International Journal of Infectious Diseases 97 (2020) 251–257

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Aetiology of bacterial meningitis in infants aged <90 days: Prospective surveillance in Luanda, Angola

Tuula Pelkonen[a,b,*], Suvi Urtti[a], Elizabete dos Anjos[b], Ondina Cardoso[b], Linda de Gouveia[c], Irmeli Roine[d], Heikki Peltola[a], Anne von Gottberg[c,e], Moe H. Kyaw[f]

[a] Children's Hospital, Helsinki University Central Hospital, Helsinki, Finland
[b] Hospital Pediátrico David Bernardino (HPDB), Luanda, Angola
[c] Centre for Respiratory Disease and Meningitis (CRDM), National Institute for Communicable Diseases (NICD), Division of the National Health Laboratory Service (NHLS), Johannesburg, South Africa
[d] Faculty of Medicine, University Diego Portales, Santiago, Chile
[e] School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[f] Sanofi Pasteur, Epidemiology, Swiftwater, PA, USA

ARTICLE INFO

Article history:
Received 13 May 2020
Received in revised form 2 June 2020
Accepted 5 June 2020

Keywords:
Bacterial meningitis
Neonatal
Angola
Africa
Surveillance
Drug resistance

ABSTRACT

Background: Despite effective antibiotics and vaccines, bacterial meningitis (BM) remains one of the leading causes of morbidity and mortality in young infants worldwide. Data from Africa on the aetiology and antibiotic susceptibility are scarce.
Objective: To describe the aetiology of BM in Angolan infants <90 days of age.
Methods: A prospective, observational, single-site study was conducted from February 2016 to October 2017 in the Paediatric Hospital of Luanda. All cerebrospinal fluid samples (CSF) from infants aged <90 days with suspected BM or neonatal sepsis were assessed. The local laboratory performed microscopy, chemistry, culture, and susceptibility testing. PCR for vaccine-preventable pathogens was performed in Johannesburg, South Africa.
Results: Of the 1287 infants, 299 (23%) had confirmed or probable BM. Of the 212 (16%) identified bacterial isolates from CSF, the most common were *Klebsiella* spp (30 cases), *Streptococcus pneumoniae* (29 cases), *Streptococcus agalactiae* (20 cases), Escherichia coli (17 cases), and *Staphylococcus aureus* (11 cases). A fifth of pneumococci (3/14; 21%) showed decreased susceptibility to penicillin, whereas methicillin-resistant *S. aureus* (MRSA) was encountered in 4/11 cases (36%). Of the gram-negative isolates, 6/45 (13%) were resistant to gentamicin and 20/58 (34%) were resistant to third-generation cephalosporins. Twenty-four percent (33/135) of the BM cases were fatal, but this is likely an underestimation.
Conclusions: BM was common among infants <90 days of age in Luanda. Gram-negative bacteria were predominant and were often resistant to commonly used antibiotics. Continued surveillance of the antibiogram is pivotal to detect potential changes without delay.

Introduction

The global burden of bacterial meningitis (BM) remains high, even with improving coverage of conjugate vaccines (GBD 2016 Meningitis Collaborators, 2018GBD 2016 Meningitis Collaborators, 2018). Although the incidence, aetiology, and outcome vary by age and region (Edmond et al., 2010; GBD 2016 Meningitis Collaborators, 2018; Oordt-Speets et al., 2018), the burden is heaviest in Sub-Saharan Africa where the problem is related to poverty (GBD 2016 Meningitis Collaborators, 2018GBD 2016 Meningitis Collaborators, 2018). Outside *Neisseria meningitidis* epidemics, the peak age of BM is in the neonatal period (GBD Meningitis Collaborators, 2018; GBD 2016 Meningitis Collaborators, 2018; Heath et al., 2011; Molyneux et al., 2015), when mortality is higher (25–58%) in low- and middle-income countries (LMICs) than in industrialized countries (6–15%) (Furyk et al., 2011; Gaschignard et al., 2011;

* Corresponding author at: Children's Hospital, PO Box 347, 00029 HUS, Helsinki, Finland.
E-mail addresses: tuulapelkonen@hotmail.com (T. Pelkonen), suvi.urtti@fimnet.fi (S. Urtti), elizabeteanjos@icloud.com (E. dos Anjos), ondinacardoso@hotmail.com (O. Cardoso), lindad@nicd.ac.za (L. de Gouveia), irmeli.roine@gmail.com (I. Roine), heiheikkipeltola@gmail.com (H. Peltola), annev@nicd.ac.za (A. von Gottberg), MKyaw11@gmail.com (M.H. Kyaw).

https://doi.org/10.1016/j.ijid.2020.06.016

Heath et al., 2011; Okike et al., 2014). Sequelae are reported in 20–58% of patients (Edmond et al., 2010; Furyk et al., 2011; Heath et al., 2011).

The World Health Organization (WHO) defines a case as 'neonatal' when it occurs at ≤28 days of age, whereas the US guidelines place the age limit at <90 days (Molyneux et al., 2015). Furthermore, neonatal BM is deemed 'early-onset' if signs appear at <7 days of age, or as 'late-onset' if signs appear at 7–89 days (Okike et al., 2014).

The diagnosis of BM is especially difficult in small infants whose symptoms can be subtle and mimic sepsis or other infections (Furyk et al., 2011; GBD 2016 Meningitis Collaborators, 2018). It is estimated that 25% of infants with sepsis also have BM (Heath et al., 2011). Although the global burden of BM at age <90 days is unknown, the WHO estimated that 402 414 deaths were due to neonatal sepsis, meningitis, or both in 2015 (World Health Organization, 2019). Efforts to reduce this burden are particularly needed in Africa and Asia where the incidence is the highest (GBD 2016 Meningitis Collaborators, 2018GBD 2016 Meningitis Collaborators, 2018).

The most common causative agents of neonatal BM in industrialized countries are *Streptococcus agalactiae* and *Escherichia coli*, whereas in LMICs other gram-negative bacteria, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae* also appear (Furyk et al., 2011; Okike et al., 2014; Oordt-Speets et al., 2018). Antimicrobial resistance is also increasing in LMICs (Furyk et al., 2011; Huynh et al., 2018; Okomo et al., 2019), where laboratory facilities may be absent or insufficient (Molyneux et al., 2015; Okomo et al., 2019). However, data on antibiotic susceptibility are critical for adopting best-treatment strategies for BM.

Angola is a country in Sub-Saharan Africa with a population of 28 million. The population growth rate is 3.3%, and the annual birth cohort is 1.2 million (Gavi, 2020). Luanda, the capital, has a population of 2.8 million and an urban area of 8.2 million. The under-5 mortality rate is 77/1000, the infant mortality rate is 52/1000, and the neonatal mortality rate is 28/1000; these indices are among the highest in the world. Approximately 36 000 neonatal deaths occurred in Angola in 2018 (Unicef, 2020). The pentavalent and the 13-valent pneumococcal conjugate vaccines were introduced in the country in 2006 and in 2013, respectively. Both are administered at 2, 4, and 6 months of age (Gavi, 2020). Due to the minimal data on neonatal BM in Central Africa (Okomo et al., 2019; Oordt-Speets et al., 2018), this study was performed to examine the aetiology of BM in Angolan children <90 days of age.

Materials and methods

Setting

Most babies in Luanda are born in maternity hospitals. If health problems arise, patients may be sent to the Hospital Pediátrico David Bernardino (HPDB), a 300-bed paediatric referral and teaching hospital in the city. Most newborn babies and small infants are taken to HPDB directly from home and only a few are transferred to this hospital from health centres or other hospitals. A diagnostic bacteriological laboratory was established in 2002. During the study, the 30-bed neonatal ward was not yet equipped with technology for mechanical ventilation of babies. Routine treatment for BM and neonatal sepsis was a combination of ampicillin and cefotaxime, and of ampicillin and gentamicin, respectively.

Study design

This prospective, observational, single-site, and descriptive study was conducted between February 1, 2016 and October 23, 2017. All infants aged <90 days presenting at HPDB who underwent lumbar puncture (LP) were included in the study. Attending physicians performed the LPs when there was a suspicion of BM according to the WHO criteria: acute onset of fever (usually >38.5 °C rectal or 38.0 °C axillary), headache, and one of the following signs: neck stiffness, altered consciousness, or other meningeal signs (World Health Organization, 2020). Newborn babies also underwent LP if they presented with a bulging fontanelle or with other more subtle and non-specific signs like prostration or irritability and if neonatal sepsis was suspected. There were no exclusion criteria. Data for older children have been published before (Urtti et al., 2019).

Macroscopic examination, leukocyte counts, and glucose and protein concentration measurements were performed for all cerebrospinal fluid (CSF) specimens. For babies <30 days old, Gram staining and bacterial culture were always performed, whereas for older infants these were performed only if CSF leukocytes were >10/mm^3 or the glucose concentration was <25 mg/dl. When available, bacterial antigen testing (latex agglutination test, Pastorex Meningitis; Bio-Rad Laboratories Inc., Marne-La-Coquette, France) was conducted if >100 leukocytes/mm^3 were present and bacterial culture was negative.

BM was considered confirmed when bacteria were identified in the CSF (by culture, Gram stain, antigen detection, or PCR) (World Health Organization, 2020). BM was considered 'probable' when the CSF was turbid or showed >100 leukocytes/mm^3. BM was also considered probable with milder leukocytosis (10–100 cells/mm^3) if the protein concentration was >100 mg/dl or glucose was <40 mg/dl (World Health Organization, 2020).

Laboratory methods

The CSF specimens were transported to the hospital laboratory within 3 h of sampling. Based on previously established guidelines (Cheesbrough, 2000), the CSF was cultured on blood and chocolate agar plates and incubated accordingly. Bacteria were identified with standard bacteriological phenotypic methods. Antimicrobial susceptibility was tested using available discs and applying Clinical and Laboratory Standards Institute standards (Clinical and Laboratory Standards Institute, 2007). Whenever possible, the remainder of the CSF sample was stored at −80 °C and later shipped for PCR identification to the Centre for Respiratory Diseases and Meningitis (CRDM), National Institute for Communicable Diseases (NICD), Johannesburg, South Africa.

DNA extraction was performed using the MagNA Pure 96 instrument (Roche, Mannheim, Germany) with the Viral NA small volume kit (Roche). Molecular detection of meningitis pathogens targeted the *ctr*A, *lyt*A, and *hpd* genes for *N. meningitidis*, *S. pneumoniae*, and *H. influenzae*, respectively, using a multiplex real-time PCR assay with the Applied Biosystems 7500 Fast real-time PCR platform (Applied Biosystems, Foster City, CA, USA) (Wang et al., 2012).

All PCR-positive samples were further serotyped/grouped by real-time PCR. *ctr*A-positive samples were confirmed using two multiplex reactions for the detection of serogroups A, W, X, B, C, and Y (Mothershed et al., 2004). Serotypes in *hpd*-positive samples were determined using three multiplex reactions (Maaroufi et al., 2007; Wang et al., 2011). *lyt*A-positive samples with cycle threshold (Ct) values ≤35 were tested by eight multiplex reactions detecting 38 serotypes (Magomani et al., 2014; Pimenta et al., 2013).

Endpoints

The primary objective of this study was to describe the aetiology of BM in infants <90 days of age. The secondary objective

was to assess the antibiotic susceptibility of isolates. Clinical information was obtained from specially designed forms completed by the study nurses and doctors.

Statistical analysis

The data were computed and analysed with StatView version 5.1 (SAS Institute, Cary, NC, USA). The results are presented descriptively with means, percentages, medians, *p*-values, and interquartile ranges (IQRs) where appropriate.

Results

Study population

A total of 4796 children (aged ≤15 years) with suspected meningitis were tapped during the study period. Of these, 1287 (26.8%) were aged <90 days (Figure 1) and the main reason for LP was specified in 1036 cases (Table 1). The CSF characteristics are summarized in Table 2. There were 299 BM cases, of which 212/1089 (19.5%) were confirmed cases and 87 (8.0%) were probable cases.

For the infants with BM, the exact age was recorded as follows: 272 (91%) were <1 month, 21 (7%) were 1 month, and six (2%) were 2 months. One hundred and sixty-five (55%) were female.

Forty percent (57/143) of infants with BM weighed <2500 g. Of the infants, 11% (21/183) were classified as premature and 26% (47/183) showed growth failure, while 63% (115/183) were born at term with appropriate weight.

The outcome was registered for 135 infants with confirmed or probable BM. Of these, 33 (24%) died.

Aetiology and organism characteristics

Bacteria were detected in CSF by culture, PCR, latex agglutination, Gram staining, or combinations thereof in 22.3% (212/949) of cases (Table 3). The CSF culture result was available in 960 cases, of which 45 (4.7%) showed contamination and 116 (12.1%) were positive. PCR was performed in 379 cases, of which 24 (6.3%) were positive and 67 (17.7%) inconclusive. Latex agglutination was performed in 68 cases, of which 14 (20.6%) were positive. Gram-staining was performed in 1005 cases, of which 168 (16.7%) were positive.

Table 1
Indications for cerebrospinal fluid sampling in infants aged <90 days.

Reason	Number analysed	%
Altered level of consciousness	261	25.2
Convulsions	99	9.5
Prostration	96	9.3
Irritability	10	1.0
Bulging fontanelle	6	0.6
Meningism	1	0.1
Neonatal sepsis	563	54.3
Total	1036	100

The most common pathogens were *Klebsiella* spp in 30 cases (14.2%), *S. pneumoniae* in 29 cases (13.7%), *S. agalactiae* in 20 cases (9.4%), *E. coli* in 17 cases (8.0%), and *S. aureus* in 11 cases (5.2%) (Table 3 and Figure 2). Of the 13 serotyped strains of *S. pneumoniae*, eight were vaccine types (three cases of 23 F, two of 19A, and one case each of 5, 7F/7A, and 19F). All of these children were too young to have received a pneumococcal vaccine. *H. influenzae* was identified in eight cases (one type a, five type b, and two non-typeable cases). These five children with *H. influenzae* type b (Hib) meningitis were also too young for scheduled vaccination.

Antimicrobial susceptibility

Resources allowed susceptibility testing of 96 isolates for most relevant antibiotics (Table 4). Among *S. pneumoniae*, 3/14 (21%) showed decreased susceptibility to penicillin and one strain was also resistant to third-generation cephalosporins. Among *S. aureus*, 4/11 (36%) strains were methicillin-resistant (MRSA). Furthermore, 20 (34%) of the 59 gram-negative bacteria tested were resistant to third-generation cephalosporins, all being extended-spectrum β-lactamase (ESBL)-producing isolates. Chloramphenicol resistance of these strains was 48% (23/48), whereas resistance to gentamicin (6/45; 13%) and ciprofloxacin (2/46; 4%) was rarer. *Klebsiella* spp and *E. coli* were resistant to third-generation cephalosporins in 11/24 (46%) and 4/13 (31%) cases, respectively, but less so to gentamicin (1/18 (6%) and 4/12 (33%), respectively).

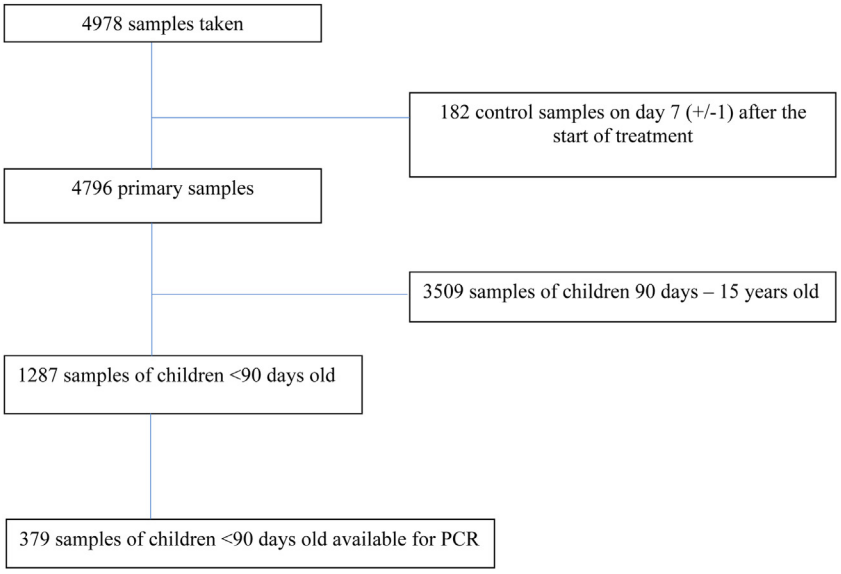

Figure 1. Flowchart for the inclusion of cerebrospinal fluid samples.

Table 2
Cerebrospinal fluid characteristics.

Characteristics	Number (%), or median (IQR)	Bacteria found by				No bacteria found	*p*-Value
		Culture	PCR only	Latex only	Gram stain only		
Gross appearance							<0.0001
Clear	376 (40%)	25	8	0	17	326	
Xanthochromism	338 (36%)	46	6	3	20	263	
Haemorrhagic	179 (19%)	27	5	1	13	133	
Turbid	52 (5%)	17	5	7	12	11	
Total	945	115	24	11	62	733	
Leukocyte number	0 (0–25) *n* = 923	0 (0–75)	0 (0–2)	779 (110–2950)	0 (0–105)	0 (0–2)	<0.0001
Majority PMN leukocytes	61/114 (54%)	23/27 (85%)	1/2 (50%)	6/8 (75%)	15/18 (83%)	16/59 (27%)	<0.0001
Glucose, mg/dl	50 (30–70) *n* = 912	34 (11–58)	49 (35–54)	18 (6–23)	26 (15–50)	53 (34–73)	<0.0001
Protein, mg/dl	85 (55–144) *n* = 805	125 (60–231)	74 (40–141)	267 (130–286)	105 (69–158)	87 (56–138)	<0.0001

IQR, interquartile range; PMN, polymorphonuclear.

Table 3
Agents identified in cerebrospinal fluid.

Causative agent	Bacteria detected by				In all	% of total
	Culture	PCR	Latex	Gram-stain		
Gram-positive bacteria	44	15	11	35	105	49.5
Streptococcus pneumoniae	14	15			29	13.7
Streptococcus pyogenes	2				2	0.92
Streptococcus agalactiae	9		11		20	9.4
Other streptococci	8				8	3.8
Staphylococcus aureus	11				11	5.2
Other gram-positive bacteria				35	35	16.5
Gram-negative bacteria	71	9		27	107	50.5
Haemophilus influenzae						
Type a		1			1	0.5
Type b	1	4			5	2.4
Non-typeable		2			2	0.92
Neisseria meningitidis		2			2	0.92
Klebsiella spp	30				30	14.2
Escherichia coli	17				17	8.0
Enterobacter spp	7				7	3.3
Pseudomonas spp	7				7	3.3
Citrobacter spp	4				4	1.9
Proteus spp	2				2	0.92
Shigella spp	2				2	0.92
Salmonella spp	1				1	0.5
Other gram-negative bacilli				27	27	12.7
Total	115	24	11	62	212	100

Discussion

The suspicion of BM among the infants <90 days old in this series was often correct, although only a quarter of children fulfilled the criteria for confirmed or probable BM. Bacteria were detected in about 20% of the microbiologically analysed CSF samples; gram-negative bacteria were more common than gram-positive. Many gram-negative strains were resistant to third-generation cephalosporins.

Considering all the LPs performed during this study, the culture identified bacteria in 12% of cases, which is more than in most other studies on neonatal BM in non-industrialized countries. In Ethiopia, neonates ≤28 days with suspected BM showed positive CSF culture in 5% of cases (56/1189) (Reta and Zeleke, 2016). In Iran, out of the 415 neonates hospitalized for sepsis, 10% (*n* = 40) were diagnosed with meningitis, whereas only 1% (*n* = 4) had positive CSF cultures (Khalessi and Afsharkhas, 2014). In Kenya, 1908 infants <60 days old with suspected sepsis or BM had a LP performed; of these, 75 (3.9%) had a positive CSF culture. Surprisingly, no more than 161 (8.4%) fulfilled the criteria for BM (Mwaniki et al., 2011). Such an overview is rarely obtained, as many studies do not report the total number of CSF samples taken.

In this study, PCR detected *S. pneumoniae*, *H. influenzae*, and *N. meningitidis*, but increased the detection rate by 11% (24/212) (Table 3). In a Chinese study on 56 neonates with BM, CSF culture identified five cases (9%), real-time PCR identified 25 cases (45%), and multiplex PCR-based reverse line blot hybridization identified 16 cases (29%) (Wang et al., 2014). The PCR-based molecular assays detected seven neonatal BM pathogens and in fact were more sensitive than culture (Wang et al., 2014). Resources permitting, rapid PCR-based assays could also be useful in LMICs where culture facilities are lacking (Bårnes et al., 2018).

The most common aetiological agent observed in the present study was *Klebsiella* spp (14.2%). This was also the predominant isolate (34%) from bacteraemic infections in a large meta-analysis of invasive bacterial infection in neonates in Central and Southern Africa (Okomo et al., 2019). For neonatal BM, the pooled pathogen prevalence was 26% for *S. agalactiae* (group B *Streptococcus*, GBS), 18% for *S. aureus*, 15% for *S. pneumoniae*, 15% for *Klebsiella*, and 15% for *E. coli* (Okomo et al., 2019). However, most data were from South Africa and there was considerable heterogeneity between regions (Okomo et al., 2019). In another meta-analysis of neonatal BM in Africa, *S. pneumoniae* was responsible for 20.4% and *E. coli* for

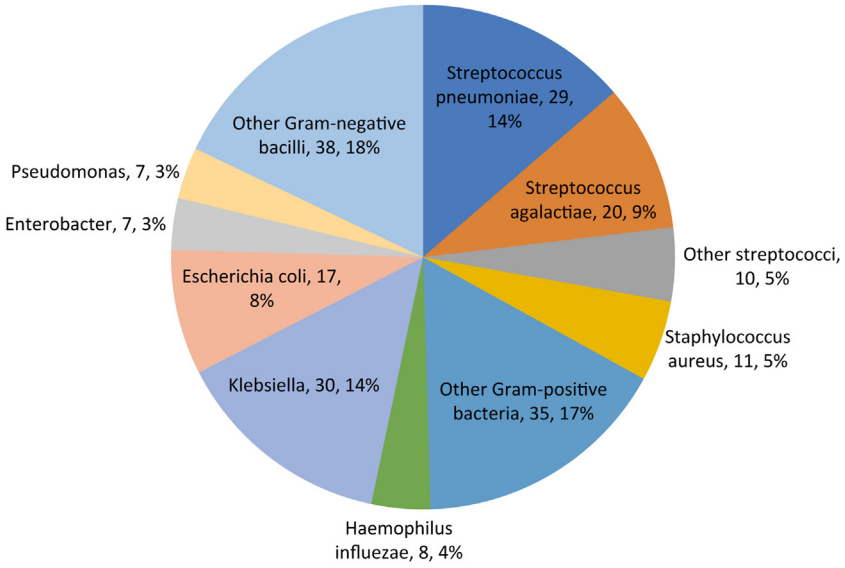

Figure 2. Causative agents of bacterial meningitis in infants <90 days of age (n = 212).

Table 4
Antibiotic susceptibility of cerebrospinal fluid isolates[a].

Bacteria	Total isolates	Penicillin	Amoxicillin	Gentamicin	Third-generation cephalosporin	Chloramphenicol	Ciprofloxacin
Gram-positive bacteria							
Streptococcus pneumoniae	14	Penicillin 11/14 (79)			9/10 (90)	9/14 (64)	1/1 (100)
Staphylococcus aureus	11	Oxacillin 7/11 (64)		2/2 (100)	5/6 (83)	10/11 (91)	8/9 (89)
Gram-negative bacteria							
Klebsiella spp	30		3/26 (12)	17/18 (94)	13/24 (54)	3/16 (19)	19/19 (100)
Escherichia coli	17		3/17 (18)	8/12 (67)	9/13 (69)	7/13 (54)	9/10 (90)
Pseudomonas spp	7		2/7 (29)	6/7 (86)	4/7 (57)	6/7 (86)	5/5 (100)
Enterobacter spp	7		1/5 (20)	2/2 (100)	6/7 (86)	3/5 (60)	3/4 (75)
Citrobacter spp	4		1/4 (25)	1/1 (100)	1/2 (50)	2/2 (100)	2/2 (100)
Other gram-negative bacteria	6		1/6 (17)	5/5 (100)	6/6 (100)	4/5 (80)	6/6 (100)
All gram-negative bacteria	71		11/65 (17)	39/45 (87)	39/59 (66)	25/48 (52)	44/46 (96)

[a] Data are number (%). Isolates may have been tested for susceptibility to different antimicrobial agents.

17.7% of cases (Oordt-Speets et al., 2018). In the present study, the corresponding figures were 13.7% and 8.0%.

Agents that cause BM (Swann et al., 2014) and other invasive infections (Okomo et al., 2019) in neonates in Sub-Saharan Africa are often resistant to the recommended antimicrobials. We observed more *S. pneumoniae* strains with reduced susceptibility to penicillin in Angola than in Malawi (21% vs. 7%) (Swann et al., 2014); one of our strains was even resistant to third-generation cephalosporins. *E. coli* showed resistance to gentamicin and to third-generation cephalosporins more often than *E. coli* in Malawi (33% vs 12%, and 31% vs 0%, respectively) (Swann et al., 2014). In the meta-analysis of invasive infections, resistance was even more common (47% and 33%, respectively) (Okomo et al., 2019). *Klebsiella*, the most common bacterium in our study, showed alarming rates of resistance to third-generation cephalosporins (46% vs. 35% in Malawi and 49–78% in the meta-analysis) (Okomo et al., 2019; Swann et al., 2014). However, resistance to gentamicin was rarer (6% vs 14% vs 66%) and all strains were susceptible to ciprofloxacin.

The introduction of conjugate vaccines against Hib and *S. pneumoniae* decreases the incidence of BM not only in those vaccinated, but also through herd immunity in non-vaccinated populations, including infants <2 months of age (GBD Meningitis Collaborators, 2018; GBD 2016 Meningitis Collaborators, 2018; Heath et al., 2011; Molyneux et al., 2015; Oordt-Speets et al., 2018). However, BM caused by serotypes not covered by vaccines are widely reported (GBD 2016 Meningitis Collaborators, 2018GBD 2016 Meningitis Collaborators, 2018). In HPDB, Luanda, 10 years after the introduction of Hib vaccination, Hib was still identified in five infants (one infant had BM due to *H. influenzae* type a and two infants had non-typeable *H. influenzae*). Three years after the introduction of the 13-valent pneumococcal vaccine, *S. pneumoniae* was the second most common agent, despite 62% of the serotypes being included in the vaccine. Improving the uptake of both vaccines would also lead to beneficial effects in neonates.

The fatality rate (24%) of BM in this study was probably an underestimation, as this information was available for only a fraction of the infants (135/299; 45%). In rural Kenya, LP was not performed in 81% (466/575) of the infants <60 days old who ultimately died from likely BM or sepsis (Mwaniki et al., 2011). In Luanda, many infants also arrived so ill that LP was considered too risky. Although a questionable decision, these children were not included in the analysis. Of note is our own finding from the same hospital that 17% of children with BM died soon after arrival at the emergency department (Pelkonen et al., 2008). These data were not available for this study.

This study has some limitations. As the series was collected in a single hospital in Luanda, the generalizability of the findings is limited. However, most children in greater Luanda with symptoms

and signs suggestive of BM primarily attend this institution or are referred here from other hospitals. We could not distinguish between community-acquired and hospital-acquired infections. The study was conducted in an urban setting, and there may be some differences (such as in aetiological agents) when compared with a rural setting. CSF culture results were not available for all patients. The PCR assay used detected *S. pneumoniae*, *H. influenzae*, and *N. meningitidis*, but no bacteria common in the neonatal period, such as *S. agalactiae* or other gram-negative bacteria. One may thus assume that the role of these other bacteria was underestimated. Furthermore, economic constraints limited the taking of blood cultures and antibiotic susceptibility testing of CSF isolates. Finally, individual patient data were obtained from the study forms and medical records, which were not always fully completed. The patients were included in the study at a very busy emergency department, which could have resulted in imprecise recording of the patient data.

Despite these limitations, we believe that the message of this study remains valid. BM is common among infants <90 days old in Luanda, and this potentially fatal disease is primarily caused by gram-negative bacteria that often are multiresistant. Continued surveillance of the agents and their susceptibility patterns is essential in order to provide the best available treatment.

Funding

This study was funded by Sanofi Pasteur. PCR at the NICD of CSF from suspected meningitis cases was funded by WHO-AFRO and NICD, as part of regional reference laboratory of vaccine-preventable invasive bacterial disease (VP-IBD) surveillance responsibilities. The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Ethical approval

The study was approved by the Ethics Committee of HPDB and the Hospital Director (January 6, 2016). Once informed by the attending physician, the guardians of enrolled infants provided consent by signing or fingerprinting a specific form.

Conflict of interest

MHK was an employee of Sanofi Pasteur when this study was conducted. SU and TP report grants from the Paediatric Research Foundation, and TP from the Päivikki and Sakari Sohlberg Foundation, Helsinki, Finland. All other authors declare no competing interests.

Author contributions

TP, AG, and MHK conceived and designed the study. TP, SU, EA, OC, and LG conducted the study. TP, EA, and LG analysed the data. TP, SU, EA, OC, LG, IR, HP, AG, and MHK interpreted the data. TP and MHK are accountable for the accuracy and integrity of the contents of the manuscript. All authors provided critical revisions of manuscript for the intellectual content. All authors approved the final draft of the manuscript.

Acknowledgements

The authors thank all participants who volunteered to take part in the study, the primary investigators, their site staff, and the Finnish Christian Medical Society for administrative assistance.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.06.016.

References

Bårnes GK, Gudina EK, Berhane M, Abdissa A, Tesfaw G, Abebe G, et al. New molecular tools for meningitis diagnostics in Ethiopia - a necessary step towards improving antimicrobial prescription. BMC Infect Dis 2018;18:684.
Cheesbrough M. District laboratory practice in tropical countries. Part 2. Cambridge, United Kingdom: Cambridge University Press; 2000.
Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Seventeenth Informational Supplement. CLSI document M100-S17. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.
Edmond K, Clark A, Korczak VS, Sanderson C, Griffiths UK, Rudan I. Global and regional risk of disabling sequelae from bacterial meningitis: a systematic review and meta-analysis. Lancet Infect Dis 2010;10:317–28.
Furyk JS, Swann O, Molyneux E. Systematic review: neonatal meningitis in the developing world. Trop Med Int Health 2011;16:672–9.
Gaschignard J, Levy C, Romain O, Cohen R, Bingen E, Aujard Y, et al. Neonatal bacterial meningitis: 444 cases in 7 years. Pediatr Infect Dis J 2011;30:212–7.
Gavi. The vaccine alliance. Angola country information. 2020 https://www.gavi.org/programmes-impact/country-hub/africa/angola. [Accessed 13 January 2020].
GBD 2016 Meningitis Collaborators. Global, regional, and national burden of meningitis, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 2018;17:1061–82.
Heath PT, Okike IO, Oeser C. Neonatal meningitis: can we do better?. Adv Exp Med Biol 2011;719:11–24.
Huynh BT, Kermorvant-Duchemin E, Herindrainy P, Padget M, Rakotoarimanana FMJ, Feno H, et al. Bacterial infections in neonates, Madagascar, 2012-2014. Emerg Infect Dis 2018;24:710–7.
Khalessi N, Afsharkhas L. Neonatal meningitis: risk factors, causes, and neurologic complications. Iran J Child Neurol 2014;8:46–50.
Maaroufi Y, De Bruyne JM, Heymans C, Crokaert F. Real-time PCR for determining capsular serotypes of *Haemophilus influenzae*. J Clin Microbiol 2007;45:2305–8.
Magomani V, Wolter N, Tempia S, du Plessis M, de Gouveia L, von Gottberg A. Challenges of using molecular serotyping for surveillance of pneumococcal disease. J Clin Microbiol 2014;52:3271–6.
Molyneux EM, Dube Q, Newberry L. Improving the outcome of bacterial meningitis in newborn infants in Africa: reflections on recent progress. Curr Opin Infect Dis 2015;28:215–20.
Mothershed EA, Sacchi CT, Whitney AM, Barnett GA, Ajello GW, Schmink S, et al. Use of real-time PCR to resolve slide agglutination discrepancies in serogroup identification of *Neisseria meningitidis*. J Clin Microbiol 2004;42:320–8.
Mwaniki MK, Talbert AW, Njuguna P, English M, Were E, Lowe BS, et al. Clinical indicators of bacterial meningitis among neonates and young infants in rural Kenya. BMC Infect Dis 2011;11:301.
Okike IO, Johnson AP, Henderson KL, Blackburn RM, Muller-Pebody B, neoMen Study Group, et al. Incidence, etiology, and outcome of bacterial meningitis in infants aged <90 days in the United Kingdom and Republic of Ireland: prospective, enhanced, national population-based surveillance. Clin Infect Dis 2014;59:e150–7.
Okomo U, Akpalu ENK, Le Doare K, Roca A, Cousens S, Jarde A, et al. Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: a systematic review and meta-analysis in line with the STROBE-NI reporting guidelines. Lancet Infect Dis 2019;19:1219–34.
Oordt-Speets AM, Bolijn R, van Hoorn RC, Bhavsar A, Kyaw MH. Global etiology of bacterial meningitis: a systematic review and meta-analysis. PLoS One 2018;13:e0198772.
Pelkonen T, Roine I, Monteiro L, Joao Simoes M, Anjos E, Pelerito A, et al. Acute childhood bacterial meningitis in Luanda, Angola. Scand J Infect Dis 2008;40:859–66.
Pimenta FC, Roundtree A, Soysal A, Bakir M, du Plessis M, Wolter N, et al. Sequential triplex real-time PCR assay for detecting 21 pneumococcal capsular serotypes that account for a high global disease burden. J Clin Microbiol 2013;51:647–52.
Reta MA, Zeleke TA. Neonatal bacterial meningitis in Tikur Anbessa Specialized Hospital, Ethiopia: a 10-year retrospective review. Springerplus 2016;5:1971.
Swann O, Everett DB, Furyk JS, Harrison EM, Msukwa MT, Heyderman RS, et al. Bacterial meningitis in Malawian infants <2 months of age: etiology and susceptibility to World Health Organization first-line antibiotics. Pediatr Infect Dis J 2014;33:560–5.
Unicef. Angola country profiles. 2020. https://data.unicef.org/country/ago/.
Urtti S, Cruzeiro ML, de Gouveia L, Peltola H, von Gottberg A, Kyaw MH, et al. Surveillance of bacterial meningitis in an Angolan pediatric hospital after the introduction of pneumococcal conjugate vaccines. J Glob Health Rep 2019;3:e2019091.
Wang X, Mair R, Hatcher C, Theodore MJ, Edmond K, Wu HM, et al. Detection of bacterial pathogens in Mongolia meningitis surveillance with a new real-time PCR assay to detect *Haemophilus influenzae*. Int J Med Microbiol 2011;301:303–9.

Wang X, Theodore MJ, Mair R, Trujillo-Lopez E, du Plessis M, Wolter N, et al. Clinical validation of multiplex real-time PCR assays for detection of bacterial meningitis pathogens. J Clin Microbiol 2012;50:702–8.

Wang Y, Guo G, Wang H, Yang X, Shao F, Yang C, et al. Comparative study of bacteriological culture and real-time fluorescence quantitative PCR (RT-PCR) and multiplex PCR-based reverse line blot (mPCR/RLB) hybridization assay in the diagnosis of bacterial neonatal meningitis. BMC Pediatr 2014;14:224.

World Health Organization. Bacterial meningitis (including *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis*, and *Streptococcus pneumoniae*). 2020. https://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/sentinel/meningitis_surveillance/en/.

World Health Organization. Defeating meningitis by 2030: baseline situation analysis. 2019. https://www.who.int/immunization/sage/meetings/2019/april/2_DEFEATING_MENINGITIS_BY_2030_baseline_situation_analysis.pdf?.