Bacterial coinfections in dengue virus disease: what we know and what is still obscure about an emerging concern

Title Page

Title: Bacterial Coinfections in Dengue Virus Disease: What We Know and What Is still Obscure about an Emerging Concern

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Abstract – 249 words:

Purpose: Dengue virus is the most frequent arthropod-borne viral infection worldwide. Simultaneously to the growth of its incidence, cases of bacterial coinfection in dengue have been increasingly reported. The clinical course of dual infections may worsen for reciprocal interactions and delays in the diagnosis, so that clinicians should be aware of this eventuality. Therefore, we reviewed literature to provide an overview of the epidemiological, clinical and physiopathological issues related to bacterial coinfections and bacteremia in dengue.

Methods: Clinical studies and case reports regarding bacteremia and bacterial coinfections in dengue and the interactions between the pathogens published on PubMed were reviewed.

Results: We found 26 case reports, only 3 studies on concurrent bacteremia and 12 studies reporting data on bacterial coinfections in dengue. According to the three available studies, the 0.18-7% of dengue infections are accompanied by concurrent bacteremia, while the 14.3-44.4% of dengue-related deaths seems associated to bacterial coinfections. Comorbidities, advanced age and more severe dengue manifestations could be risk factors for dual infections. A longer duration of fever and alterations in laboratory parameters such as procalcitonin, hyponatremia, leukocyte count and renal function tests can raise the suspicion.

Conclusions: Despite the real burden and consequences of this emerging concern is still not computable accurately due to the lack of a significant number of studies on large cohorts, clinicians need a greater awareness about it to early recognize warning signs, to properly use available diagnostic tools and to readily start antibiotic treatment able to prevent worsening in mortality and morbidity**.**

KeyWords: Dengue; Bacteremia; Coinfection; Bacteria; Innate Immunity; Pathogenesis.

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Introduction

Dengue virus (DEV) infection is the most frequent arthropod-borne viral disease worldwide, transmitted mainly by *Aedes* spp mosquitoes and caused by one of four different serotypes belonging to the *Flaviviridae* family together with *West Nile virus* and many others. The global burden of DEV has grown dramatically in the last decades and one recent estimate reports 390 million of DEV infections per year, of which 96 million clinically manifesting [1]. The clinical presentation of dengue can range from asymptomatic infections to serious life-threatening manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2]. The severity of the infection depends on a large number of factors related to the virus and to the host. Moreover, two sequential infections by different serotypes of DEV can predispose to DHF and DSS due to an antibody-dependent enhancement of DEV infection which leads to the generation of a large amount of infected cells [2]. In developed countries the disease is currently sporadic and occurs mainly in travellers, especially those returning from Southeast Asia [3]. It has been estimated that about 2% of all diseases among travellers returning from endemic regions it is caused by DEV [3], but more surveillance data are required to assess the real burden of the disease, especially nowadays considering the increase in intercontinental travels and globalization.

There are different reports in literature regarding dual infections with DEV and bacteria such as *Leptospira* spp, *Staphylococcus* spp and *Enterobacteriaceae* [4-6]. Depending on the studies and on the severity of dengue, it seems that from 0.18% to 7% of DEV infections are associated with concurrent bacteremia (CB) [7-9]. Although the overall proportion of dual infections may be small, the absolute number can become awesome considering the above data, especially during major DEV outbreaks. Moreover, the clinical course of dual infections may worsen for dangerous interactions between pathogens, for missed diagnosis due to unusual clinical presentations and for delays in the beginning of the most appropriate therapy, so that clinicians should be aware of this eventuality. It is not clear yet whether and how DEV can predispose to super-infection and to

bacteremia. Different hypothesized mechanisms are the induced weakened immunity, the severe neutropenia and the microbial translocation observed during the disease [10-12]. On the other hand, also bacterial infections may increase susceptibility to DEV [13]. To date there is a dearth of studies on this issue, but what seems rational is that concurrent bacterial infections can not always be a mere coincidence. Herein we review the literature about CB and bacterial coinfections in dengue to evaluate the burden of the phenomenon and the possible pathophysiological mechanisms that can explain it and to point out the issues and the limits in managing and in recognition of dual infections.

Materials and Methods

A PubMed search from January 1943, when Kimura and Hotta first isolated DEV, through March 2016 was performed to identify case reports and studies addressing the bacterial coinfection and CB issue in DEV infection. We made our search combining *bacteremia*, *coinfection*, *immunosuppression*, *innate immunity*, *case reports* and *bacteria* with *dengue* as Mesh terms and *concurrent bacteremia*, *microbial translocation*, *case report* and *dual infection* with *dengue* as keywords. We considered all case reports with at least an english written abstract. For case reports of which we were not able to read more than the abstract we reported the missing data as not available. Conversely, we considered only english written published or accepted manuscripts of studies on adults and with a bacterial coinfection diagnosis made on the basis of culture tests, considering serological diagnosis of bacterial coinfections unreliable due to cross-reactivity issues, as explained further below. The search was augmented by review of bibliographic references from the included studies and case reports to identify additional relevant papers.

Since it is epidemiologically and clinically fundamental to differentiate DEV cases with CB from those with bacterial coinfections without bacteremia or with a positive blood culture collected without stringent temporal limits with respect to dengue diagnosis, data reported by studies that isolated bacteria from blood within a maximum of 72 hours of patient's admission for dengue were considered as data concerning CB, whilst all the studies in which the previous timeframe for blood

126 culture samples collection is missing or exceeded were considered as studies on bacterial
127 coinfections (BC) in dengue. Therefore, BC include also dual infections without bacteremia,
128 infective complications of dengue and nosocomial infections.

129 **Results**

130 We found 26 case reports, 3 studies specifically focused on CB [7-9] and 12 studies [7-9, 11, 14-21]
131 reporting data on CB or BC in DEV disease fulfilling the inclusion criteria. We then summarized
132 the evidences to performe a review of the literature providing an overview of the epidemiological,
133 clinical and physiopathological issues related to BC and CB in DEV infection.

134 **Epidemiological Issues**

135 Only three studies have been addressed to investigate on the CB issue in dengue and they were all
136 retrospective [7-9]. The main characteristics of these studies and of the enrolled populations are
137 summarized in Table 1. The reported CB rates were 0.18% [7], 1.2% [8] and 7% [9]. The first two
138 studies also reported BC rates of 0.3% [7] and 4% [8], which are almost twice and more than triple
139 the CB rates in the same cohorts respectively. Two out of the three studies were conducted on
140 patients presenting a positive laboratory confirmation of DEV infection [7, 8], while Lee et al.
141 evaluated CB in patients affected by DHF or DSS only [9]. This difference may explain the
142 significant gap between the rate they found and those reported by the other two studies. In
143 agreement with the hypothesis that CB and BC rates increase with the increasing severity of DEV
144 infection, as corollary of their main objective, a few studies on smaller cohorts addressing risk
145 factors and outcomes exclusively for DHF reported CB rates similar to those reported by Lee,
146 precisely 7% [14], 7.3% [15] and 8.1% [16]. Solely one out of the three studies on CB in dengue,
147 by Thein et al. [7], has specified that only patients with clinical deterioration despite treatment for
148 DEV were tested with blood cultures, whilst in the other two studies it is not stated whether all the
149 included patients underwent a blood sample collection for bacterial cultures [8, 9]. Therefore, in
150 addition to the limitations related to the retrospective design, it is also possible that some of the dual
151 infection cases were not diagnosed and that the reported rates underestimate the real amount of CB

in dengue. To date, only one prospective study has been conducted on bacteremia in dengue, but its aim was not to evaluate CB rates [17]. They examined secondary bacteremia rates in DEV-infected adults with a duration of fever superior to the usual 5 days [17]. They reported a 25% of secondary bacteremia in a small cohort of 40 patients, without providing the timeframe for blood cultures collection and they concluded that an average longer duration of fever respect to the usual lenght of dengue fever could be a warning sign of BC [17]. Actually, considering DEV-infected cohorts selected for specific features, such as the duration of fever or the most severe manifestations of dengue, CB and BC rates may increase, identifying categories of patients at greater risk of dual infections. More specifically, from 26.5% to 45.4% of cases admitted to an intensive care unit for dengue can develop BC [18, 19] and 22.7% of all the admitted cases requires treatment for septic shock [19]. Furthermore, up to 17.4% of elderly patients, i.e. patients with 65 years or more, presenting with DHF may experience CB [15] and the 42.8% of DHF cases who develops acute renal failure has also CB [16]. These data reinforce the hypothesis that CB and BC rates may increase with increasing severity of dengue and that certain categories such as the elderly, the patients requiring intensive care or those developing organ dysfunction could be at greater risk of dual infection during dengue.

Among the most frequently isolated bacteria responsible for CB in dengue, as shown in Table 1, there are *Staphylococcus aureus, Escherichia coli, Klebsiella spp, Salmonella spp* and *Streptococcus* spp, while rarely reported are *Pseudomonas aeruginosa*, *Moraxellaceae*, *Enterococcaceae* and *Aeromonas* spp [7-9]. It is interesting to note that a substantial portion of these bacteria are capable of colonizing parts of human body and that when the source of bacterial infection was investigated, no organ localization with primary bacteremia was found to be the most frequent condition. In Table 2 we listed all dual infection case reports found in literature. In case reports a different set of bacteria prevails; the majority of them does not usually colonize human body and it is characterized by peculiar modes of transmission, such as *Mycoplasma pneumoniae* or *Orientia tsutsugamushi*. The difference between the bacterial isolates reported by the previous

studies and those reported by case reports may be due at least in part to publication bias and to our inclusion criteria, which are not the same for the two types of scientific report.

Although the available reports show that a significant portion of DEV infections could be associated to a bacterial infection, to date there are too few studies on CB and BC in DEV disease to define with certainty the real burden of this emerging concern. Besides, to our knowledge, prospective studies on large sample size of patients are missing and they would help to define more confidently the CB and BC rates in dengue. The available data are also difficult to compare and to analyze together due to the lack of uniformity with which the studies have been conducted and it should be pointed out that all the available informations related to this issue were obtained from cohorts with special features of settings in tropical and subtropical regions [7-9, 14-19] and this may be a limitation to the use of all these data in Western clinicians reality.We need local, national and international surveillance systems for CB and BC in DEV disease and a shared systematic approach to the analysis of the phenomenon. Moreover, we need studies on large cohorts with different features than of those carried out so far, for example studies with a prospective design and with the aim of evaluating the dual infection issue among migrants and travellers in Western countries too.

Clinical Issues

DEV infection fatality rate ranges from 0.5% to 5% and though it may increase twentyfold when DHF and DSS develop, DHF and DSS cases alone account for less than 50% of all DEV-related deaths [14]. Regarding dengue mortality due to CB or BC, the available data are scarce, are provided by a few studies on small cohorts, with just 8-28 fatal cases and a large variability in the reported rates, however, to date what they show is that from 14.3% to 44.4% of DEV-related deaths could be associated to bacterial coinfections [14, 19-21] and that an increased leucocyte count and cell band percentage have been associated with a higher risk of CB and BC and of death in DEV infected patients [14, 19]. If further studies on larger cohorts would confirm the previous rates, the dual infection issue would be certainly not of secondary importance in the management of DEV disease, starting as early as from the triage of patients.

A first problem in recognizing dual infections in DEV cases is the perfect overlap of the clinical and laboratory presentation between DEV disease and some of the others infections with which it may present in association. As it is known, most if not all of the signs and symptoms found in DEV disease are not specific [2]. Considering typhoid fever (TF), as example, the diarrhea, the gastrointestinal bleeds, the singular pattern of increase in transaminases for which AST level rises more quickly and reachs a higher value than ALT and then reverts to normality first, the leukopenia with neutropenia, the thrombocytopenia and even the relative bradycardia may all be found also in DEV infection [2, 22-24].

Few studies have attempted to describe how DEV clinical presentation changes in conjunction with bacterial infections and what are the risk factors for CB. The first study was conducted by Lee et al. [9] on adults with DHF and DSS only. Patients with dual infections were older, with a longer lasting fever (an average of 8 vs 4 days) and with higher frequencies of DSS, acute renal failure, gastrointestinal bleed, altered consciousness, unusual DEV manifestations and mortality [9]. Acute renal failure and a fever lasting for more than 5 days were found to be independent risk factors for CB [9]. These conclusions agree with the previously reported studies on DEV-infected patients with a long lasting fever or developing acute renal failure, in whom dual infection rates were higher compared to those found in patients without these complications [16, 17].

See et al. found that patients with DEV and CB were more likely to have several comorbidities, in particular diabetes mellitus, hypertension, hyperlipidemia, chronic renal failure and cancer and that they have a higher hospital mortality [8]. Besides, they created and validated a Dengue Dual Infection Score (DDIS) for early identification of DEV infected patients in need of empirical antibiotic treatment [8]. The DDIS can range from 0 to 5 and it is obtained from the attribution of one point for each of the following parameters if present within 24 hours from admission: pulse rate $\geq$ 90 beats/min, total white cell count $\geq$ 6.000/µL, hematocrit < 40%, sodium < 135 mmol/L and urea $\geq$ 5 mmol/L [8]; a *DDIS* $\geq$ 4 was found to be associated to CB in 94.4% of cases [8]. It is interesting to note that the same cut-off of 6.000 white blood cells has been associated with a higher

risk of BC and with a risk of death increased by almost 10 times [19]. Moreover, studies on severe DEV infections identified in the increased leukocyte and cell band count a significant warning sign of serious dengue, sugesting the possibility of a superimposing bacterial infection [14, 19]. Lastly, Thein et al. compared CB cases with only DEV-infected cases and found that at admission dual infected patients have higher mean temperatures (38.4°C vs 37.6°C) and neutrophil count, more frequently a Pitt Bacteremia Score (PBS) ≥ 4, hematocrit change ≥ 20% and DSS, while they have lower serum albumin levels, lymphocyte and platelet count and surprisingly lower rates of hemorrhagic manifestations [7]. DEV-infected patients with CB need also more volume of fluids for a longer period [7]. They concluded proposing the PBS as a valuable resource to detect early CB in DEV infections, but not all the dual infections evolve in severe sepsis and even less start so severely, while PBS only distinguishes between patients critically ill or not [7].

A promising contribution to identify BC and CB among patients with confirmed DEV infection could come from the use of procalcitonin. Currently only one study investigated on that and it was carried out on patients admitted to intensive care unit for dengue [18]. The patients with bacteremia showed significantly higher procalcitonin level than those without, so that they suggested that procalcitonin assessment could help to exclude bacteremia in DEV cases, considering its high sensitivity and negative predictive value [18].

Once the dual infection is suspected, it is fundamental to use the correct diagnostic tools to confirm the suspicion. Depending on the available DEV serology test, sensitivity and specificity can range considerably and false positivity for DEV in case of leptospirosis, brucellosis and TF has been described, probably due to polyclonal activation or cross-reactivity occurrence [25, 26]. Moreover, it is possible also the contrary. For example, the Widal serodiagnosis used to detect *Salmonella typhi* may result falsely positive in patients affected by DEV [27]. As shown in Table 2, a large part of dual infections is diagnosed by physicians using only DEV serology. Cases considered as coinfections may actually be a single infection with a false positive serology for one of the two implicated pathogens and solely a positive bacterial culture associated with a direct diagnostic

method for DEV, such as PCR or NS1 antigen detection, would give the certainty of the dual infection.

Physiopathological Issues

DEV pathogenic mechanisms have been investigated in detail, but little is known about the pathogenesis of BC and CB in dengue. The majority of case reports and studies [4-9, 17] cite as the possible cause of this clinical concern the vascular leakage and the associated disintegration of the mucocutaneous barrier described during dengue [5, 12, 28, 29]. Consistent with this hypotesis are the previously reported data on bacterial isolates from DEV-infected patients which show that a large portion of the bacteria involved in coinfection are usual colonizing of human body [7-9]. Considering that one of the main DEV cellular target are monocytes/macrophages and that a large number of these cells resides in the gut [28], the replication of DEV in them may produce an inflammatory milieu, where the breakdown of the digestive epithelial barrier occurs [12, 28, 29], followed by the microbial translocation (MT) of resident bacteria from the enteric lumen into the bloodstream [12, 28, 29]. The same event has been hypothesized also for *Staphylococcal* bacteremia, following disruption of the cutaneous endothelial lining in patients with predisponing skin comorbidities and dengue [5]. Recent studies reported higher plasma levels of microbial translocation markers in DEV infected patients compared to healthy controls [28]. It also seems that MT correlates with DEV infection severity [12, 28]. However, this pathogenic model has yet to be demonstrated in vivo. If we consider the MT as the only mechanism whereby explaining dual infections, we should expect a higher incidence of bacterial infections in patients with greater vascular damage and hemorrhagic signs, but evidences are still conflicting. If CB and BC rates seem to increase with increasing severity of DEV and coinfected patients seem to develop more frequently DSS [7], it is also true that lower rates of hemorrhagic manifestations has been noted in dual infections compared to only DEV-infected controls [7]. Finally, the MT model cannot explain all bacterial coinfections in dengue. For instance, especially in high-incidence countries for TF, an undetermined number of chronic carriers of *Salmonella typhi* could face *Salmonella typhi*

bacteremia if infected by DEV through MT, but *Salmonella* spp and some of the other bacteria involved in dual infections, such as *Leptospira* spp, don not usually represent part of the normal flora of the gut, protagonist of MT. Furthermore, it should be state that some of the reported coinfections such as those with *Leptospirosis* spp, *Burkholderia pseudomallei*, *Mycoplasma pneumoniae* or *Orientia tsutsugamushi* could merely be a co-occurrence by chance of both the pathogens in the same individual.

Hypothetically, another possible mechanism to explain bacterial coinfections might be the severe absolute neutropenia, which may develop due to bone marrow suppression induced by DEV [11]. Despite this hypotesis could be reasonable, in a retroscpecitve study on a large cohort of DEV-infected patients, a neutrophil count ≤ 500 cells/μL was not found to be a predictor of nosocomial bacterial infections nor it was associated with a more frequent antibiotic use, probably because of the short and transient duration of the neutropenia [11].

It seems that DEV can cause a transitory immune suppression affecting the immune system cells during acute infection [10], so much so that during and after the infection immune system is less effective in mounting a defensive response also against secondary bacterial threats. In fact, DEV seems able to diminish response to proliferative stimuli in T cell populations by impairing antigen-presenting cells functions [30], to reduce the phaghocitic and migratory skills of splenic and peritoneal-cavity macrophages [31] and to suppress the interferon signaling pathway through the down-regulation of different genes [32]. Moreover, in mosquitoes DEV seems capable of increasing the susceptibility to *Staphylococcus aureus* and *Pseudomonas aeruginosa* septic injury [33] and of down-regulating the expression of different genes involved in the major innate immunity pathways, including some genes coding for receptors of viral and bacterial pathogen-associated molecular patterns and for antimicrobial peptides, the production of which was shown to be reduced in response to bacterial challenges [34]. Considering the notable overlap between the innate immune system of diptera and human [33, 34], the explanation of bacterial and DEV coinfections may be found by studies on interactions between DEV and the human innate immune systems. Actually, in

human myeloid/plasmocytoid dendritic cells and monocytes DEV can affect the expression of some co-stimulatory molecules and of the Toll-Like Receptors (TLRs), proteins with a pivotal role in the innate immune system [35]. The modulation of the expression of TLRs may influence not only the development of a specific immune response against the virus, but also the dendritic cells activation [35], thereby influencing immune responses involved in antibacterial defenses as well. This effect seems to depend on the severity of DEV infection [35] and consistent with these findings, the presence of subneutralizing antibodies induced by previous exposure to a different DEV serotype has been linked not only to a higher risk of severe form of dengue, but also to a more prominent down-regulation of TLRs expression and up-regulation of suppressors of the NF-kB signaling pathway, crucial for cytokine production [36]. Considering these results, the aforementioned higher CB and BC rates in DHF and DSS cases should not surprise. A summary of the main mechanisms through which DEV may induce CB and BC is represented in Figure 1.

Finally, if it is possible that DEV can facilitate CB and BC, it is also possible that bacterial contagion could increase susceptibility to more symptomatic and severe forms of dengue. It has been described a modulating effect of LPS, the Gram-negative outer membrane endotoxin, on DEV replication [13]. Chen et al. observed that when LPS was added to in vitro cultures of human monocytes and macrophages after DEV infection, DEV replication was enhanced and prolonged [13] and similar conclusions were also reached by one study in *Aedes aegypti* cells cultures [34]. These findings are strongly suggestive of a modulation over the viral load and the immune response carried out by concurrent Gram-negative coinfections during dengue, they seem to agree with the previously cited study reporting a correlation between dengue severity and LPS plasma levels [28] and if they were confirmed in human models, we could even expect that in Gram-negative coinfections sings and symptoms related to DEV active replication could temporarily worsen or be prolonged right after the beginning of the antibiotic therapy because of the release of a large amount of LPS from killed bacteria.

We are clearly far from understanding the physiopathology of CB and BC in dengue, but certainly we can note that there is a mutual life-threatening strengthening influence between DEV and bacteria.

Conclusion

A significant portion of dengue cases could be associated to a bacterial infection, but the real burden of this emerging concern is still not computable accurately due to the lack of a shared approach to the study of this issue and of a surveillance system monitoring and reporting systematically the dual infections, also in western countries. Clinicians need a greater awareness about CB and BC in dengue since that in addition to be potentially more serious and with a higher risk of complications, dual infections can put clinicians in front of management problems and can predispose to delays in the diagnosis and in the beginning of the most appropriate therapy, able to prevent aggravation in mortality and morbidity. We encourage clinicians to suspect CB and BC in any DEV case, especially in patients with comorbidities, elderly, with a long lasting fever or more severe forms of dengue. In such cases, the DDIS and the procalcitonin may prove useful diagnostic tools, if their high specificity and sensitivity respectively will be confirmed by further studies [8, 18]. Moreover, not to prescribe unnecessary antibiotics because of false positive results, when it is possible, we recommend to prefer biological sample culture tests over serology to confirm a suspicion of bacterial coinfection in dengue, considering yet that some of the involved microorganisms could be difficult to culture. Nevertheless, we do not recommend the indiscriminate use of biological sample cultures nor the administration of an empiric antibiotic treatment to each suspected or confirmed DEV case, since that the former would result in a huge waste of human and economic resources, especially in developing countries and the latter may lead to the selection of multiresistant bacteria. Evaluating the risk factors, the laboratory, the clinical presentation and its evolution, clinicians should be able to identify DEV-infected patients in need of appropriate further diagnostic investigations and of an empiric antibiotic therapy to reduce mortality and morbidity.

Conflict of Interest: The authors have no conflict of interest to declare.

Fig. 1 The hypothesized mechanisms whereby *Dengue Virus* may induce Concurrent Bacteremia and Bacterial Coinfections

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451 Table 1. Main features of the three published studies focused on Concurrent Bacteremia in Dengue

	Lee IK et al, Am J Trop Med Hyg 2005	See KC et al, Am J Trop Med Hyg 2013	Thein TL et al, J Microbiol Immunol Infect 2015
Study population	100	2065	9553
Study design	Retrospective	Retrospective	Retrospective
Age	>18 years	>16 years	>18 years
Female	46 (46%)	860 (42%)	NA
Country	Taiwan	Singapore	Singapore
DEV cases	DHF or DSS	All types	All types
CB	7 (7%)	25 (1,2%)	18 (0,18%)
BC	NA	83 (4%)	29 (0,3%)
Fatality rate	2/7 (28,5%)	16/83 (19,3%)	3/18 (16,7%)
Source of Bacteremia	1 Meningitis 1 Facial cellulitis 5 Primary bacteremia	3 Endocarditis 2 Vascular infections 1 Limb cellulitis 6 Bile ducts infections 4 UTI 9 Primary bacteremia	NA
Isolated Pathogens	3 *Klebsiella pneumoniae* 1 *Klebsiella ozaenae* 1 *Rosemonas* spp 1 *Moraxella lacunata* 1 *Enterococcus faecalis*	8 *Staphylococcus aureus* (5 MSSA and 3 MRSA) 6 *Escherichia coli* 4 *Klebsiella pneumoniae* 2 *Salmonella typhi* 1 *Salmonella enteritidis* 1 *Streptococcus agalactiae* 1 *Group A streptococcus* 1 *Aeromonas maltophilia* 1 *Kluyvera cryocrescens*	5 *Staphylococcus aureus* 4 *Salmonella typhi* 3 *Escherichia coli* 2 *Klebsiella pneumoniae* 2 *Streptococcus* spp 1 *Pseudomonas aeruginosa* 1 Unspecified anaerobe

CB Diagnosis	Any positive blood culture within 72 hours of admission for DEV	Any positive blood culture within 48 hours of admission for DEV or Any clinical diagnosis	Any positive blood culture within 72 hours of admission for DEV
Blood Culture testing Criteria	NA	NA	Patients presenting clinical deterioration despite DEV treatment
DEV Diagnosis	PCR, IgM capture ELISA or fourfold increase of HIT	PCR, IgM ELISA or NS1 antigen	RT-PCR or Rapid Dengue Duo Strip Test
Exclusion Criteria	Prior antibiotic treatment Contamination of cultures	Contamination of cultures	NA

Legend: DHF Dengue Hemorragic Fever; DSS Dengue Shock Syndrome; CB Concurrent Bacteremia; BC Bacterial Coinfections including also CB; NA Not Available for missing or unspecified data; UTI Urinary Tract Infections; MSSA Methicillin-Sensitive *Staphylococcus aureus*; MRSA Methicillin-Resistant *Staphylococcus aureus*; HIT Hemagglutination inhibition titers; RT-PCR Reverse Transcriptase-Polymerase Chain Reaction.

466 Table 2. Bacterial Coinfections and Concurrent Bacteremia in Dengue: case reports from literature

Age & Sex	Associated Bacteria	Diagnostic tests	Possible DB	Outcome	Reference
NA	*Salmonella typhi*	NA	No	Recovery	Bansal R et al, Trop Doct 2015
10 F	*Leptospira* spp	DEV and *Leptospira* IgM serology	Yes	Recovery	Nunez-Garbin A et al, Rev Peru Med Exp Salud Publica 2015
52 M	*Leptospira* spp	DEV and *Leptospira* serology	Yes	Death	Wijesinghe A et al, BMC Res Notes 2015
10 M	*Salmonella typhi*	Blood cultures for *S typhi*, DEV NS1 and IgM ELISA	No	Recovery	6
22, 64, 67 M	*Leptospira* spp	*Leptospira* spp antigen, IHC and PCR on autoptic samples, DEV RT-PCR	No	Death	4
25 F	*Orientia tsutsugamushi*	Weil-Felix and PCR for *O tsutsugamushi*, DEV NS1 and IgM	No	Recovery	Kumar S et al, J Vector Borne Dis 2014
30 F	*Stenotrophomonas maltophilia*	Blood culture for *S maltophilia*, DEV NS1 antigen	No	Recovery	Sriranaraj S et al, Australas Med J 2014
48 F	*Enterococcus faecium*	Blood cultures for *E faecium*, DEV IgG serology	Yes	Death	Tsai JJ et al, Southeast Asian J Trop Med Public Health 2013
24 M	*Salmonella typhi*	Blood cultures for *S typhi*, DEV NS1 and serology	No	Recovery	Vaddadi S et al, Int J Res Dev Health 2013

17 M	*MRSA*	Blood culture for *MRSA*, DEV IgM ELISA	Yes	Death	Sunderalingam V et al, Case Rep Infect Dis 2013
42 M	*Leptospira* spp	*Leptospira* spp antigen IHC on kidney autoptic samples, DEV NS1 on blood	No	Death	Sharp TM et al, Emerg Infect Dis 2012
46 NA	*Leptospira* spp	NA	No	NA	Cadelis G, Rev Pneumol Clin 2012
40 F	*Orientia tsutsugamushi*	Weil-Felix and IgM for *O tsutsugamushi*, DEV IgM	Yes	Recovery	Iqbal N et al, Trop Med Health 2012
15 M	*Staphylococcus aureus*	Sputum cultures for *S aureus*, DEV ELISA serology	Yes	Recovery	Nagassar RP et al, BMJ Case Rep 2012
28 M	*Burkholderia pseudomallei*	Ascitic fluid culture for *B pseudomallei*, DEV PCR on autoptic samples	No	Death	Macedo RN et al, Rev Soc Bras Med Trop 2012
14 M	*Staphylococcus aureus*	Autoptic samples cultures for *S aureus*, DEV IHC on autoptic samples	No	Death	Araujo SA et al, Am J Trop Med Hyg 2010
23 M	*Brucella melitensis*	Blood culture for *B melitensis*, DEV serology	Yes	Recovery	26
23 M	*Leptospira* spp	*Leptospira* and DEV IgM ELISA	Yes	Recovery	Behera B et al, J Infect Dev Ctries 2009
36, 39, 39, 42, 43 M	*Staphylococcus aureus*	Blood, intraoperative and wound specimens cultures for *S aureus*, DEV PCR on serum	No	Recovery	5

6 F	*Streptococcus pyogenes*	Blood cultures for *S pyogenes*, DEV serology	Yes	Recovery	Vitug MR et al, Int J Dermatol 2006
8 F	*Mycoplasma pneumoniae*	*Mycoplasma* agglutination test, DEV IgM rapid test, RT-PCR and hemoagglutination test	Yes	Recovery	Likitnukul S et al, Southeast Asian J Trop Med Public Health 2004
6, 9 F, 9, 11 M	*Salmonella typhi* *Salmonella paratyphi*	Blood cultures for *Salmonella* spp, DEV IgM rapid test and hemagglutination test	Yes	Recovery	Basuki PS, Folia Med Indon 2003
44 F	*Shigella sonnei*	Stool culture for *S sonnei*, DEV IgM rapid test and Duo IgM IgG-capture ELISA	Yes	Recovery	Charrel RN et al, Emerg Infect Dis 2003
NA	*Leptospira* spp	NA	No	NA	Kaur H et al, Indian J Gastroenterol 2002
2 F	*Leptospira* spp	*Leptospira* and DEV IgM ELISA	Yes	Recovery	Rele MC et al, Indian J Med Microbiol 2001
19 F, 32 M	*Salmonella typhi*	Blood cultures for *S typhi*, DEV serology	No	Recovery	Sudjana P et al, Southeast Asian J Trop Med Public Health 1998

Legend: DB Diagnostic Bias; DEV *Dengue virus*; NA Data Not Available; IHC Immunohistochemistry; *MRSA* Methicillin-resistant *Staphylococcus aureus*; RT-PCR Reverse-Transcriptase Polymerase Chain Reaction.

Figure 1 The hypothesized mechanisms whereby dengue virus may induce concurrent bacteremia and bacterial coinfections