Seven hours to adequate antimicrobial therapy in urosepsis using isothermal microcalorimetry

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Running Head: Rapid antibiogram using microcalorimetry

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Abstract:

Urosepsis can progress toward severe sepsis, septic shock and ultimately death. Rapid antimicrobial susceptibility testing is crucial to decrease mortality and morbidity. This study shows that isothermal microcalorimetry can provide an antibiogram within 7 hours with a sensitivity of 95% and specificity of 91% when using VITEK-2® system as reference.

Main text:

Urinary tract infections (UTIs) are the second most common infection. Patients at risk of urosepsis are elderly, diabetics, immunocompromised and patients with obstruction in the urinary tract. Urosepsis might progress to severe sepsis and septic shock both associated with a high mortality rate ranging between 22% and 76% (8,10). Treatment of urosepsis includes empirical broad spectrum antimicrobial therapy and timely de-escalation when antimicrobial susceptibility testing (AST) results become available (15-17). Identification of the pathogen and determination of its susceptibility patterns takes at least 48 hours on average. Moreover, several methods currently used for AST have additional drawbacks (12,13). Isothermal microcalorimetry (IMC) that measures metabolic heat production by microbes was recently identified as a near-future promising alternative to conventional methods for AST (12,13). As urosepsis is virtually always accompanied by UTIs with a high density of uropathogens, urine specimens could be used directly for AST by IMC. Other arguments also advocate for such approach. Firstly, urine is a potent growth medium (6). Secondly, in 95% of uroseptic cases, urine culture and positive blood culture lead to similar pathogen isolation (18). And thirdly, polymicrobial infections are rare in bacteraemic UTIs (5 to 11 %), thus ensuring that only the targeted pathogen is investigated (2,14). Before introducing IMC for AST in the clinic or performing a clinical study, one needs to determine its sensitivity, specificity and accuracy. For this study we used 15 uropathogens (9 *Escherichia coli*, 3 *Enterococcus faecalis*, and 3 *Enterococcus faecium*) previously identified by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Susceptibility patterns were obtained by the VITEK-2® system (an automated system for AST). Among the 9 *E. coli* strains, 3 were sensitive to all antimicrobials tested, 3 were resistant to at least ciprofloxacin and 3 strains were extended spectrum beta-lactamase (ESBL) producing strains. Among enterococci, all *E. faecium* strains and one *E. faecalis* strain were resistant to amoxicillin. Culture of these strains were diluted in modified artificial urine (3) to obtain an optical density (OD) of 0.1. 10µl of this dilution were added to a 4ml microcalorimetric vial prefilled with 3ml of artificial urine with or without antimicrobial (using the EUCAST guidelines for breakpoint concentration). For AST with *E. coli*, the following antimicrobials and their respective concentrations were used: ciprofloxacin 0.5 mg/l, cotrimoxazole 2 mg/l, ceftriaxone 1 mg/l, amoxicillin 8 mg/l, piperacillin 8 mg/l and ertapenem 0.5 mg/l. For enterococci, only amoxicillin 4 mg/l, and cotrimoxazole 0.03 mg/l were tested. After inoculation, the vials were sealed and introduced in the microcalorimeter (TAM48, Waters/TA, Delaware, USA). The metabolic heat production rate was recorded until returning close to the baseline. Data were extracted and the
maximum growth rate ($\mu$), the lag phase duration ($\lambda$) and the total heat produced ($Q$) were calculated as described in (5). An inhibition index was calculated as:

$$I = \frac{(1 - \frac{\mu_s}{\mu_c}) + (1 - \frac{Q_s}{Q_c})}{2}$$

Where $I$ is the inhibition index expressed in percent. The $s$ and $c$ indices indicate samples and controls respectively. The results were obtained in 7 hours including 25-30 minutes for sample handling and preparation, 6 hours of measurements, and finally 30 minutes for curve fitting and data processing using the R statistical package (7,9). The calculated inhibitions were concordant with the VITEK-2® system results in 95% of cases. Similarly, IMC results were confirmed by a final OD measurement of the microcalorimetric ampoule content after 48 hours (Table 1). For ciprofloxacin and cotrimoxazole, there was a clear-cut difference (Mann-Whitney’ test: $p < 0.05$) in the inhibition index of susceptible strains showing very few growth and resistant strains showing rapid growth (Figure 1). On the other hand, for beta-lactams (amoxicillin, ceftriaxone, and piperacillin) the inhibition index calculated for susceptible strains using IMC was lower (Figure 1) but still exhibited a significant difference compared resistant strains in all cases (Mann-Whitney’ test: $p < 0.05$). This is explained by the mode and speed of action of this antimicrobial class. Early in-vitro time-kill curves studies have shown that decrease of CFU or OD after addition of these antimicrobials in the medium can take between 2 and 4 hours at concentrations up to 10x higher than MIC (1,11,19).

Such delayed action results in an apparently lower inhibition. However, for both ceftriaxone and amoxicillin in most cases the resistant strains had an inhibition index of 0%. Only one amoxicillin resistant strain had an inhibition index of 6% (Figure 1). None of the tested strains was resistant to piperacillin but one can expect that resistant strains will also have an inhibition index very close to 0%. Similarly, none of the tested strains was resistant to ertapenem, however the very narrow distribution of inhibition index suggests that clear discrimination will be possible as well (Figure 1). For AST determination with IMC, the data of ciprofloxacin, cotrimoxazole, ceftriaxone and amoxicillin showed a sensitivity of 95%, a specificity of 91% and an accuracy of 93% using the VITEK-2® results as reference. To avoid bias, the data of piperacillin and ertapenem were not used since only susceptible strains were investigated. These results warrants for IMC to be used in larger clinical studies focusing on AST in UTIs and urosepsis. For such studies, additional antibiotics such as pivmecillinam that has been reported to be valuable against ESBL strains, might be considered. Accurate and reproducible results can be obtained by diluting the original sample in artificial urine to allow bacterial growth (needed to clearly observe an inhibition). Artificial urine also improves the reproducibility as urine composition varies over patients and time. In addition, artificial urine closer matches the in vivo situation, as urine exhibits different growth characteristics compared to artificial growth media. Moreover, the concentration of bacteria used as inoculum here is rather low ($\sim 10^5$ CFU/ml) compared to concentrations observed in severe infections ($\sim 10^9$ CFU/ml). Increased inoculum concentration might reduce the measuring time by an additional 2 hours (4) thus allowing an antibiogram to be delivered in 5 hours or less. Finally, cost wise we anticipate...
consumables cost for 1 antibiogram with 6 antibiotics of $28 USD (personal and overhead were not included because of large variations between institutions).

Reference List


Table 1: Numbers and percentages in parentheses of antimicrobial testing results for the type of measurements performed. The = sign indicates that similar results are obtained by both methods as a ≠ indicates that discordant results are obtained by the 2 methods.

<table>
<thead>
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<th></th>
<th>IMC = VITEK-2</th>
<th>IMC ≠ VITEK-2</th>
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<tbody>
<tr>
<td>IMC = OD*</td>
<td>55 (92%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>IMC ≠ OD*</td>
<td>2 (3%)</td>
<td>1 (2%)</td>
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* Measured from the microcalorimetric ampule content after 48 hours.
Figure 1: Raw data (i.e., heat flow curves) from an E. coli isolate resistant to ciprofloxacin and amoxicillin (A). Note the similarity between the control and ciprofloxacin and amoxicillin heat flow patterns as for the other antimicrobials the heat flow curve shows a rapid decrease. Boxplot showing the inhibition index obtained by IMC (see equation 1) for antimicrobial resistant and susceptible E. coli, E. faecalis and E. faecium strains previously investigated using the VITEK-2 system (B,C,D,E,F,G). R: resistant, S: susceptible.