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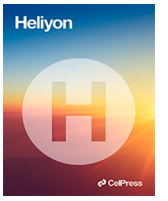

Research article

Early assessment of blood culture negativity as a potential support tool for antimicrobial stewardship

Giulia Menchinelli [a], Alice Oliveti [b], Barbara Fiori [a], Tiziana D'Inzeo [a,b], Teresa Spanu [a,b], Rita Murri [a,c], Massimo Fantoni [a,c], Maurizio Sanguinetti [a,b,*], Brunella Posteraro [b,d,1], Giulia De Angelis [a,b,1]

[a] *Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, 00168, Rome, Italy*
[b] *Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, 00168, Rome, Italy*
[c] *Dipartimento di Sicurezza e Bioetica, Università Cattolica del Sacro Cuore, 00168, Rome, Italy*
[d] *Dipartimento di Scienze Mediche e Chirurgiche Addominali ed Endocrino Metaboliche, Fondazione Policlinico Universitario A. Gemelli IRCCS, 00168, Rome, Italy*

A R T I C L E I N F O

Keywords:
Antimicrobial stewardship
Bloodstream infection
Blood culture
Incubation time

A B S T R A C T

Objective: To assess whether 48-h negative blood culture (BC) bottles are still negative at the classic 120-h incubation endpoint and whether 48 h might be the time to make antimicrobial therapy decisions.
Methods: Data from the first collected bottles from bloodstream infection (BSI) episodes of single patients were retrospectively analyzed. Probabilities of bottles being negative at the classic endpoint were calculated from 0 to 120 h of incubation.
Results: Among BC-negative episodes (4018/4901 [82.0%]), most (2097/4018 (52.2%) occurred in medicine patients. At 48 h, probability was 100.0% (95% CI, 99.9–100.0) for all 4018 patients. Of these, 1244 (31.0%) patients remained on antibiotics until 120 h. Excluding 401 (32.2%) patients who received antibiotics for another (non-bloodstream) infection, 843 (67.8%) of 1244 patients could have merited early (48-h) discontinuation of antibiotics. Stopping treatment in these patients would have led to saving 5201 days of access (943 [18.1%] days), watch (3624 [69.7%] days), or reserve (634 [12.2%]) AWaRe groups' antibiotics, which correspond to 65.6% (5201/7928) of days of administered antibiotics in all 1244 patients.
Conclusion: As an early indicator of BC negativity, the 48-h endpoint could reliably support antimicrobial stewardship, but the clinical judgment remains imperative especially when BSI is highly suspected.

1. Introduction

Blood culture (BC) remains the key diagnostic method for bloodstream infections (BSIs), which may result in substantial patient

* Corresponding author. Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Largo Gemelli, 8 00168, Rome, Italy.
E-mail address: maurizio.sanguinetti@unicatt.it (M. Sanguinetti).
[1] Brunella Posteraro and Giulia De Angelis are co-senior authors.

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morbidity and mortality [1]. Especially when sepsis or septic shock is suspected [2], initiation of empirical broad-spectrum antimicrobial therapy (i.e., before antimicrobial susceptibility results for the infecting pathogen(s) are available) is required [3]. Unnecessary antimicrobial administration (e.g., until BCs are confirmed negative) or the failure to deescalate empirical broad-spectrum antimicrobial therapy is, however, associated with adverse effects, mainly infections by *Clostridioides difficile* [4] or by antibiotic-resistant pathogens [5], which may be consequences of antibiotic-induced disruption of the gut microbiome [6]. While the optimal time window inside which patients should receive empirical antimicrobial therapy has not yet been established [7], the benefit-to-risk ratio of receiving empirical antimicrobials depends on the pre-test probability for BSI (e.g., general medicine patients with isolated fever) [8].

Consistent with the occurrence of clinical syndromes in which BCs exhibit low yield (e.g., pneumonia or the skin and soft tissue infection) [8], incubation of BC bottles for up to 5–7 days may increase detection of slow-growing BSI pathogens (e.g., *Candida*, strict anaerobes, or *Brucella species*) [9]. However, confirming previous evidence [10], 92%–98% of BCs become positive for most common

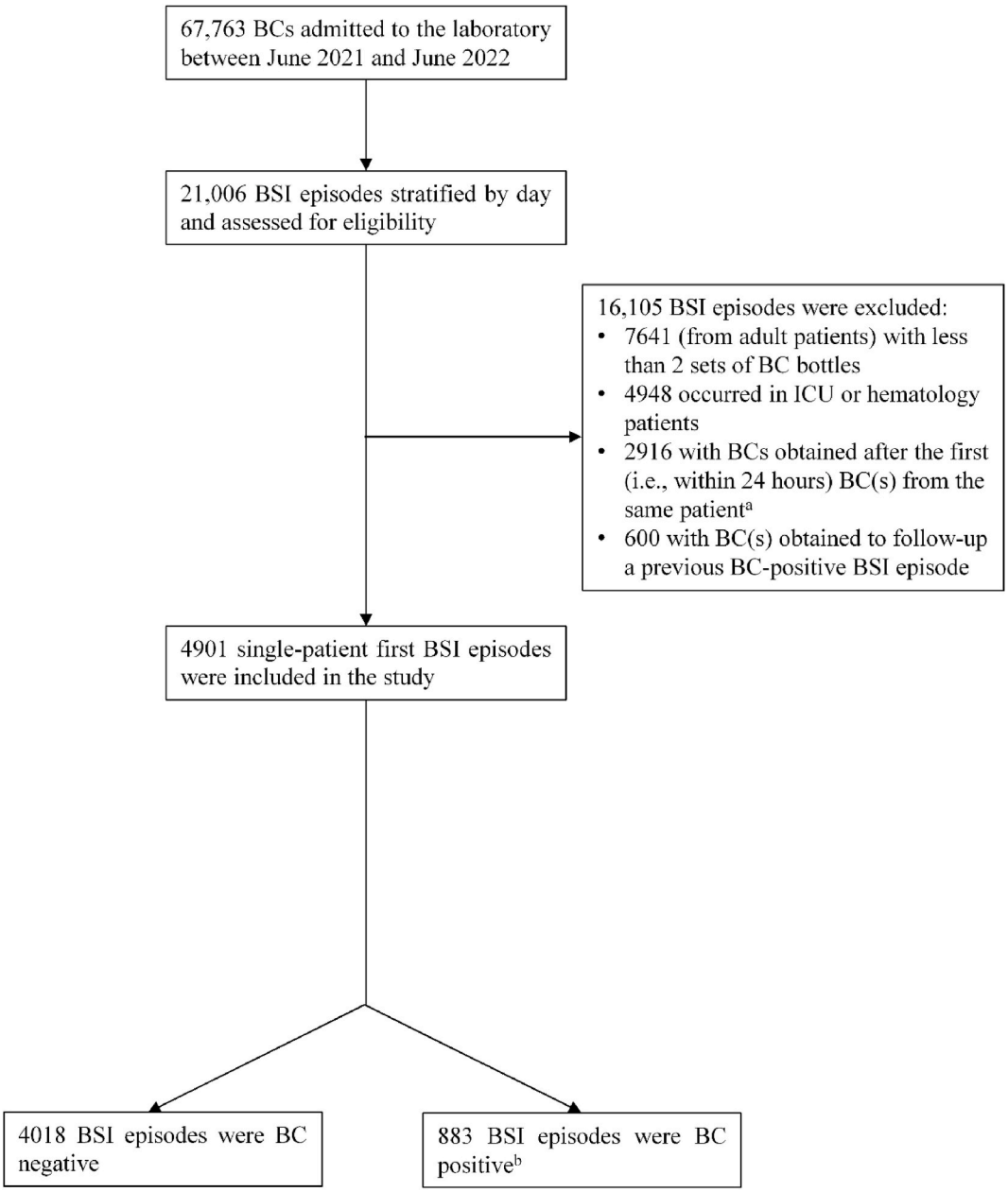

Fig. 1. Flow chart of the study. [a] This number comprises 437 episodes of patients who had a negative BC 48–120 h after the first (BC-negative) episode. [b] This number comprises 19 episodes of patients who had a positive BC 48–120 h after the first (BC-negative) episode. BC, blood culture; BSI, bloodstream infection.

BSI pathogens within 24–48 h of incubation in an automated BC instrument [11,12]. Notably, current BC media (e.g., resin-containing media) allow recovery of BSI pathogens even with clinically relevant antimicrobial concentrations in the blood sample with which the BC is inoculated [13,14].

Thus, the 48-h endpoint could be a decision point to deescalate/stop antimicrobial therapy for negative BCs. Here, using a large sample of clinical BCs, we assessed at several endpoints the probability that BCs were negative at the completion of 5-day incubation period, and we discussed the antimicrobial stewardship implications of our findings.

2. Methods

2.1. Design

We conducted a retrospective cohort study of BCs obtained between June 2021 and June 2022 from adult or pediatric patients in medical or surgical wards and in the emergency room (ER) of the Fondazione Policlinico Universitario Agostino Gemelli IRCCS, a tertiary-care teaching hospital in Rome, Italy. At this hospital, an infectious disease consultation team (IDCT) works to assist physicians in all (except intensive care unit [ICU] or hematology) wards in optimizing the antimicrobial treatment of patients with BSI [15]. Since we aimed to assess the potential therapeutic implications of the above 48-h endpoint, including multiple (i.e., more than one) BSI episodes per patient would have led to double/triple counting of antimicrobial days (see below). According to the study flow chart (Fig. 1), only the first BC bottles, i.e., collected within the first 24 h of the onset of signs and symptoms (as defined elsewhere [16]) per single patient were therefore assessed for eligibility, whereas follow-up BCs (i.e., collected after a positive BC to document clearance of the pathogen(s) from the blood) were *a priori* excluded. Episodes with BCs obtained after the first BC(s) (i.e., repeated/new episodes) were excluded unless than patients (n = 19) had a positive BC collected 48–120 h after the first BC-negative episodes. In these cases, the first BC bottles were considered false negatives and were therefore excluded. For adult patients only, we included those with at least 2 sets of (1 aerobic and 1 anaerobic) bottles, which resulted in a single patient/episode total cultured blood volume that met the BSI diagnostic standard [1]. Furthermore, ICU or hematology patients were excluded due to unavailability of data on antimicrobial administration for these wards (see below). Part of this study has been presented at the 33rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) held in Copenhagen, Denmark (15–April 18, 2023).

2.2. Data collection and definitions

For each BC, the following variables were collected: bottle type (aerobic, anaerobic, or pediatric); sampling date; time of insertion into the (automated) instrument; time of (positive or negative) flagging by the instrument; time (hour) to positivity (TTP; i.e., the time interval between insertion of the bottle into the instrument and detection of the bottle as positive); and the number and/or type of BSI pathogen(s) identified (only for positive bottles). For each patient from whom BC bottles were obtained, variables included the type of hospital ward (e.g., medicine, surgery, etc.) and the type and duration (i.e., assessed from 48 h before to 120 h after bottles were inserted into the instrument) of antimicrobial (i.e., antibiotic) treatment (Table S1). We categorized the antibiotics administered to patients in stewardship groups (i.e., access, watch, or reserve) according to the 2021 WHO AWaRe classification database [17]. All variables assessed in the study were retrieved from the "microbiology" and "antimicrobial administration" databases and cross-referenced automatically. Additional data were retrieved from the IDCT database to identify potential patients for whom the treating physician should have considered the 48-h endpoint to stop antibiotics.

A positive-BC BSI episode was defined as one or more bottles that grew one or more clinically relevant organisms (Table S2). In the case of monomicrobial episode, TTP was recorded for the first of bottles that grew the same organism. In the case of polymicrobial episode, when two or more organisms originated from the same or different bottles, TTP was recorded for the last of bottles that grew the additional organism(s) (i.e., other than the first organisms(s) isolated). A negative-BC BSI episode was defined as all bottles that were negative or only one set of bottles or only one of the two bottles of a set that grew organisms considered to be contaminants (e.g., coagulase negative staphylococci, oral streptococci, diphtheroid organisms, etc. [10]).

2.3. Data analysis

Variable (categorical or continuous) comparisons were performed using the two-sample test of proportions or Student's *t*-test, as appropriate. At predefined endpoints, i.e., 0, 5, 10, 15, 20, 24, 30, 36, 48, 72, and 120 h since BC bottles were inserted into the automated instrument, we calculated the probability (expressed as percent with 95% interval confidence [CI]) for bottles to be negative at the end of the 5-day incubation period dividing the number of negative episodes at 120 h (numerator) by the number of episodes that were negative at the endpoint (denominator). At each endpoint, probability was calculated stratifying the episodes by bottles from all patients or bottles from patients in specific (e.g., medicine, surgery, etc.) hospital wards. In all analyses, the Intercooled Stata program version 13 and GraphPad Prism 8 were used, and $p < 0.05$ was considered statistically significant.

3. Results

Of BC bottles potentially eligible for the study (Fig. 1), 11,575 (17.1%) had a mean TTP of 18.1 h (standard deviation [SD], ±14.3) and the remaining 56,188 (82.9%) had a negative result. According to above specified criteria, we included BC bottles corresponding to the first episodes of suspected or confirmed BSI (n = 4901), of which 883 (18.0%) were positive and 4018 (82.0%) were negative.

Mean bottle numbers were 3.9 (SD, ±1.4), 4.5 (SD, ±1.3), and 3.9 (SD, ±1.4) for total, BC-positive, or BC-negative episodes, respectively, with values in the last two groups differing significantly ($p < 0.001$). Of 804 monomicrobial episodes, 448 (55.7%), 304 (37.8%), 36 (4.5%), and 16 (2.0%) were caused by Gram-positive aerobic, Gram-negative aerobic, *Candida*, or (Gram-positive/Gram-negative) anaerobic organisms, respectively (Table S2). The remaining 79 episodes were polymicrobial, with two (62; 78.4%), three (13; 16.5%), or four (4; 5.1%) causative pathogens identified. Only considering BC-negative episodes, 2097 (52.2%), 584 (14.5%), 578 (14.4%), 430 (10.7%), and 329 (8.2%), respectively, occurred in medicine, pediatric, surgery, ER, or oncology patients.

Only one (0.0%) of 4901 BSI episodes studied in total had BC bottles flagged as positive at >48 h of incubation (i.e., 49 h), with the causative pathogen identified as *Candida glabrata*. Excluding this episode, TTP for the remaining 35 *Candida*-positive episodes ranged from 6 to 43 h.

Table 1 shows the probabilities of BC bottles being negative at 120 h (i.e., the end of 5-day incubation period), as assessed from 0 to 120 h of incubation, stratified by patient categories. The probability after only 24 h was 98.6% (95% CI, 98.2–98.9) and reaches 100.0% (95% CI, 99.9–100.0) at 48 h for all patients, whereas highest (above 99.0%) probabilities at 24 h were observed in pediatric (99.5%; 95% CI, 98.5–99.9), ER (99.1%; 95% CI, 97.7–99.7), or oncology (99.1%; 95% CI, 97.4–99.8) patients. At 36 h, probabilities were 100.0% for both pediatric (95% CI, 99.4–100.0) and ER (95% CI, 99.1–100.0) patients.

Fig. 2 depicts the above probabilities stratified by patients who had or had not received antibiotics (≤48 h) prior to BC collection. No statistically significant differences were observed for BCs incubated from 0 to 120 h of incubation ($p > 0.05$), with probabilities at 48 h reaching 99.9% and 100% for patients with or without previous antibiotic treatment, respectively. The *C. glabrata*-positive BSI episode mentioned above belongs to a patient who was on antibiotics at the time of BC sampling.

Of 4018 patients with BC-negative BSI episodes, 1762 (43.9%) received antibiotics at 48 h (Table S1) and 1244 (31.0%) received antibiotics between 48 and 120 h (i.e., remained on antibiotics until day 5 of incubation of their BC bottles) (Table 2). A detailed examination of the IDCT database showed that 401 (32.2%) of 1244 patients had a documented (primary) infection at one/multiple site(s) other than bloodstream (e.g., lower respiratory tract), for which administration of (targeted, pathogen-specific) antibiotics was required. This resulted in 843 (67.8%) patients for whom the treating physician (with advice from the IDCT) might have considered an early (48-h) discontinuation of antibiotics.

As shown in Table 2, stratifying administered antibiotics by WHO-designed stewardship groups (access, watch, or reserve) revealed that the 843 patients received antibiotics for 5201 (access, 943 [18.1%]; watch, 3624 [69.7%]; and reserve, 634 [12.2%]) days in total. These amounts correspond to 65.6% (5201/7928) of days of administered antibiotics in the 1244 patients included in the analysis. If antibiotic treatments would have stopped before completion of the 5-day incubation period, this could have led to saving 39.4% (3126/7928) days of antibiotics from WHO-designed stewardship groups (access, 575 days [7.3%]; watch, 2176 days [27.4%]; and reserve, 375 days [4.7%]).

4. Discussion

We showed that BC bottles from single (non-ICU/non-hematology medical/surgical or ER) patients with suspected BSI that were negative at 48 h (2 days) had almost 100% probability of remaining negative at 120 h (5 days), the widely accepted incubation time after which a BC bottle can be considered negative. We reasoned that shortening the 5-day time could have a substantial impact on antimicrobial stewardship decisions (deescalating, stopping, or starting antibiotics) and their direct consequences (saving antibiotic days or antibiotic exposure).

A study conducted during a COVID-19 patient surge in New York city hospitals, in March 2020, found that reducing BC incubation

Table 1
Probabilities of having a negative result after 5 days of incubation, as calculated at several endpoints, for blood cultures at a large hospital in Rome, Italy, June 2021–June 2022.

Endpoints (hours)	Values (expressed as percent [95% CI]) for categories[a]					
	All patients	Medicine patients	Surgery patients	Pediatric patients	Oncology patients	ER patients
0	82.0 (80.9–83.1)	80.5 (78.9–82.0)	82.3 (79.3–85.1)	96.4 (94.6–97.7)	80.8 (76.7–84.5)	74.0 (70.2–77.5)
5	82.6 (81.5–83.6)	80.7 (79.1–82.2)	82.7 (79.7–85.4)	96.4 (94.6–97.7)	81.8 (77.7–85.5)	76.6 (72.9–80.1)
10	87.3 (86.3–88.2)	85.1 (83.6–86.5)	87.2 (88.8–93.4)	97.2 (95.5–98.3)	88.2 (84.5–91.3)	85.5 (82.1–88.4)
15	94.3 (93.5–95.0)	93.1 (92.0–94.1)	94.4 (92.3–96.1)	98.3 (96.9–99.2)	95.1 (92.2–97.1)	93.9 (91.3–95.9)
20	97.4 (96.8–97.8)	96.8 (95.9–97.5)	97.5 (95.9–98.6)	98.8 (97.6–99.5)	99.1 (97.4–99.8)	97.1 (95.0–98.4)
24	98.6 (98.2–98.9)	98.1 (97.5–98.7)	98.8 (97.6–99.5)	99.5 (98.5–99.9)	99.1 (97.4–99.8)	99.1 (97.7–99.7)
30	99.2 (98.9–99.5)	98.8 (98.3–99.2)	99.7 (98.8–100.0)	100.0 (99.4–100.0)	99.1 (97.4–99.8)	99.5 (98.3–99.9)
36	99.7 (99.4–99.8)	99.5 (99.1–99.7)	99.8 (99.0–100.0)	100.0 (99.4–100.0)	99.7 (98.3–100.0)	100.0 (99.1–100.0)
48	100.0 (99.9–100.0)	100.0 (99.7–100.0)	100.0 (99.4–100.0)	100.0 (99.4–100.0)	100.0 (98.9–100.0)	100.0 (99.1–100.0)
72	100.0 (99.9–100.0)	100.0 (99.8–100.0)	100.0 (99.4–100.0)	100.0 (99.4–100.0)	100.0 (98.9–100.0)	100.0 (99.1–100.0)
120	100.0 (99.9–100.0)	100.0 (99.8–100.0)	100.0 (99.4–100.0)	100.0 (99.4–100.0)	100.0 (98.9–100.0)	100.0 (99.1–100.0)

Blood culture bottles (BacT/Alert FA PLUS [aerobic], BacT/Alert FN PLUS [anaerobic], or BacT/Alert Pediatric FAN [pediatric]; bioMérieux, Marcy l'Étoile, France), obtained from hospitalized or ER patients (set(s) of bottles for adult patients or single bottle(s) for pediatric patients) in the case of bloodstream infection (i.e., episode), had been inserted into a BacT/Alert VIRTUO (bioMérieux) instrument and incubated for up to 5 days (120 h) or until microbial growth was detected (i.e., bottle flagged positive). At completion of 5-day incubation period, BC bottles had been considered negative for any microorganism(s) (i.e., bottle flagged negative).

[a] At each endpoint (from 0 to 120 h), probabilities were calculated for all or type (i.e., hospital ward) of patients. ER, emergency room.

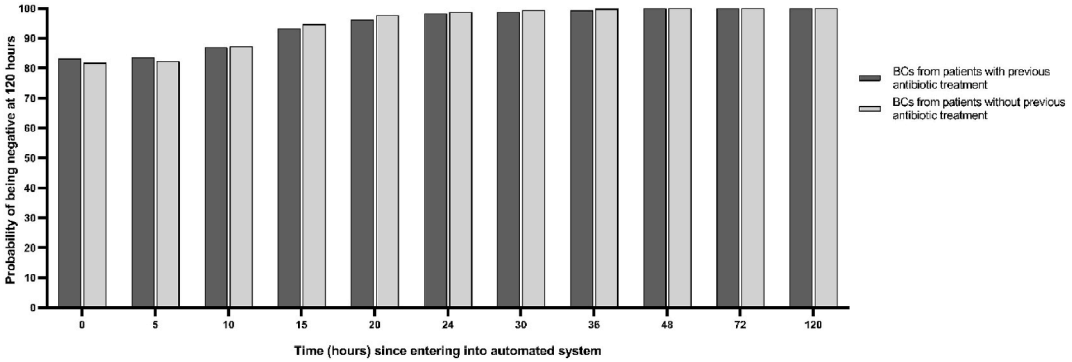

Fig. 2. Probabilities of bottles being negative at 120 h as determined at sequential endpoints after BC collection. Values were stratified by whether BC bottles had been collected from patients with previous antibiotic treatment or not.

Table 2
Antibiotic administration in patients with BC-negative BSI episodes, as assessed for two selected time intervals, at a large hospital in Rome, Italy, June 2021–June 2022.

WHO-designed antibiotic groups	Days of antibiotics for patients (all; n = 1244), as administered:		Days of antibiotics for patients (without any infection; n = 843)[a], as administered:	
	From 0 to 120 h of BC incubation	From 48 to 120 h of BC incubation	From 0 to 120 h of BC incubation	From 48 to 120 h of BC incubation
Access	1732	1058	943	575
Watch	5243	3144	3624	2176
Reserve	953	570	634	375
All groups	7928	4772	5201	3126

[a] Of 1244 patients included in the analysis, 401 patients had at least one of documented infections in total (n = 444), of which lower respiratory tract (n = 129), gastrointestinal tract (n = 90), urinary tract (n = 86), skin and soft tissue (n = 48), bone and joint (n = 30), cardiovascular system (n = 28), central nervous system (n = 18), or surgical site (n = 15) infections, according to CDC infectious disease classification guidelines [16]. The remaining 843 patients had no documented infection(s).

to 4 days likely had minimal impact on patient care [18]. All but two positive BCs from COVID-19 patients were detected within 3 days of incubation, with one positive on day 4 for *Cutibacterium acnes* (a normal skin microbiota organism) and one positive on day 5 for *Candida albicans* (a slow growing organism). In that historical moment, according to Sepulveda et al. [18], a 4-day incubation would have allowed to preserve the functionality while enhancing the diagnostic capacity of the clinical microbiology laboratory. Was that endpoint too conservative? Or is our proposed 48-h endpoint safe enough?

To answer the question, we first excluded from the analysis all follow-up BCs (i.e., performed to monitor the efficacy of antimicrobial treatment in patients with previously documented BSI). It is known that growth of BSI pathogens can be delayed (usually, >48 h) in BC obtained after the initial (first) BC from patients, possibly due to partial (not yet complete) efficacy of administered antimicrobials. Second, we included BSI episodes from (adult) patients with at least 4 bottles (i.e., 2 sets) collected. This allowed us to reach (in 3702 episodes) or even overcome (in 609 episodes) the acceptance limit (i.e., 4 bottles, if properly filled) currently recommended by BSI diagnostic standard procedures [1] and to minimize the risks of false negative or late positive episodes. Third, the days between 48 h (in our study, TTP was ~32 h at most) and 5 days (i.e., the current endpoint for a negative BC result) could have been antibiotic-free days if antibiotics had not been administered for a documented clinical syndrome (i.e., pneumonia) other than BSI. Thus, excluding patients who continued antibiotic therapy for 5 days regardless of BC results (for example, ~30% received targeted antibiotics for a lower respiratory tract infection) resulted in a patient population of ~70% that might have merited the early discontinuation of antibiotics.

Substantially, apart from 401 episodes where indisputable (clinical, laboratory, or radiological) evidence made antibiotic treatments necessary, 843 episodes could have been considered and, therefore, managed by physicians as negative at the 48-h BC incubation endpoint. We found that in these episodes, patients were treated with antibiotics and that, not negligibly, these antibiotics belonged to the WHO-defined reserve group, which includes ceftazidime-avibactam, colistin, fosfomycin, linezolid, and polymyxin B. Ideally, the AWaRe classification provides the basis for antibiotic stewardship tools aimed at reducing antimicrobial resistance globally. Thus, the 48-h endpoint may be a valuable starting point towards increasingly responsible use of antibiotics in daily clinical practice. The magnitude of our proposal may be larger in other world regions or clinical contexts. For example, in USA, the proportion of patients who are on antibiotics on day 1 of BC incubation should be much higher due to SEP-1 bundle (a national quality measure on sepsis), based on which administering immediate (within 3 h) antibiotics to patients with signs and symptoms of sepsis is mandatory [19].

While algorithms have been proposed to guide BC ordering in nonneutropenic adult patients, more patients with no antibiotic

exposure within 72 h of BC collection are likely to have a positive result for BC [8]. In our study, previous antibiotic treatments (i.e., within 48 h prior to BC collection) did not increase the likelihood that BC bottles were negative at 120 h compared with BC bottles from untreated patients. This observation is not only consistent with the well-documented antibiotic neutralization efficiency of BC media [13,14], but also makes the number of episodes with negative BC bottles in our study highly plausible. If we look at the pediatric context [20], where TTP for BC can be < 24 h [11], we are too cautious in stating that empirical antibiotics in BC-negative pediatric patients should not be administered for more than 48 h. Aside from the emphasis on clinical and therapeutic implications, shortening the BC incubation period would imply potential resource savings for the clinical microbiology laboratory. This idea was pioneered in two companion studies (published about two decades ago) by Bourbeau et al. [21,22], where older (less efficient) versions of the BC media used in our study allowed to detect 97.5% of all clinically relevant bacterial and fungal isolates in the first 3 days of incubation. At that time, the authors were refrained to shorten the 5-day BC incubation period partly due to regulatory requirements and partly due to an instrumentation capable of holding bottles for 5 days. About twenty years later, the COVID-19 pandemic led Sepulveda et al. [18] to mitigate the overutilization of BCs through a 4-day incubation. Similarly, the capacity of the BC instrument(s) in our laboratory could be increased by stopping the incubation of the BC bottles for all patient situations judged no longer deserving of antimicrobial treatment.

Agreeing with Sepulveda et al. [18], changing the time for incubation of routine BCs may have scarce effect on the likelihood of documenting clinically relevant bacteremia or candidemia. A closer examination of the BSI pathogens identified in our study showed that bacterial species known not to grow too rapidly (e.g., the anaerobe *Eggerthella lenta*) or all but one *Candida* species grew within 48 h of BC incubation. However, we acknowledge that *Brucella* or other (uncommon) fastidious microbes would not be detected in the BC by even a 5-day incubation or would require the use of an alternative BC method [22]. This suggests that BC ordering should be motivated by a well-founded or, when possible, pathogen-specific suspicion of BSI, and that collecting more than 2 sets of BCs should be considered whenever an infective endocarditis is suspected [8].

This study has some limitations because we focused on non-ICU, non-hematology patients. So, our findings could not reflect more challenging scenarios (e.g., involving critically ill patients or neutropenic patients), where a range of clinical, laboratory, and radiological criteria are used to guide antimicrobial therapy [23]. We were unable to collect and, consequently, analyze demographic (age, gender, etc.) or clinical data, such as major comorbidities (diabetes, end-stage renal disease, cirrhosis, cancer, solid organ transplant, etc.) or genetic factors, which are known to influence antimicrobial treatment decisions. This analysis could have strengthened the significance of our findings. When analyzing retrospectively a large data sample, we were unable to control the BC pre-analytical step parameters (e.g., bottle filling) [1] that could have influenced the results for all BC bottles (and relative BSI episodes) included in the study. However, to overcome potential biases, we excluded episodes with less than 2 sets of bottles collected (obviously only for adult patients), as well as we included 19 (BC-positive) episodes for patients with first (apparently false) negative BC bottles. It is plausible that, in these patients, some problem arose during BC collection, probably affecting the volume of blood inoculated per bottle [1], or that bacteremia was apparent in a new (BC-positive) episode occurring shortly after the first (BC-negative) episode. Finally, our analysis was based on the BC results obtained with the use of last-generation BC bottles (bioMérieux BacT/Alert FA PLUS [aerobic], BacT/Alert FN PLUS [anaerobic], and BacT/Alert Pediatric FAN [pediatric]) and their incubation in a last-generation instrument (bioMérieux BacT/Alert VIRTUO), which have been adopted in our laboratory since 2019 [13,14] but cannot be part of the equipment of other clinical microbiology laboratories in the world.

5. Conclusion

Our findings suggest that the 48-h endpoint may be an early indicator of BC negativity and therefore a target for future antimicrobial stewardship interventions. The adoption of this endpoint could be particularly important in patients without documentation of clinical, laboratory, or radiological features of a localized infection and those with a low clinical suspicion of infective endocarditis or candidemia. However, caution is necessary in all situations where BSI is highly suspected as well as imperative is the clinical judgment, which also depends on knowing the prevalence of slow-growing pathogens in the hospital. Further studies, possibly involving all seriously ill patients, are needed to strengthen the value of our findings.

Ethical approval statement

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Fondazione Policlinico Universitario A. Gemelli IRCCS (approval code, 39616/2022).

Data availability statement

Data may be available upon reasonable request.

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CRediT authorship contribution statement

Giulia Menchinelli: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Alice Oliveti:** Writing – original draft, Resources, Investigation, Conceptualization. **Barbara Fiori:** Writing – original draft, Investigation. **Tiziana D'Inzeo:** Writing – original draft, Investigation. **Teresa Spanu:** Writing – review & editing. **Rita Murri:** Writing – review & editing. **Massimo Fantoni:** Writing – review & editing. **Maurizio Sanguinetti:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Brunella Posteraro:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Giulia De Angelis:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Maurizio Sanguinetti reports financial support was provided by European Union.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27849.

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