PLoS one

Rapid Discrimination of *Salmonella enterica* Serovar Typhi from Other Serovars by MALDI-TOF Mass Spectrometry

Martin Kuhns[1,9], Andreas E. Zautner[1,9], Wolfgang Rabsch[2], Ortrud Zimmermann[1], Michael Weig[1], Oliver Bader[1*¶], Uwe Groß[1¶]

1 Institute for Medical Microbiology and International Health Network Göttingen, University Medical Center Göttingen, Göttingen, Germany, 2 German National Reference Center for Salmonella and other Enteric Pathogens, Robert Koch Institute, Wernigerode, Germany

Abstract

Systemic infections caused by *Salmonella enterica* are an ongoing public health problem especially in Sub-Saharan Africa. Essentially typhoid fever is associated with high mortality particularly because of the increasing prevalence of multidrug-resistant strains. Thus, a rapid blood-culture based bacterial species diagnosis including an immediate sub-differentiation of the various serovars is mandatory. At present, MALDI-TOF based intact cell mass spectrometry (ICMS) advances to a widely used routine identification tool for bacteria and fungi. In this study, we investigated the appropriateness of ICMS to identify pathogenic bacteria derived from Sub-Saharan Africa and tested the potential of this technology to discriminate *S. enterica* subsp. *enterica* serovar Typhi (*S.* Typhi) from other serovars. Among blood culture isolates obtained from a study population suffering from febrile illness in Ghana, no major misidentifications were observed for the species identification process, but serovars of *Salmonella enterica* could not be distinguished using the commercially available Biotyper database. However, a detailed analysis of the mass spectra revealed several serovar-specific biomarker ions, allowing the discrimination of *S.* Typhi from others. In conclusion, ICMS is able to identify isolates from a sub-Saharan context and may facilitate the rapid discrimination of the clinically and epidemiologically important serovar *S.* Typhi and other non-*S.* Typhi serovars in future implementations.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: obader@medizin.uni-goettingen.de

9 These authors contributed equally to this work.

¶ These authors also contributed equally to this work.

Introduction

Fever is a leading cause for hospital admission in Sub-Saharan Africa. Often malaria is thought to be the underlying disease; however bacterial blood stream infections (BSI) contribute to a significant proportion of febrile illness [1,2,3,4]. Bacterial BSI is an important cause of morbidity and mortality [5], and in case of septic shock mortality can be as high as 60% [6]. It was well demonstrated that time between onset of septic shock and start of adequate therapy is essential for survival [7]. However, as microbiological diagnostics is often not available in African countries due to infrastructure, budget and personnel constraints, clinicians have to rely on syndrome-based empirical approaches to treat febrile illness [8,9]. Consequently, fever in Africa is often treated sequentially: first with anti-malarial drugs and then with antibiotics, risking poor clinical outcome and development of resistance [10,11].

Whereas *Staphylococcus aureus* and *Escherichia coli* continue to be the most common causative agents of true BSIs in developed countries [12], Gram-negative bacteria, in particular *Salmonella enterica*, are the main cause of BSI in African countries [3,13,14].

Both, *S. enterica* serovar Typhi (*S.* Typhi) and non-typhoid *Salmonella* are frequently isolated from blood cultures in African countries [15] and typhoid fever, caused by *S.* Typhi, is estimated to annually cause about 21 million cases and approximately 217.000 deaths [16]. Varied manifestations of typhoid fever are observed especially in pediatric patients, including septicemia, diarrhea and lower respiratory tract infections [17]. In the sub-Saharan regions of Africa multidrug-resistant typhoid fever (MDRTF) is becoming a serious problem [17]. Since the 1980s repeated outbreaks with MDRTF associated with increased morbidity and mortality have been reported, particularly in malnourished children and children below an age of five years [17]. The MDRTF rate in Kenya increased from 5% to 77.2% within 1988–2008 [18,19] and is similar in Nigeria 61% [20] and Ghana 63% [21].

The laboratory diagnosis of *S. enterica* relies on bacterial culture using different selective media [22]. As most *S. enterica* strains produce hydrogen sulfide with exception of *S.* Paratyphi A and some *S.* Typhi strains, they can generally be distinguished from other *Enterobacteriaceae* using thiosulfate containing agar (10). However, some species like *Citrobacter freundii* are also able to

produce hydrogen sulfide and differentiation between *S. enterica* and *C. freundii* can therefore be challenging. Consequently, various other chromogenic media have been developed to discriminate between *C. freundii* and *S. enterica* [22,23,24]. For further subtyping of *S. enterica* the White-Kauffmann-Le Minor classification scheme [25] or phage typing [26] are in use, however the latter technologies are not available in most laboratories.

Currently, intact cell mass spectrometry (ICMS) advances to a widely used routine identification tool for bacteria and fungi [27,28]. Here, mass spectra from whole bacterial or fungal cell lysates are used for identification [27]. This method was previously shown to identify Salmonellae at the species and subspecies level [29]. Additionally, it was shown that serovar-

specific biomarker ions can be found in ICMS spectra allowing the distinction of *S.* Enteritidis, *S.* Typhimurium/4, 5, 12:i:-, *S.* Virchow, *S.* Infantis, *S.* Hadar, *S.* Choleraesuis, *S.* Heidelberg, and *S.* Gallinarum. However, the clinically most important serovar *S.* Typhi was not included in those particular analyses [29,30].

In this study, we used blood culture isolates taken from a study population suffering from febrile illness in Ghana [3] and additional *S. enterica* reference strains to investigate the suitability of ICMS to (i) identify pathogenic bacteria derived from sub-Saharan Africa (spectrum databases were generated with isolates originating mainly in the Western World) and (ii) test the

Table 1. Concordance of species identification by conventional and ICMS methods.

Species	N°	conventional ID	ICMS – ID
Enterobacteriacae			
Citrobacter freundii complex	3	ok	ok
Enterobacter cloacae complex	9	ok	ok
Escherichia coli	10	ok	ok
Klebsiella oxytoca	1	ok	ok
Klebsiella pneumoniae	6	ok	ok
Klebsiella pneumoniae [a]	1	Enterobacter spec.	ok
Klebsiella variicola [a]	1	Pantoea agglomerans	ok
Proteus mirabilis	3	ok	ok
Proteus vulgaris	1	ok	ok
Providencia rettgeri	1	ok	ok
Salmonella ssp.	160	ok	ok
Shigella flexneri [a]	1	ok	Escherichia coli
gram+ cocci			
Enterococcus casseliflavus	1	ok	ok
Enterococcus faecalis	3	ok	ok
Lactococcus lactis [a]	1	Enterococcus faecium	ok
Staphylococcus aureus	26	ok	ok
non-fermenter			
Achromobacter xylosoxidans	1	ok	ok
Acinetobacter baumanii complex	8	ok	ok
Acinetobacter junii	1	ok	ok
Acinetobacter junii [a]	1	Acinetobacter haemolyticus	ok
Acinetobacter junii [a]	1	Acinetobacter lwofii	ok
Chryseobacterium gleum [a]	1	Chrysobacterium indologenes	Chryseobacterium indologenes
Comamonas aquatica [a]	1	Achromobacter xylosoxidans	Comamonas testosterone
Pseudomonas aeruginosa	17	ok	ok
Pseudomonas putida complex	1	ok	ok
Psychrobacter pulmonis [a]	1	Pseudomonas stutzeri	Psychrobacter ssp.
Stenotrophomonas maltophilia	2	ok	ok
gram+ rods			
Brevibacterium casei [a]	2	Rhodococcus equi	ok
Microbacterium arborescens [a]	1	Corynebacterium ssp.	ok
Paenibacillus spp. [a]	1	no reliable identification	no reliable identification
Rhodococcus pyridinivorans [a]	1	Corynebacterium ssp.	Rhodoccus rhodochrous
Sinomonas flava [a]	1	Brevibacterium ssp.	Sinomonas atrocyanea

[a]In the case of absence or discordance of identifications by conventional and ICMS, the correct species was identified by sequencing of the 16S rDNA locus.
doi:10.1371/journal.pone.0040004.t001

potential of this technology to discriminate *S.* Typhi from other serovars.

Materials and Methods

Strains

Isolates used for our analyses were taken from a previous epidemiologic study done in three independent locations in Ghana [3]. In that study, isolates were obtained from blood cultures of patients with fever of unknown origin and differentiated by conventional means (microscopy, API systems, agglutination with antisera using the White-Kauffmann-LeMinor scheme). Further *Salmonella S.* Typhi subtyping was done with the Vi phage typing scheme [31]. To exclude a bias towards potential clonal outbreaks in the Ghanaian study centers, we included 44 additional pseudonymized isolates obtained and archived during routine diagnostic procedures in Göttingen or the Salmonella Reference Center.

In total, our set contained 160 *Salmonella enterica* subsp. *enterica* isolates of 12 different serovars (84x *S.* Typhi, 51x *S.* Typhimurium, 14x *S.* Enteritidis, 2x *S.* Typhimurium var. Copenhagen 2x *S.* Paratyphi, one each of serovars Albany, Brandenburg, Infantis, Hadar, Tennessee, and two not further characterized non-*S.* Typhi serovars) as well as other species present in the blood cultures (Table 1), as described previously [3].

Intact Cell Mass Spectroscopy

Cells were grown over night on sheep blood agar (Oxoid, Wesel, Germany) at 37°C under safety conditions as required, prepared in duplicate for ICMS by smear preparation and overlaid with HCCA matrix, both under a safety cabinet and after drying transported to the MALDI device. ICMS was done by standard procedures recommended for the BioTyper 3.0 system (Bruker Daltonics, Bremen, Germany). For analysis, 600 spectra from 2–20 kDa were gathered in 100-shots steps. Results with score values >2.000 were considered correct. Analyses for isolates not yielding a significant score were repeated once by smear preparation and in the case of 22 (all non-Salmonella) isolates subsequently by formic acid-acetonitrile extraction. All ICMS identification experiments were done in a blinded form. In 15 cases 16 S rDNA sequencing was used as a tie-breaker for discordant or unclear results.

At the time of investigation, the Biotyper 3.0 and SR databases together contained 29 spectra from the genus *Salmonella* (*S.* Typhi (10), *S.* Paratyphi (3), S. *enterica* subsp. *Arizonae* (2), and one spectrum each of *S.* Bongori, *S.* Anatum, *S.* Choleraesuis, *S. enterica* subspec. *diarizonae*, *S.* Dublin, *S.* Enteritidis, *S.* Gallinarum, *S.*

Figure 1. Value of BioTyper hit scores for *S.* Typhi identification. Negative values indicated higher false hit scores, positive values indicated higher correct hit scores. A value near zero indicated a similar score distribution between correct and false hits.
doi:10.1371/journal.pone.0040004.g001

Hadar, *S. enterica* subspec. *houtenae*, *S. enterica* subspec. *indica*, *S. enterica* subspec. *salamae*, *S.* Stanley, *S.* Typhimurium, and one untyped). For the global identification procedure all of these were counted as "*Salmonella enterica* spp.". Phyloproteomic analyses were done using Flexanalysis and PCA algorithms implemented into the BioTyper 3.0 software (both Bruker Daltonics). Spectra were pre-processed by baseline subtraction and smoothing, for PCA-based hierarchical clustering distance measurement was set to 'correlation'; the linkage algorithm to 'average'.

Results

Classification Results

A total of 225 blood culture isolates plus 44 *S. enterica* control strains were re-typed using ICMS (Table 1). No major misidentifications were observed. Where discordant results were obtained, ICMS identifications were either correct or at least phylogenetically closer to the species eventually identified by 16 S rDNA sequencing, with the exception of one *Shigella flexneri* isolate. Discrimination of *S. enterica* spp. from other Enterobacteriaceae including *Citrobacter* spp. (as well as other genera) was 100%.

To analyze the usability of Biotyper score values for the discrimination of *S.* Typhi from other *Salmonella* serovars, score values for all *Salmonella* isolates were obtained for all *S. enterica* spp. spectra contained in the database and a "delta mean score" (geometric mean [correct hits] - geometric mean [false hits]) calculated (Figure 1). Due to the lack of multiple spectra for each of the different serovars in the database, all non-*S.* Typhi isolates were considered as one group and all *S.* Typhi isolates as the other. This analysis showed that spectra from *S.* Typhi isolates did not reproducibly give higher score values with *S.* Typhi database entries. Although a certain number of spectra, for which most high ranking hits were correct, were observed, false hits were always present with scores >2.000. Similarly, non-*S.* Typhi isolates also produced high score values with *S.* Typhi database entries.

### Phyloproteomic Analysis of *Salmonella* Isolates

To further determine whether the different *Salmonella* serovars can be differentiated by their ICMS-spectra, the spectra were clustered and the phyloproteomic nearness was analyzed. Surprisingly, the three major *Salmonella* serovars tested (Typhi, Enteritidis and Typhimurium) clustered into several well-separated groups (Figure 2A). With only five outliers (5.9%), *S.* Typhi isolates fell into only two distinct sets (Figure 2B, clusters 1b and 2b). This clustering was independent of the isolate origin, indicating that this nearness did not reflect a clonal outbreak (Figure 2B). A correlation of the *S.* Typhi clusters with the Vi phage type was not observed; however this may have been missed as the vast majority of the isolates (75%) were of phage type D1 (data not shown). In an overlay of spectra from the four major clusters at least three major and several smaller peaks can be identified, which separate *S.* Typhi isolates from other serovars (Figure 2C). These differences in biomarker ions separating *S.* Typhi from other serovars were present independently of the cluster the spectrum was contained in.

Discussion

Today, the laboratory diagnosis of *S.* Typhi is predominantly based on the White-Kauffmann-Le Minor classification scheme [25] or phage typing [31] following bacterial culture. Although there are several approaches to substitute bacterial culture and SV determination by PCR [32], these assays have a limited sensitivity
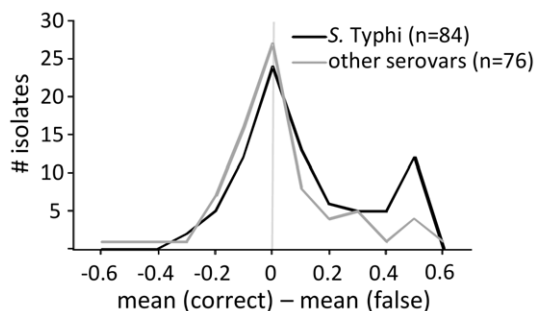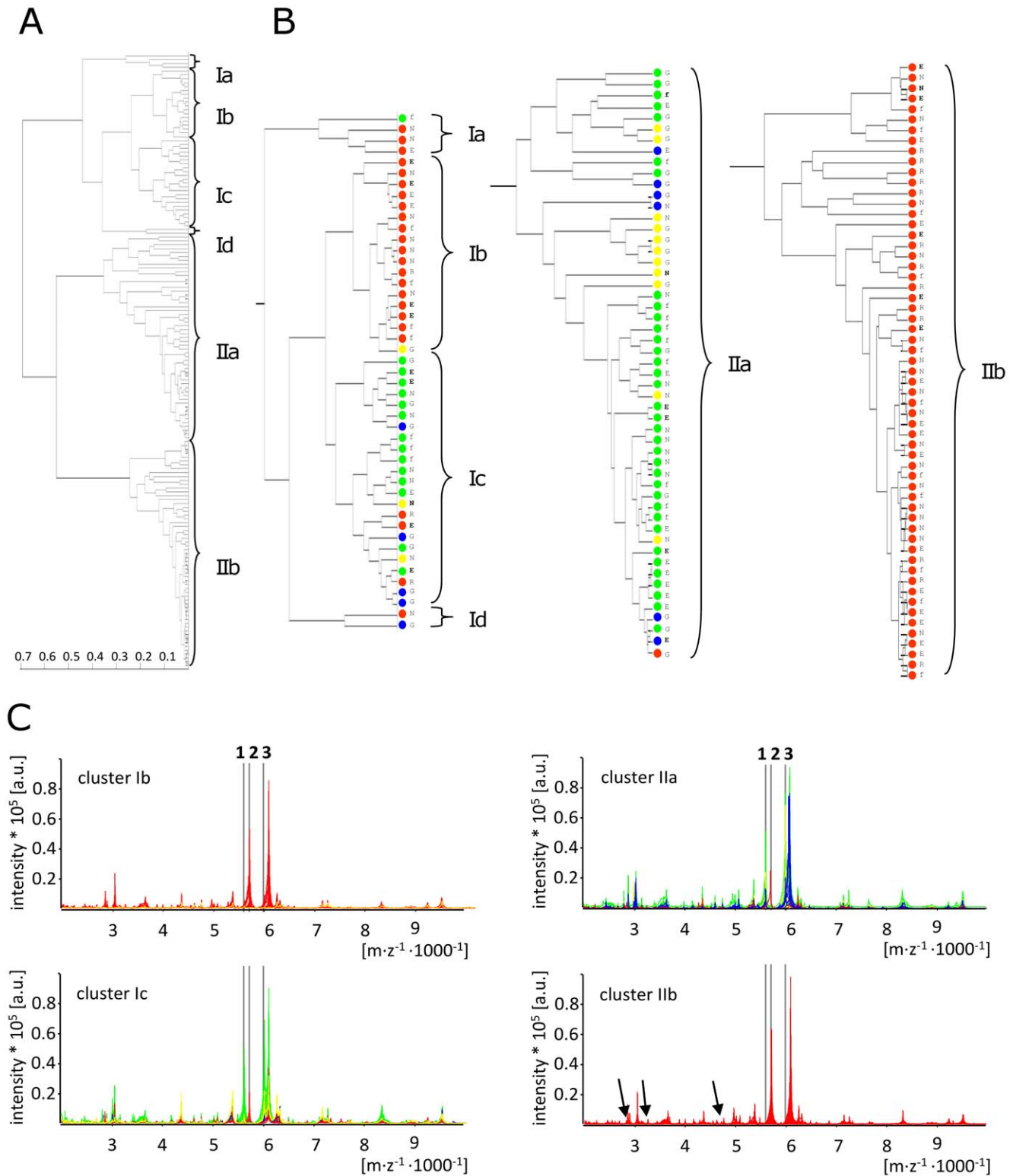
Figure 2. Relatedness of *Salmonella enterica* ICMS spectra reflects serotype. (A) Global cluster analysis of *S. enterica* isolates. (B) Enlargement of major clusters from (A). Serovars: *S.* Typhi (red), *S.* Typhimurium (green), *S.* Enteritidis (yellow), others (blue). Isolate sources: G:Göttingen; R:Salmonella Reference Center; E:Eikwe; N:Nkawkaw; f:Fosso. Isolation time points in Ghana (E, N, and f only): not bold = 2006; bold = 2009 (C) Overlay of ICMS spectra contained in the four major clusters identifies at least one major peak (peak 2; m/z = 5713.9) specific to *S.* Typhi (red) and two major peaks (peaks 1 and 3; m/z = 5616.7 and m/z = 6009.7 respectively) specific for non-*S.* Typhi isolates (green, yellow and blue). Several other small peaks specific for *S.* Typhi were also seen (three example peaks indicated in cluster IIb by arrows, m/z = 2856.4, m/z = 3258.0, and m/z = 4716.3, respectively).

doi:10.1371/journal.pone.0040004.g002

and offer no substitution for antibiotic susceptibility testing [33] making culture-based approaches still indispensable.

In this context, MALDI-TOF MS-based ICMS has recently advanced to a widely used routine species identification tool for cultured bacteria and fungi [27,28]. To analyze whether this method was also applicable to isolates from a sub-Saharan context, we retyped a previously established collection of Ghanaian blood culture isolates [3] by ICMS. This collection included a significant number of *S. enterica* isolates.

With the exception of one *Shigella flexneri* isolate, no clinically important errors were observed. Also, discrimination of *S. enterica* spp. from other Enterobacteriaceae was 100%. As demonstrated here and also by others [27,28] species identification from ICMS spectra is very robust and generally only dependant on the presence of the respective spectrum in the database. As shown here, it is applicable not only to isolates obtained in developed countries, but also to countries from sub-Saharan Africa.

In contrast to species identification, subtyping within a single species (or differentiation between extremely close related species) is a more subtle process. In our study this was demonstrated by the inability of the system to discriminate *E. coli* and *S. flexneri* or to type inside the genus *Salmonella*. This lack of implementation is also officially stated by the manufacturer. Nevertheless, previous phyloproteomic analyses have shown spectrum clusters of *S.* Typhi isolates among other Enterobacteriaceae [34] and several biomarker ions that differentiate non-*S.* Typhi isolates from each

other [30]. In our analysis, smear spectra obtained from *S.* Typhi isolates were of such difference from other serovars that they could be clustered into distinct sets. Furthermore, we were able to identify at least six biomarker ions that differentiate *S.* Typhi from non-*S.* Typhi spectra. Thus, we were able to discriminate *S.* Typhi from other *S. enterica* serovars using ICMS.

In conclusion, our study demonstrates that (i) ICMS-based species identification is applicable to isolates from sub-Saharan Africa and (ii) that it is possible to discriminate clinically important subtypes, such as the serovars inside the *S. enterica* subspecies even using smear spectra. This finding should be of special interest in areas where enteric bacteria, particularly *Salmonella enterica*, are highly prevalent as causative agent of BSI and other severe infections and together with new enrichment technologies [35], this should lead to significant speed increase in Salmonella diagnostics. Future research will therefore be directed to implement this in the respective commercial ICMS technologies using weighted pattern matching and specific reference spectra.

Author Contributions

Conceived and designed the experiments: OB UG. Performed the experiments: AEZ MK. Analyzed the data: MW OB. Contributed reagents/materials/analysis tools: WR OZ. Wrote the paper: MK AEZ OB.

References

1. Bahwere P, Levy J, Hennart P, Donnen P, Lomoyo W, et al. (2001) Community-acquired bacteremia among hospitalized children in rural central Africa. Int J Infect Dis 5: 180–188.

2. Berkley JA, Lowe BS, Mwangi I, Williams T, Bauni E, et al. (2005) Bacteremia among children admitted to a rural hospital in Kenya. N Engl J Med 352: 39–47.

3. Groß U, Amuzu SK, de Ciman R, Kassimova I, Groß L, et al. (2011) Bacteremia and antimicrobial drug resistance over time, Ghana. Emerg Infect Dis 17: 1879–1882.

4. Peters RPH, Zijlstra EE, Schijffelen MJ, Walsh AL, Joaki G, et al. (2004) A prospective study of bloodstream infections as cause of fever in Malawi: clinical predictors and implications for management. Trop Med Int Health 9: 928–934.

5. Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, et al. (1997) The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis 24: 584–602.

6. Brun-Buisson C, Doyon F, Carlet J, Dellamonica P, Gouin F, et al. (1995) Incidence, risk factors, and outcome of severe sepsis and septic shock in adults. A multicenter prospective study in intensive care units. French ICU Group for Severe Sepsis. JAMA 274: 968–974.

7. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, et al. (2006) Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 34: 1589–1596.

8. English M, Berkley J, Mwangi I, Mohammed S, Ahmed M, et al. (2003) Hypothetical performance of syndrome-based management of acute paediatric admissions of children aged more than 60 days in a Kenyan district hospital. Bull World Health Organ 81: 166–173.

9. Petti CA, Polage CR, Quinn TC, Ronald AR, Sande MA (2006) Laboratory medicine in Africa: a barrier to effective health care. Clin Infect Dis 42: 377–382.

10. Perkins BA, Zucker JR, Otieno J, Jafari HS, Paxton L, et al. (1997) Evaluation of an algorithm for integrated management of childhood illness in an area of Kenya with high malaria transmission. Bull World Health Organ 75 Suppl 1: 33–42.

11. Shears P (2001) Antibiotic resistance in the tropics. Epidemiology and surveillance of antimicrobial resistance in the tropics. Trans R Soc Trop Med Hyg 95: 127–130.

12. Pien BC, Sundaram P, Raoof N, Costa SF, Mirrett S, et al. (2010) The clinical and prognostic importance of positive blood cultures in adults. Am J Med 123: 819–828.

13. Archibald LK, Kazembe PN, Nwanyanwu O, Mwansambo C, Reller LB, et al. (2003) Epidemiology of bloodstream infections in a bacille Calmette-Guerin-vaccinated pediatric population in Malawi. J Infect Dis 188: 202–208.

14. Gordon MA, Walsh AL, Chaponda M, Soko D, Mbvwinji M, et al. (2001) Bacteraemia and mortality among adult medical admissions in Malawi–

predominance of non-Typhi Salmonellae and *Streptococcus pneumoniae*. J Infect 42: 44–49.

15. Reddy EA, Shaw AV, Crump JA (2010) Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. Lancet Infect Dis 10: 417–432.

16. Kothari A, Pruthi A, Chugh TD (2008) The burden of enteric fever. J Infect Dev Ctries 2: 253–259.

17. Zaki SA, Karande S (2011) Multidrug-resistant typhoid fever: a review. J Infect Dev Ctries 5: 324–337.

18. Kariuki S, Revathi G, Kiiru J, Mengo DM, Mwituria J, et al. (2010) Typhoid in Kenya is associated with a dominant multidrug-resistant *Salmonella enterica* serovar Typhi haplotype that is also widespread in Southeast Asia. J Clin Microbiol 48: 2171–2176.

19. Mengo DM, Kariuki S, Muigai A, Revathi G (2010) Trends in *Salmonella enterica* serovar Typhi in Nairobi, Kenya from 2004 to 2006. J Infect Dev Ctries 4: 393–396.

20. Akinyemi KO, Smith SI, Oyefolu AO, Coker AO (2005) Multidrug resistance in Salmonella enterica serovar Typhi isolated from patients with typhoid fever complications in Lagos, Nigeria. Public Health 119: 321–327.

21. Marks F, Adu-Sarkodie Y, Hunger F, Sarpong N, Ekuban S, et al. (2010) Typhoid fever among children, Ghana. Emerg Infect Dis 16: 1796–1797.

22. Cooke VM, Miles RJ, Price RG, Richardson AC (1999) A novel chromogenic ester agar medium for detection of *Salmonellae*. Appl Environ Microbiol 65: 807–812.

23. Browne NK, Huang Z, Dockrell M, Hashmi P, Price RG (2010) Evaluation of new chromogenic substrates for the detection of coliforms. J Appl Microbiol 108: 1828–1838.

24. Kodaka H, Mizuochi S, Honda T, Yamaguchi K (2000) Improvement of mannitol lysine crystal violet brilliant green agar for the selective isolation of H2S-positive *Salmonella*. J Food Prot 63: 1643–1647.

25. Guibourdenche M, Roggentin P, Mikoleit M, Fields PI, Bockemühl J, et al. (2010) Supplement 2003–2007 (No. 47) to the White-Kauffmann-Le Minor scheme. Res Microbiol 161: 26–29.

26. Rabsch W, Truepschuch S, D W, Gerlach RG (2011) Typing phages and prophages of Salmonella. In: Porwollik S, editor. Salmonella: From Genome to Function: Caister Academic Press. 25–48.

27. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, et al. (2009) Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 49: 543–551.

28. Bader O, Weig M, Taverne-Ghadwal L, Lugert R, Groß U, et al. (2011) Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Microbiol Infect 17: 1359–1365.

29. Dieckmann R, Helmuth R, Erhard M, Malorny B (2008) Rapid classification and identification of *Salmonellae* at the species and subspecies levels by whole-cell

matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 74: 7767–7778.

30. Dieckmann R, Malorny B (2011) Rapid screening of epidemiologically important *Salmonella enterica* subsp. enterica serovars by whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 77: 4136–4146.

31. Rabsch W (2007) Salmonella typhimurium phage typing for pathogens. Methods Mol Biol 394: 177–211.

32. Wain J, Hosoglu S (2008) The laboratory diagnosis of enteric fever. J Infect Dev Ctries 2: 421–425.

33. Nga TV, Karkey A, Dongol S, Thuy HN, Dunstan S, et al. (2010) The sensitivity of real-time PCR amplification targeting invasive *Salmonella* serovars in biological specimens. BMC Infect Dis 10: 125.

34. Conway GC, Smole SC, Sarracino DA, Arbeit RD, Leopold PE (2001) Phyloproteomics: species identification of *Enterobacteriaceae* using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Mol Microbiol Biotechnol 3: 103–112.

35. Sparbier K, Weller U, Boogen C, Kostrzewa M (2012) Rapid detection of *Salmonella* sp. by means of a combination of selective enrichment broth and MALDI-TOF MS. Eur J Clin Microbiol Infect Dis 31: 767–773.