Epidemiol. Infect. (2011), **139**, 772–776. © Cambridge University Press 2010
doi:10.1017/S095026881000169X

SHORT REPORT
Changes in blood culture methodology have an impact on time trends of bacteraemia: a 26-year regional study

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(*Accepted 15 June 2010; first published online 12 July 2010*)

SUMMARY

The incidence of bacteraemia has increased considerably during the last decades. This may be related to population ageing, increased use of invasive procedures, and increased ascertainment of bacteraemia. Generalized additive and generalized linear models were used to analyse the impact of four successive improvements in blood culture methodology on the recovery of prevalent blood culture isolates while simultaneously controlling for underlying time trend and seasonal variation. Between 1981 and 2006, 20 091 bacteraemias comprising 22 800 blood culture isolates were diagnosed. The changes in methodology increased the recovery of some bacterial groups; the greatest impact was observed for Enterobacteriaceae, pneumococci and *Staphylococcus aureus* whereas recovery of β-haemolytic streptococci, *Pseudomonas aeruginosa*, other Gram-negative aerobes, and fungi was not affected. Changes in blood culture methodology should be taken into account when assessing time trends of bacteraemia.

Key words: Bacteraemia, blood culture, epidemiology, fungaemia, modelling, time trend.

Bacteraemia and fungaemia are severe infections with 30-day mortality ranging from 20% to 40%. Currently, population-based bacteraemia incidence rates average 150/100 000 person-years and have increased notably during the last decades [1, 2]. This increase may relate to demographic changes, e.g. an ageing population and increasing longevity of patients with chronic diseases. At the same time, patients are submitted to more invasive and immunomodulatory procedures and the diagnosis of bacteraemia has become more sensitive through the development of automated blood culture systems and refinements of blood culture media. It is uncertain to which extent each of these changes has affected long-term trends. To our knowledge, no previous studies have addressed the impact of consecutive improvements of blood culture methods *per se* on long-term trends of bacteraemia and fungaemia. We therefore conducted this study to analyse the impact of four changes in blood culture methods on the recovery of prevalent pathogens.

The study was conducted in the North Denmark region between 1 January 1981 and 31 December 2006 (population: 1981, 485 501; 2006, 495 090). All residents had access to tax-supported healthcare from general practitioners and public hospitals (10 hospitals in 1981, seven in 2006). The Department of Clinical Microbiology, Aalborg Hospital provided

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Table 1. *Blood culture methods in the North Denmark region between 1981 and 2006*

	1981–1991	1992–1996	1996–1998	1999–2003	2004–2006
Blood culture system	12-tube system	Colorbact	BacT/Alert	BacT/Alert	BacT/Alert
Period of incubation	7 days	7 days	6·7 days	6·7 days	6·7 days
Representation of change in the statistical model	–	TECH92	TECH96	TECH99	TECH04
No. of 'units' per blood culture	2 vacuum tubes	3 bottles	3 bottles	3 bottles	3 bottles
Aerobic bottles	–	2	2 standard	1 standard + 1 FAN	1 SA + 1 FA (non-vented)
Anaerobic bottle	–	1	1 standard	1 standard	1 SN
Nominal volume of blood (adults)	16–18 ml	20–22 ml	28–32 ml	28–32 ml	28–32 ml
Paediatric bottle	No	No	Yes	Yes	Yes

FA, FAN media; SA, standard aerobic; SN, standard anaerobic.

diagnostic services, including blood cultures, to all public hospitals in the region. We retrieved information on the annual numbers of blood cultures processed during 1981–2006 from annual statistical reports (1981–1994) and from the electronic laboratory information system (ADBakt, Sweden) (1995–2006); during the 26-year study period a total of 253 000 blood cultures were obtained based on the attending physician's clinical assessment (annual numbers increased from 3327 in 1981 to 17 047 in 2006).

During the study period, three different blood culture systems were used (Table 1). Until 1991, blood samples were drawn into Vacutainer® tubes (Becton Dickinson, USA), transported to the laboratory, and inoculated into a set of 12 tubes of bacteriological media (four with nutrient broth, four with semi-solid nutrient agar, and four with semi-solid thioglycollate agar) [1]. During 1991, this 12-tube system was gradually replaced by a broth culture system (Colorbact®, Copenhagen) inoculated at bedside [3]. The BacT/Alert system (bioMérieux, France) has been used since 1996 [4] and its use has been modified twice; in 1999 by introduction of aerobic fastidious antimicrobial neutralization (FAN) bottles [5], and in 2004, the glass bottles were replaced by polycarbonate bottles that do not require manual venting before incubation in order to obtain aerobic growth conditions [6]. Growth was detected by visual inspection in the 12-tube system and the Colorbact system; in the BacT/Alert system growth readings were performed automatically. Positive blood cultures were subcultured and identified by a combination of conventional and commercial methods [7].

All bacteraemias and fungaemias in the region are registered in the North Denmark Region Bacteraemia Research Database [8]. Data covering 1981–1991 was retrieved from archived blood culture reports, but since 1992 the information has been recorded concurrently with the clinical episode. In patients with multiple positive blood cultures, one or more of the following criteria were used to distinguish a new episode: (1) a blood culture isolate that differs from the previous with regard to species and/or antibiogram, (2) a different focus of infection, or (3) an interval of at least 30 days between two positive blood cultures [8, 9]. Coagulase-negative staphylococci, *Bacillus* spp., *Corynebacterium* spp., and *Propionibacterium acnes* were regarded as contaminants unless isolated from two or more separate blood cultures [10]. Polymicrobial bacteraemia was defined as an episode with more than one clinically important blood culture isolate detected within 48 h.

For the statistical analysis, the monthly number of blood culture isolates was used as the analytical unit; during the 26-year study period there were $26 \times 12 = 312$ count observations for each genus or group ('X'). The recovery of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* was analysed separately. The remaining isolates were grouped as β-haemolytic streptococci (including Lancefield groups A, B, C, G), enterococci, miscellaneous Gram-positives (mostly coagulase-negative staphylococci, non-haemolytic streptococci and Gram-positive rods), Enterobacteriaceae other than *E. coli*, aerobic Gram-negatives other than *P. aeruginosa*, anaerobic bacteria, and fungi. First, we

https://doi.org/10.1017/S095026881000169X Published online by Cambridge University Press

Table 2. *The impact of four successive changes in blood culture methodology on the recovery of prevalent species and groups assessed by generalized additive and generalized linear models*

	Change (%) in the general trend (95% confidence interval)			
	Change to bottle system (TECH92)	Change to BacT/Alert (TECH96)	Advent of FAN medium (TECH99)	Non-venting of aerobic bottles (TECH04)
S. aureus	↑ 33·1 (10·9–59·6)	*	*	*
β-haemolytic streptococci	*	*	*	*
Pneumococci	↑ 46·5 (2·8–108·8)	*	*	↑ 40·2 (6·8–84·1)
Enterococci	*	↑ 60·1 (20·6–112·6)	*	*
Miscellaneous Gram-positive aerobes	↑ 103·2 (62·7–153·9)	*	*	*
E. coli	↑ 50·5 (28·6–76·1)	↑ 24·6 (9·3–42·1)	↑ 27·1 (12·6–43·5)	*
Other Enterobacteriaceae	↑ 43·2 (14·7–78·7)	*	↑ 29·7 (8·3–55·3)	*
P. aeruginosa	*	*	*	*
Miscellaneous Gram-negative aerobes	*	*	*	*
Anaerobes	↑ 153·4 (83·0–250·8)	*	*	*
Fungi	*	*	*	*

* Not included in the model because the change was not statistically significant.

examined the temporal trend of the count data graphically and used moving averages of order 13 to smooth seasonal variation. Second, to study the effect of changes in blood culture methods on the long-term trends of bacteraemia, a generalized additive (GA) model was used with a maximum of 21 degrees of freedom to model smooth time relationships [11]. Each change in blood culture method was defined as an independent variable, TECH (Table 1). TECH92 was 0 until 31 December 1991 and 1 after 31 August 1992; during the 8-month period from December to August TECH92 increased linearly from 0 to 1 in order to reflect the gradual shift from the 12-tube system to the Colorbact system. TECH96 was 0 until 31 December 1995 and 1 thereafter, TECH99 was 0 until 31 December 1998 and 1 thereafter, and TECH04 was 0 until 31 December 2003 and 1 thereafter.

It was assumed that the count at time t (X_t) in each genus or group followed a Poisson distribution with mean μ_t, where

$$\log(\mu) = s(\text{time}) + \alpha_{92}\text{TECH92} + \alpha_{96}\text{TECH96} + \alpha_{99}\text{TECH99} + \alpha_{04}\text{TECH04},$$

TECH variables were deleted stepwise if not statistically significant ($P<0{\cdot}05$). The fit of each GA model was evaluated on the basis of the unbiased risk estimator score, R^2 and the estimated degree of freedom of the general trend [11]. If an appropriate GA model was not found, we fitted a generalized linear (GL) model with a polynomial trend over time. From the model fitted, we estimated the increase in bacteraemias with 95% confidence intervals (CI) associated with each change in blood culture method that was statistically significant ($P<0{\cdot}05$). Statistical analyses were performed using R statistical software (R Foundation for Statistical Computing, Austria).

Between 1981 and 2006, a total of 20 091 bacteraemias and fungaemias was diagnosed, of which 18 034 (89·8%) episodes were monomicrobial and 2057 (10·2%) were polymicrobial. The total number of isolated pathogens was 22 800 with *E. coli* being the most prevalent (7064, 31·0%) followed by *S. aureus* (3229, 14·2%) and pneumococci (1948, 8·5%). In the groups, Enterobacteriaceae other than *E. coli* were most prevalent (3872, 17·0%) followed by the miscellaneous Gram-positives (2081, 9·1%) and anaerobic bacteria (1172, 5·1%). Table 2 summarizes the statistically significant changes associated with the changes in blood culture methodology. First, replacement of the 12-tube blood culture system with the Colorbact system (TECH92) was associated with increased numbers of bacteraemias caused by six of the 11 species or groups studied. The largest increase was seen for anaerobic bacteria (153·4%, 95% CI 83·0–250·8) and the miscellaneous Gram-positives (103·2%, 95% CI 62·7–153·9). Introduction of the BacT/Alert broth culture system (TECH96) further

https://doi.org/10.1017/S095026881000169X Published online by Cambridge University Press

augmented the numbers of *E. coli* and was associated with an increased number of enterococcal bacteraemias. Similarly, introduction of the aerobic FAN bottle (TECH99) increased the yield of all Enterobacteriaceae and finally, the non-vented plastic bottles (TECH04) increased detection of pneumococci.

Our analyses have shown that the successive improvements of blood culture techniques were associated with improved sensitivity for detection of bacteraemia. However, this impact was differential in the species and pathogen groups. The changes had the greatest impact on *E. coli*, Enterobacteriaceae other than *E. coli*, pneumococci and *S. aureus*, whereas the recovery of β-haemolytic streptococci, *P. aeruginosa*, other Gram-negative aerobes, and fungi was not materially affected by any of the changes.

Introduction of a broth culture system in 1992 had the greatest effect both in terms of microbial spectrum and absolute increase. This is probably due to the larger volume of blood obtained per blood culture; several studies have reported a relationship between the volume of blood cultured and the diagnostic yield of the culture [12, 13]. Additionally, the increased recovery of anaerobic bacteraemias suggests that the new anaerobic broth medium was superior to the thioglycollate medium used previously. We have no explanation for the increase in enterococcal bacteraemia associated with the introduction of the BacT/Alert system in 1996, and we cannot preclude that this observation occurred by chance. Replacement of one of two standard aerobic bottles with a FAN bottle increased detection of all Enterobacteriaceae. The FAN bottle was designed to enhance the recovery of fastidious microorganisms from blood and to improve the detection of bacteraemia and fungaemia in patients receiving antimicrobial agents [5]. Finally, the improved detection of pneumococcal bacteraemia associated with the non-vented plastic bottles was unexpected, but may be explained by the larger headspace in these bottles that increases the absolute volume of oxygen [6].

Because we used GA and GL models to fit smooth terms for the trend over time, we could assess the impact of changes in blood culture methodology while simultaneously controlling for underlying time trend and associated seasonal variation. Moreover, the study's longitudinal population-based design, based on data from the bacteraemia database, enabled us to identify all patients diagnosed with bacteraemia and fungaemia during a 26-year period, and limited the risk of referral and diagnostic bias. The stringent definitions of bacteraemia and fungaemia in the database reduced the risk of repeatedly positive blood cultures which are likely to occur with some pathogens, e.g. *S. aureus*.

Limitations to our study include the fact that we treated each blood culture isolate as an independent observation although 11% of the episodes were polymicrobial. Our baseline assumption was that each change in blood culture methodology was likely to improve detection of both monomicrobial and polymicrobial cultures. Had the analysis been restricted to monomicrobial bacteraemias, this could have reduced numbers of foremost enterobacteria, enterococci, anaerobes, and *Candida* spp. Additionally, the observed changes may not only relate to the shift in blood culture methodology in itself as all hospital wards were notified about changing procedures. This can have increased the awareness of the utility of blood cultures and the physicians may have lowered their threshold for ordering cultures. Similarly, in 1992 when trained phlebotomists took over the sampling of blood from junior physicians, the annual number of blood cultures increased by about 30% in subsequent years. Finally, besides the major changes in methodology reported in this study, minor formula modifications of the blood culture media could have occurred.

In conclusion, we have demonstrated that improvements of blood culture methods contributed to the increased frequency of some bacterial groups. Thus, future long-term studies of bacteraemia and fungaemia should take note of the blood culture methods used, especially to changes which can have a differential and profound impact on particular pathogen groups.

ACKNOWLEDGEMENTS

Malene C. Engebjerg was supported by a grant from the Graduate School in Biostatistics, Copenhagen University, Denmark.

DECLARATION OF INTEREST

None.

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