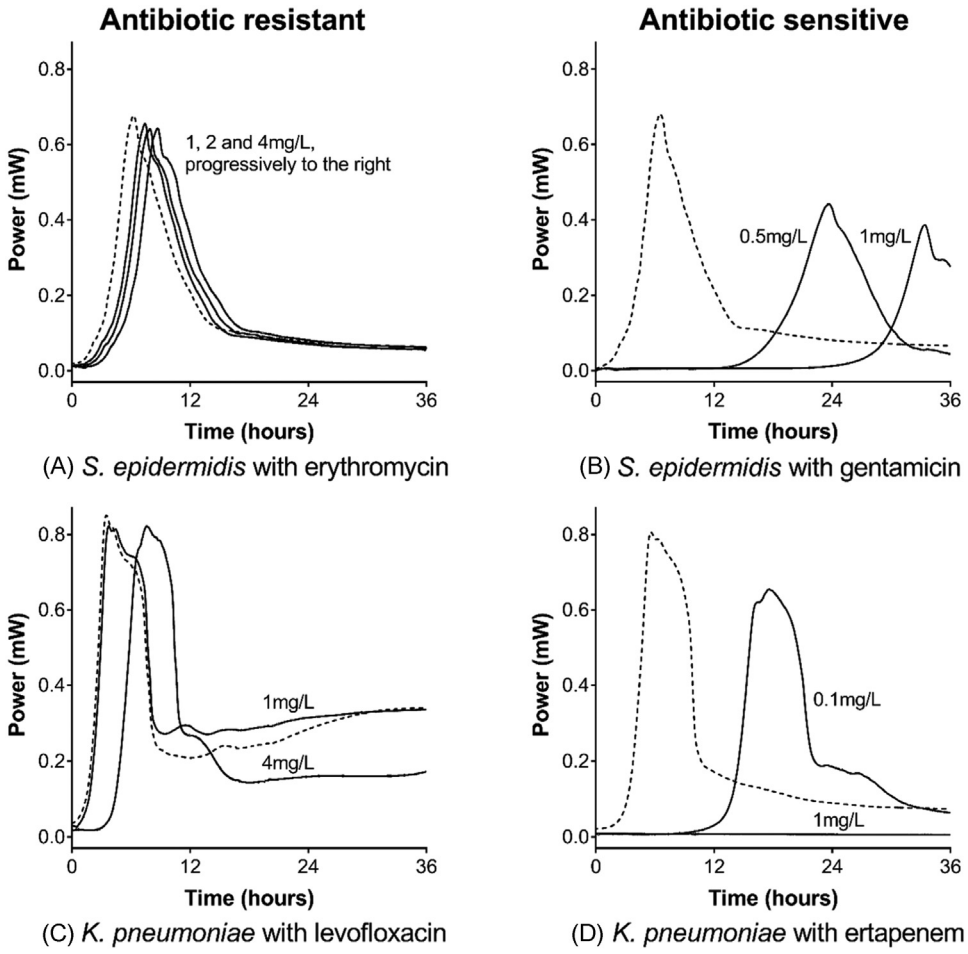

Fig. 5. The effect of different concentrations of antibiotics on the isothermal microcalorimetry of wild-type gram-positive (*Staphylococcus epidermidis*) and gram-negative (*Klebsiella pneumoniae*) bacteria isolated from blood culture in routine clinical practice. Dashed lines indicate that microcalorimetry was carried out in the absence of antibiotics (control) and solid lines in the presence of antibiotics. On the *left* (**A** and **C**) the bacteria are resistant to the test antibiotic, and on the *right* (**B** and **D**) sensitive to the test antibiotic: (**A**) *S. epidermidis* resistant to erythromycin, (**B**) *S. epidermidis* sensitive to gentamicin, (**C**) *K. pneumoniae* resistant to levofloxacin, and (**D**) *K. pneumoniae* sensitive to ertapenem. All curves are shown from curve baseline at time = 0 h.

carbohydrates. It metabolizes glucose before lactose, which in turn is metabolized before other sugars in a hierarchical manner [24]. Using M9 medium containing glucose and lactose, Braissant et al. [19] ascribed the first *E. coli* microcalorimetry peak to glucose oxidation, the second to glucose fermentation, and the third to lactose fermentation. Therefore, the composition of broths can have important effects on thermograms, especially since the type of broths used to obtain *E. coli* thermograms have varied considerably (tryptic soy broth, as in this study and Zaharia et al. [21], M9 [19], Luria–Bertani broth [20,22,23], and Mueller-Hinton II broth [11], as well as filtered sterile urine of unspecified composition [17] and "artificial urine" [9]). These broths do not generally contain lactose, and unlike the M9 broth, which contains no amino acids/peptides, most other broths contain amino acids/peptides as a major or the only energy source. The broths also vary in their micronutrient content and pH, which could also affect growth patterns and associated heat flow curves. The availability of oxygen and its distribution between broth and headspace can also have a major influence on the heat flow curves of *E. coli* [21] and other gram-negative and gram-positive organisms [25]. In this study, 5 mL of broth and bacterial suspension/control fluid was inserted into a nominal 20-mL ampoule (22.2 mL actual volume). However, in other studies, it varied from 2.97 mL in a 4-mL ampoule [11] to 5 mL in a 20-mL ampoule [20]. Zaharia et al. [21] reported that the first of the two peaks of their *E. coli* thermograms were related to the utilization of dissolved oxygen in tryptic soy broth and the second to the diffusion of oxygen from the headspace into the broth. Taken together, these observations suggest that the use of microcalorimetry for strain/species identification strongly depends on the methodology used, and this should be standardized as much as possible.

Antibiotic sensitivity

The sensitivity of BI/CBI microorganisms to antibiotics was established using isothermal calorimetry. Modern multichannel instruments allow simultaneous testing of organisms without and with antibiotics at different concentrations. However, as with other techniques, the prolongation of the lag phase in antibiotic-sensitive organisms delays the result (Fig. 5).

Reproducibility

Several characteristic features of thermograms (e.g., peak height, time to peak height, and the time interval between peaks) were found to be highly reproducible (%CV about $\leq$1%), which means small disparities between different thermograms can be elicited, e.g., small disparities produced by different strains of the same species or by exposure of microbes to different

concentrations of the same medicine. The between-batch variability, typically greater than the within-batch variability, may be related to minor differences in the composition/concentration of different batches or in oxygen dissolved in the broths. However, potential differences in sampling or machine performance may also be involved. However, there are different ways of expressing precision and %CV. Precision of heat flow measurements at regular time points throughout the microcalorimetry studies were found to vary considerably, and worse when heat flow is changing rapidly. Better precision and %CV can be established using the heights of peaks (or troughs) when heat flow is not changing. Alternatively, the *time* at which rapid change in heat flow occurs is highly reproducible. In examining the relative heights of different *E. coli* peaks, it was found that %CV was better when expressed as a ratio than a difference. This may be because of systematic effects within thermograms (e.g., when a lower heat flow at one peak is associated with lower heat flow at the other peaks; Fig. 4) affect height ratios less than height differences. The same applies to precision expressed as a ratio of the time taken for two peaks to appear compared with the time interval between peaks. An additional explanation applies to the %CV of the relative heights of two peaks with about the same height. For example, *E. coli* peaks 1 and 2 have approximately the same height, so their ratio is close to 1 and their difference close to zero. Because these values are the denominators of %CV, dividing the precision (the numerator) by a mean value close to zero could elevate the %CV substantially. The high %CV for the early period of thermography (Fig. 2) may also be due to the small mean heat flow values (denominators of %CV).

Conclusion

This study found isothermal microcalorimetry to be a highly sensitive and reproducible method for identifying the growth of microorganisms responsible for CBI. It demonstrated distinct features of heat flow curves from the same organism, facilitating species identification and antibiotic sensitivity. The possible application of isothermal microcalorimetry to routine clinical practice of PN administration could potentially result in clinical and economic benefits. However, there is a need to expand the evidence base. Future studies should examine additional species and strains under different conditions and consider using additional analytical variables for the analysis of heat flow curves, such as kinetic constants and doubling times. They should examine the sensitivity (presence of organism correctly identified) and specificity (absence of organism correctly identified) of the technique in this potential application, cost–benefit, speed of analysis, and clinical outcome comparisons to traditional methods used in clinical practice.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Peter David Austin reports financial support was provided by National Institute for Health and Care Research.

CRediT authorship contribution statement

Peter David Austin: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing, Resources. **Simon Gaisford:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Validation, Writing – review & editing, Resources. **Marinos Elia:** Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing – review & editing.

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