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An autochthonous case of Zika due to possible sexual transmission, Florence, Italy, 2014

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We report a case of Zika virus infection imported in Florence, Italy ex-Thailand, leading to a secondary autochthonous case, probably through sexual transmission. The two cases occurred in May 2014 but were retrospectively diagnosed in 2016 on the basis of serological tests (plaque reduction neutralisation) performed on stored serum samples. Our report provides further evidence that sexual transmission of Zika virus is possible.

Case reports

At the beginning of May 2014, an Italian man is his early 30s (patient 1) returned to Florence, Italy, after a 10-day holiday in Thailand. On the day after his arrival, he developed a confluent maculopapular rash, on the face, trunk, arms, and legs, with fever (maximum temperature 38 °C), conjunctivitis, and frontal headache with retroocular pain.

Four days later, patient 1 was admitted to the Infectious and Tropical Diseases Unit of the Florence Careggi University Hospital. Blood tests revealed leucopenia (3,000 cells/µL; reference: 4,000–10,000/µL) while creatinine, platelet count and transaminases were normal. Serological investigation two days after (i.e. 6 days after symptoms onset), showed past exposure to measles and parvovirus, negative results for human immunodeficiency virus (HIV) 1–2 Ab/Ag and chikungunya IgM, a positive result for dengue virus (DENV) IgM, and negative results for DENV IgG, as well as DENV NS1 Ag (Table).

The symptoms subsequently rapidly resolved (total duration of fever and rash: 6 days) and he was discharged nine days after admission with a probable diagnosis of DENV infection.

Perifocal vector control activities (including spraying adult mosquitoes and destruction of larval breeding sites) were implemented the day after the availability of DENV IgM positive results, around the patient's residence and workplace, even though the period of activity of *Aedes albopictus* in Italy is usually considered to start in June and end in October [1]. A second and third blood test using enzyme-linked immunosorbent assay (ELISA), performed 38 and 109 days after symptoms onset, showed DENV IgG seroconversion and IgM negativisation in the third sample.

Nineteen days after the onset of symptoms in patient 1, his girlfriend (patient 2), who was in her late 20s developed diffuse pain, associated to both wrists and oedema on fingers of each hand, maculopapular rash on the trunk, arms, and legs, without fever. Four days later she was evaluated at the outpatient facility of the same hospital. Patient 2 had not travelled to tropical areas during the previous year. Blood tests performed on the next day (5 days after her symptoms started) showed normal white blood cells and platelet count, normal C-reactive protein, creatinine, transaminases, and undetectable beta-human chorionic gonadotropin (HCG). The patient had IgG antibodies against cytomegalovirus, Epstein–Barr virus, parvovirus and rubella, while she was seronegative for coxsackie A, coxsackie B, echovirus and DENV (IgG, IgM and NS1 Ag). Serological tests were repeated 39 and 93 days after symptoms onset, respectively, showing a slight positivity for DENV IgG, with IgM and NS1Ag persistently negative (Table).

Retrospective testing of serum samples in 2015 and 2016

Serum samples of both patients were sent to the Istituto Superiore di Sanità (ISS), Rome, Italy, to perform confirmatory tests (Table) for DENV in June and September 2015, respectively. Plaque reduction neutralisation tests (PRNTs) for DENV gave inconclusive results for both patients: indeed, a 50% of plaque reduction was

Table

Laboratory diagnostic test results for dengue virus and Zika virus in two patients, Italy, 2014–2016

Patient	Days from onset of symptoms	Dengue virus tests						Zika virus tests	
		ELISA IgM[a,b]	ELISA IgG[a,b]	ELISA NS1[a,b]	ELISA IgM[a,c]	PRNT50[a,c] titre	Real-time PCR[a,c]	PRNT80[c,d] titre	Real-time PCR[a,c]
1	6	24.2	5.21	2.23	2.01	Neg	Neg	1:10	Neg
	38	12.3	16.6	NC	2.89	1:10 (b.l.)	NC	≥1:160	NC
	109	3.23	16.4	1.84	0.87	1:10 (b.l.)	NC	≥1:160	NC
2	5	1.34	4.63	3.81	0.46	Neg	Neg	1:10	Neg
	39	3.23	15.5	2.63	0.40	1:10 (b.l.)	NC	≥1:160	NC
	93	2.51	13.2	2.77	0.34	1:10 (b.l.)	NC	≥1:160	NC

b.l.: borderline; ELISA: enzyme-linked immunosorbent assay; NC: not conducted; Neg: negative; PRNT: plaque reduction neutralisation tests; PCR: polymerase chain reaction.

[a] Test performed in 2014.

[b] Tests performed at Azienda Ospedaliero Universitaria Careggi, Florence (Italy). Commercial ELISA (VIRCELL Granada-Spain). Reference values (index): ›11: positive; 9–11: inconclusive; ‹9: negative. Positive results are highlighted in bold.

[c] Tests performed at the Istituto Superiore di Sanità, Rome (Italy). Commercial IgM-capture ELISA system (Focus Diagnostics dengue Virus IgM Capture, DxSelect, California, US). Reference values (index): ›1: positive; ‹1: negative. Positive results are highlighted in bold. Real-time PCRs were conducted on RNA from serum samples, as described in [29] and [30]. Dengue virus for PRNT: serotype 2 dengue virus (New Guinea B strain). PRNT80 titres ≥1:10 are considered positive, while PRNT50 titres ≥1:10 are considered as borderline.

[d] Test retrospectively performed in 2016 on stored samples. Zika virus for PRNT was kindly provided by Dr Isabelle Leparc-Goffart of the French National Reference Center on Arboviruses in Marseille. The test was performed as described in detail for tick-borne encephalitis virus [31], except that Vero cells were used here.

observed at a 1:10 serum dilution in the second and third serum samples of both patients, while we consider the cut-off for a positive result to be at least 80% of plaque reduction. Real-time polymerase chain reaction (PCR) tests for DENV, chikungunya virus (CHIKV), and Zika virus (ZIKV), as well as viral isolation in Vero E6 cell, were also performed on samples collected in the acute phase of the disease, all with negative results.Even though DENV PRNT results were inconclusive, patient 1 was counselled as having had dengue infection, given the history of travel and the classical kinetic of IgG and IgM antibodies measured by ELISA, while we were not able to state a definitive diagnosis for patient 2.After ZIKV for PRNT became available to us, the samples were reanalysed in February 2016 (the patients had given their informed consent for further tests), and showed positive results for ZIKV neutralising antibodies, as reported in the Table, with a clear increase in the antibody titre between the first and the second serum sample for both patients.

Background

ZIKV is an *Aedes*-borne virus (Flaviviridae family), identified in 1947 in monkey rhesus in Uganda [2,3]. Sporadic human cases were reported in Asia and Africa until 2007, when a ZIKV outbreak occurred in Yap, Micronesia [4]. Subsequently, in October 2013, ZIKV reached French Polynesia, causing a large outbreak [5]. In early 2015, autochthonous cases of ZIKV were reported in Brazil [6], and the virus subsequently spread throughout South America, Central America, and the Caribbean [7-9]. An increasing number of imported cases has been observed in Europe and United States (US) [10-13]. The presumed association of ZIKV infection during pregnancy with increased number of babies born with microcephaly in Brazil [14] convinced the World Health Organization to declare ZIKV a 'Global Emergency of Public Health Concern' in February 2016 [15].

Discussion and conclusions

Even if ZIKV transmission is mostly vectorial, transplacental and perinatal transmission have been reported; transmission through blood transfusion may also occur [16-18].

Little evidence supports the possibility of ZIKV sexual transmission to date. In December 2013, ZIKV was isolated from the semen of a patient with haematospermia in Tahiti [19]. Further in 2014, ZIKV RNA was detected 62 days after onset of febrile illness in the semen of a person with ZIKV infection, imported into the United Kingdom from the Cook Islands [20]. Sexual transmission from a man who acquired ZIKV infection in Senegal, to his wife was reported in Colorado, US, in 2007 [21], and more recently from a person who had travelled to Latin America, to his partner in Texas [22].

Possible sexual transmission of ZIKV is of particular concern during pregnancy, and specific guidelines for prevention of ZIKV infection through this route have been published recently [23].

Because patient 2 had not travelled to tropical areas during the previous year and had unprotected sexual intercourse with patient 1 during a 20 day period between his return to Italy and her own onset of symptoms, transmission by semen was suggested. Exact dates of sexual intercourse could not be recalled by the patients, who reported several sexual contact events before patient 2's symptom onset. Other transmission modalities (i.e. direct contact with other bodily fluids)

are unlikely to play a role but may not be completely ruled out.

Transmission through local potentially competent vectors, *Ae. albopictus*, can likely be excluded considering that patient 1 came back to Italy outside the usual period of vector activity and vector control measures were implemented within eight days after his arrival to Italy, possibly before the estimated extrinsic incubation period could be completed [1,24].

Failure to detect viral RNA even in samples collected few days after the onset of symptoms, and an early detection of ZIKV-specific neutralising antibodies, are consistent with previous reports [10,19,25]; however, limits in the sensitivity of the real-time PCR method used in this study cannot be definitively excluded. Serological test results confirm the broad cross-reactivity between DENV and ZIKV. With respect to PRNT results, borderline results for DENV are likely to be due to a low degree of residual cross-reactivity which may not be eliminated even using this test, which is considered highly specific. Another possible limit of our study consists in the fact that only serotype 2 DENV PRNT could be performed; however, this is not likely to affect the interpretation of the results, which clearly show a pattern consistent with ZIKV infection.

Current evidence supports the combined use of PCR and serological tests for the diagnosis of ZIKV infection. PCR can be positive in early serum and saliva samples (‹ 8 days after symptoms onset), with saliva showing higher detection rates, while PCR on urine seems to enlarge the window of detection of ZIKV RNA up to ca 30 days after symptoms onset [26,27]. Five days after disease onset, serological investigations can be conducted by detection of ZIKV-specific IgM antibodies and confirmation by neutralisation [28].

In conclusion, we provide additional evidence for sexual transmission of ZIKV. Further studies are needed to estimate the probability of sexual transmission and its role as a secondary route of transmission of ZIKV in epidemic and non-epidemic areas.

Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: LZ, GR, GV, AB; performed laboratory investigations: AM, CF, MER, EB, CF, GV; revised the manuscript: GR, MT, CR; managed the patients: LZ, MT.

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Impact of food animal trade on the spread of *mcr-1*-mediated colistin resistance, Tunisia, July 2015

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We report a high prevalence of MCR-1 and CTX-M-1-producing *Escherichia coli* in three Tunisian chicken farms. Chickens were imported from France or derived from French imported chicks. The same IncHI2-type plasmid reported to carry those genes in cattle in France and in a food sample in Portugal was found in Tunisian chickens of French origin. This suggests a significant impact of food animal trade on the spread of *mcr-1*-mediated colistin resistance in Europe.

Horizontal transfer was found to play a major role in the spread of colistin resistance in *Enterobacteriaceae* when a plasmid-located *mcr-1* gene was reported to be circulating in livestock, foodstuff and human beings in China in late 2015 [1]. A few weeks later, *mcr-1* was recognised in Europe among extended-spectrum beta-lactamase (ESBL)- or AmpC-producing *Escherichia coli* isolated from chicken meat and humans [2]. In January 2016, the worldwide distribution of the *mcr-1* gene was highlighted [3,4].

The plasmid type first identified as a *mcr-1* vehicle in China was an IncI2-like plasmid, but several different *mcr-1*-positive plasmids have now been reported, including IncHI2-type plasmids. Indeed, Tse et al. reported *mcr-1* on an IncHI2-type plasmid in a *Salmonella enterica* isolate from a food sample in Portugal in 2011 [5]. Interestingly, IncHI2-type plasmids were also recognised to spread $bla_{CTX\text{-}M\text{-}1}$ and *mcr-1* in *E. coli* in food animals in France [6]. These data suggest a specific epidemiology of *mcr-1* plasmids in the European animal reservoir that pose a risk for humans. This prompted us to investigate 37 *E. coli* strains recovered from 29 Tunisian chickens imported from France or derived from French imported chicks and harbouring resistance to colistin and broad-spectrum cephalosporins.

Detection of the $bla_{CTX\text{-}M\text{-}1}$ and *mcr-1* genes in healthy chickens in Tunisia

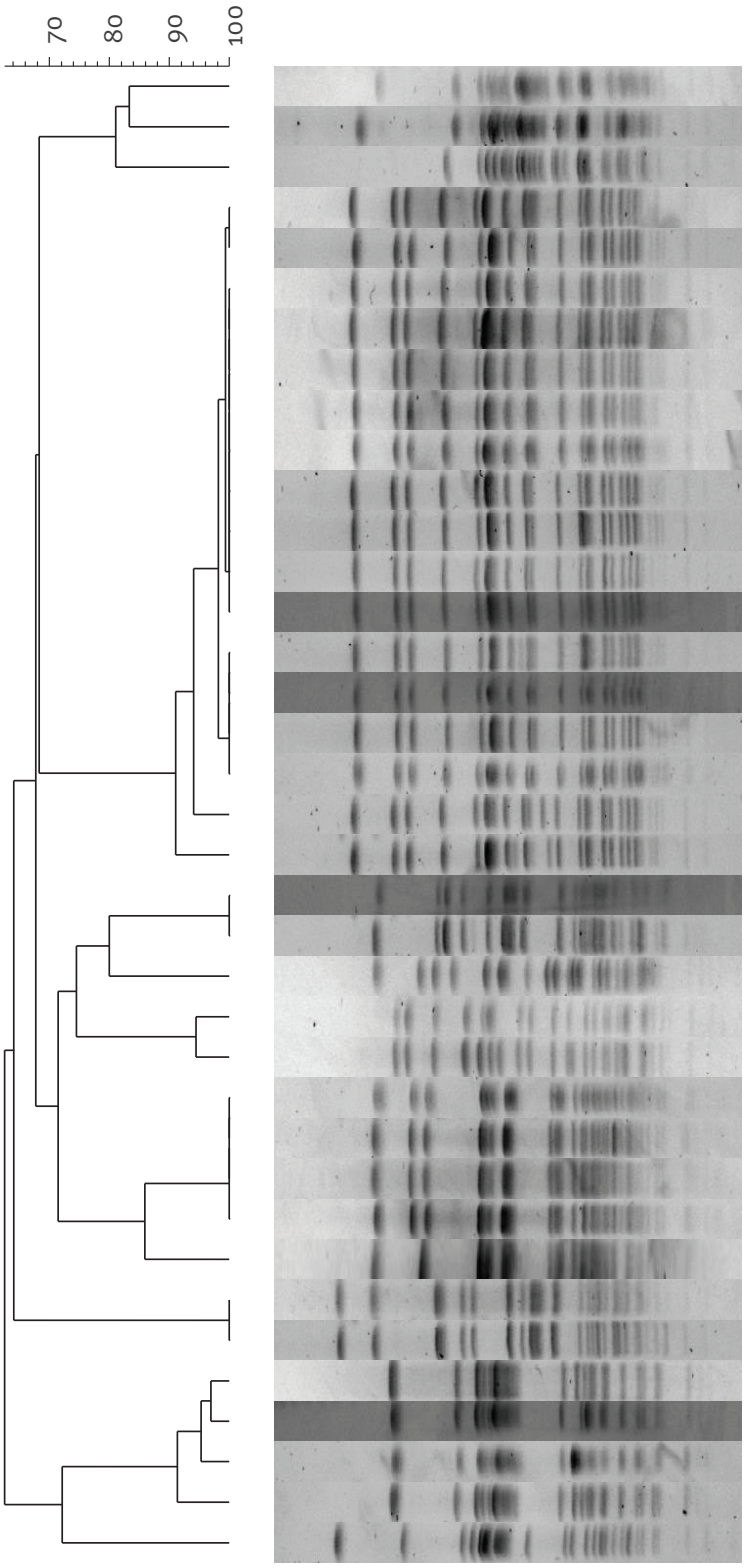
In July 2015, 52 randomly chosen healthy birds were collected on three different Tunisian farms: 10 on farm A, 12 on farm B and 30 on farm C with the initial purpose to investigate the prevalence of ESBL-positive chickens. A faecal sample of each individual was plated on MacConkey agar containing 4 mg/L cefotaxime and one colony per morphology was picked up. This resulted in the identification of 37 *E. coli* isolates harbouring resistance to broad-spectrum cephalosporins and originating from 29 birds (Table).

Those 29 birds were from farm A (2/10), farm B (2/12) and farm C (25/30). All 37 isolates produced an ESBL as attested by the synergy test, and the $bla_{CTX\text{-}M\text{-}1}$ gene was identified in all isolates by PCR and sequencing. All isolates expressed additional co-resistances to phenicols, tetracyclines, sulfonamides, trimethoprim, quinolones and fluoroquinolones as determined by disk diffusion against 32 antibiotics. Surprisingly, disk diffusion also revealed small colistin diameters (16–17 mm). We were in the course of investigating these non-susceptible isolates when the publication by Liu et al. [1] drew a different light on our results and prompted us to further investigate colistin resistance.

All isolates presented a minimum inhibitory concentration (MIC) of 2–6 µg/mL to colistin by E-test. PCR and sequencing using published primers [1] revealed the newly described *mcr-1* gene in all of the ESBL-positive *E. coli* with 100% homology to the published sequence (GenBank: KP347127.1). Isolates from farm A presented two closely related but not identical *Xba*I pulsed-field gel electrophoresis (PFGE) patterns (one band difference) belonging to cluster G, while isolates from farm B presented two distinct patterns belonging to the clusters F and K (Figure 1).

Figure 1

Pulsed-field gel electrophoresis-based dendrogram and *Xba*I macrorestrictions, Tunisia, July 2015 (n = 37)



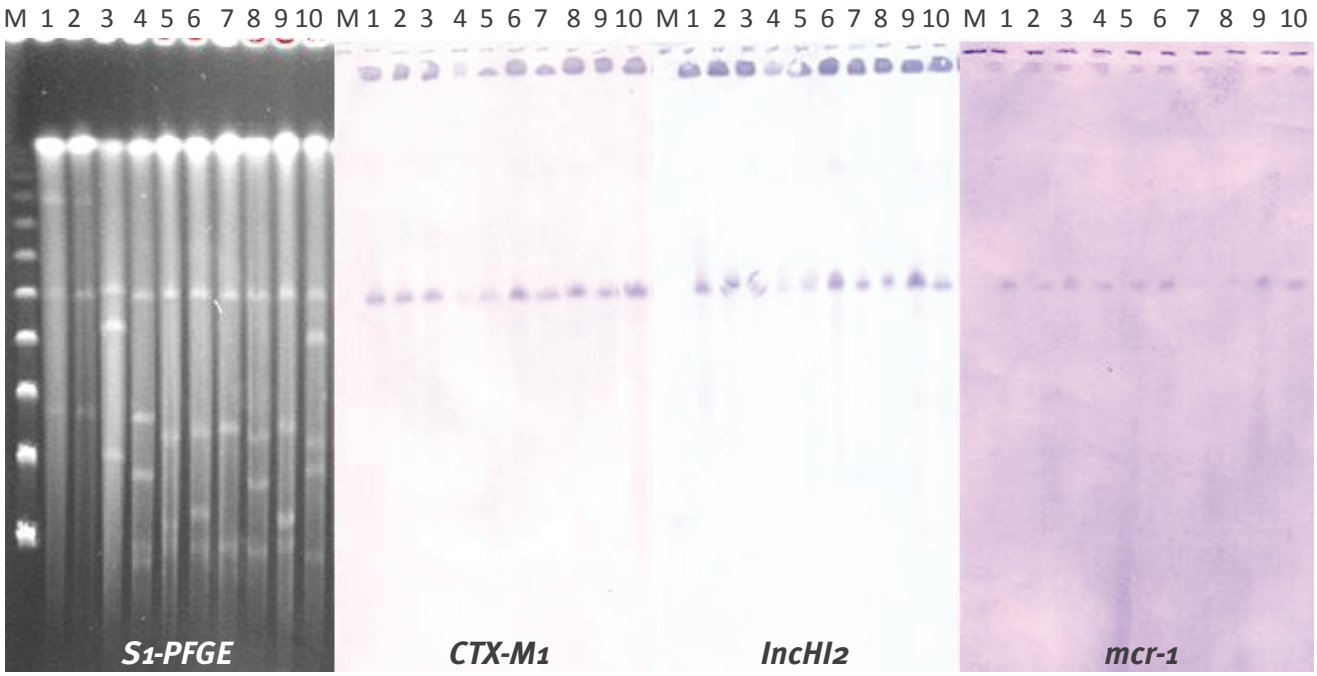
Isolate	Farm	MIC (µg/mL)	PFGE cluster
24B	C	3	A
33B	C	6	B
39B	C	3	C
19A	C	3	D
33A	C	3	D
14	C	6	D
18B	C	4	D
22	C	3	D
23	C	3	D
24A	C	3	D
26	C	4	D
32B	C	2	D
40	C	5	D
41	C	5	D
32A	C	3	D
42A	C	5	D
36A	C	3	D
39A	C	3	D
25A	C	6	D
30	C	4	D
21A	C	4	E
25B	C	3	E
10	B	6	F
MF5	A	3	G
MF8	A	5	G
15	C	3	H
16A	C	4	H
17A	C	3	H
20	C	4	H
18A	C	3	I
16B	C	3	J
31A	C	3	J
12	B	3	K
42B	C	3	K
36B	C	3	K
38B	C	4	K
38A	C	3	L

MIC: minimum inhibitory concentration; PFGE: pulsed-field gel electrophoresis.

Analysis was performed using the Dice coefficient with optimisation set at 0.5% and tolerance at 1.5%.

Figure 2

Southern blot hybridisations on S1 nuclease-pulsed-field gel electrophoresis gels using specific probes for the detection of $bla_{CTX\text{-}M\text{-}1}$, IncHI2 and *mcr-1*, Tunisia, July 2015 (n = 10)



PFGE: pulsed-field gel electrophoresis

M: size marker (Lambda ladder 0.05-1 Mb, Bio-Rad); Lane 1: isolate MF5; Lane 2: isolate MF8; Lane 3: isolate 10; Lane 4: isolate 12; Lane 5: isolate 14; Lane 6: isolate 16A; Lane 7: isolate 16B; Lane 8: isolate 18A; Lane 9: isolate 21A; Lane 10: isolate 24B.

All 37 isolates presented the same profile, so that only a subset of 10 isolates is presented here.

Isolates from farm C presented one main cluster (cluster D encompassing 17 isolates presenting patterns with › 90% similarity) and nine additional clusters (A–C, E, H–L) presenting patterns with ‹ 90% similarity. Antibiotics used were colistin, sulfonamides and enrofloxacin on farms A and C, and chloramphenicol and enrofloxacin on farm B.

Co-localisation of $bla_{CTX\text{-}M\text{-}1}$ and *mcr-1* on IncHI2-type plasmids

Replicon typing and hybridisation experiments proved that $bla_{CTX\text{-}M\text{-}1}$ and *mcr-1* co-localised in all isolates on a single and large (250–280 kbp) IncHI2-type plasmid (Figure 2).

According to the plasmid double locus sequence typing (pDLST) scheme [7], these IncHI2-type plasmids belonged to the ST4 subtype and presented positive amplification of the *hipA* gene and no amplification of the *smr092* and *smr0183* genes [7]. Interestingly, the IncHI2-type plasmids recently found in food animals in France also belonged to the very same ST4 subtype (data not shown) [6]. Hence, IncHI2-type plasmids were responsible for the spread of $bla_{CTX\text{-}M\text{-}1}$ and *mcr-1* in different chicken farms in Tunisia, in the bovine sector in France and in foodstuff in Portugal.

High prevalence of *mcr-1*-positive chickens on Tunisian farms

Data on *mcr-1* from the poultry reservoir are lacking except for one single case in Algeria [8]. However, *mcr-1* reports from chicken meat have been recurrent [1,2,9,10]. Here, farms A and C (counting 7,500 and 8,500 chickens, respectively) host grandparent flocks and import one-day-old chicks from France (Table). Farm B is located 80 km apart from the others and rears 200,000 broilers deriving from one-day-old chicks sold by a Tunisian hatchery also importing birds from France. Thus, the estimated true prevalence (with confidence intervals at 95%) of *mcr-1*-positive chickens reaches 20% (3–56%) on farm A, 17% (4–49%) on farm B and 83% (65–94%) on farm C. This last figure is even higher than recently reported from food animals in China [1].

Conclusion

From this study, we conclude that the live chicken population in Tunisia is heavily colonised by *mcr-1*-positive *E. coli* with subsequent possible contamination

Table

Epidemiological and molecular features of $bla_{CTX\text{-}M\text{-}1}$/mcr-1-positive *Escherichia coli*, Tunisia, July 2015 (n = 37)

Farm	Location	Number of birds on farm	Age of birds	Origin of the birds	Number of birds sampled	Number of ESBL-positive birds	Number of *mcr1* and bla_{CTXM_1}-positive *E. coli*	Plasmid type carrying bla_{CTXM_1} and *mcr1*
Farm A	Moknine	8,500	17–18 weeks	France	10	2	2	IncHI2/ST4
Farm B	Enfidha	200,000	35 days	Tunisia/France	12	2	2	IncHI2/ST4
Farm C	Moknine	7,500	62 weeks	France	30	25	33[a]	IncHI2/ST4

ESBL: extended-spectrum beta-lactamase.

[a] One colony per morphology was picked up, resulting in a higher number of *E. coli* isolates than the number of samples.

of chicken products [11,12]. Multilocus sequence typing (MLST) was not performed in this study since PFGE demonstrated the presence of numerous clusters of *E. coli* (A to L) so that the *mcr-1* dissemination was clearly a consequence of the spread of the unique IncHI2/ST4 plasmid in various *E. coli* backgrounds.

Contamination of both the poultry production pyramid and the food chain is undoubtedly of public health relevance. It is now crucial to determine the prevalence of the *mcr-1* gene in poultry and poultry meat as well as in other livestock (live animals or meat) in Tunisia and other African countries in order to estimate the risk to human health.

In addition, the finding of a single IncHI2-type plasmid spreading the $bla_{CTX\text{-}M\text{-}1}$/*mcr-1* genes in the food sector in different European and non-European countries makes us believe that global imports and exports of food animals and foodstuff are a major determinant of *mcr-1* dissemination. Global chicken meat production is forecast to dramatically increase in the future because of rising demands worldwide and subsequent rising production volumes in the major exporting countries. European countries already faced a major spread of ESBL/pAmpC genes in animals that subsequently became ESBL sources for humans, mostly as a result of poultry trades [13,14]. Worryingly, genes providing resistance to broad-spectrum cephalosporins and colistin have been shown to be tightly linked on the same plasmids, indicating that urgent international attention is necessary on the global market of veterinary drugs for food animals.

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Conflict of interest

None declared.

Authors' contributions

RG collected the isolates, collected the data and performed the molecular analysis. MH, WM, and JYM coordinated the work and participated to the data analysis. MH and JYM drafted the manuscript, WM and RG participated in the writing of the manuscript, and all authors have read and accepted the submitted manuscript.

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Review

Neurological sequelae of healthcare-associated sepsis in very-low-birthweight infants: Umbrella review and evidence-based outcome tree

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Sepsis is a frequent cause of death in very-low-birthweight infants and often results in neurological impairment. Its attributable risk of sequelae has not been systematically assessed. To establish an outcome tree for mapping the burden of neonatal sepsis, we performed systematic literature searches to identify systematic reviews addressing sequelae of neonatal sepsis. We included cohort studies and performed meta-analyses of attributable risks. Evidence quality was assessed using GRADE. Two systematic reviews met inclusion criteria. The first included nine cohort studies with 5,620 participants and five outcomes (neurodevelopmental impairment, cerebral palsy, vision impairment, hearing impairment, death). Pooled risk differences varied between 4% (95% confidence interval (CI):2–10) and 13% (95% CI:5–20). From the second review we analysed four studies with 472 infants. Positive predictive value of neurodevelopmental impairment for later cognitive impairment ranged between 67% (95% CI:22–96) and 83% (95% CI:36–100). Neonatal sepsis increases risk of permanent neurological impairment. Effect size varies by outcome, with evidence quality being low to very low. Data were used to construct an outcome tree for neonatal sepsis. Attributable risk estimates for sequelae following neonatal sepsis are suitable for burden estimation and may serve as outcome parameters in interventional studies.

Introduction

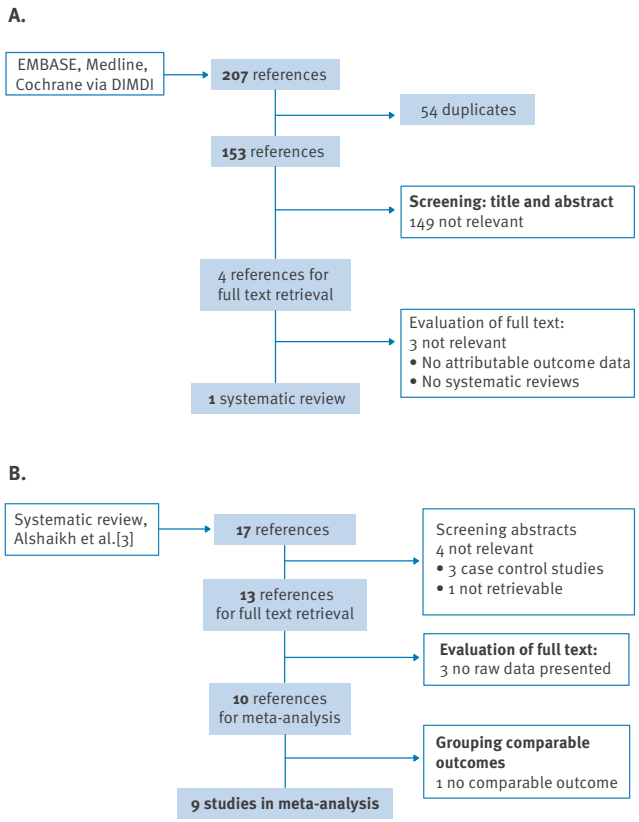
Sepsis is a major cause of death in neonates [1]. The majority of sepsis episodes (>80%) occurs in preterm neonates [2]. Among very low birth weight infants (VLBW; <1,500g), rates of sepsis range between 11% and 46% [3]. Sepsis in this high-risk population is mostly acquired during hospital stay with a late onset beyond 48–72 hours of life. Early onset sepsis, which becomes apparent within the first 48–72 hours of life is 'nosocomial' in the sense that it occurs in the hospital but should not be considered healthcare-associated because its origin is linked to childbirth and/or maternal-fetal transmission of pathogens [4,5].

Neonatal sepsis and systemic inflammatory response syndrome (SIRS) are associated with brain damage that results in disability, particularly among preterm and VLBW infants [6-9]. However, adverse neurological outcomes frequently occur in VLBW infants for reasons other than sepsis [10]. Therefore, the impact of healthcare-associated neonatal sepsis on adverse outcome is difficult to establish.

A European consortium, as part of a project initiated and funded by the European Centre for Disease Prevention and Control (ECDC), recently developed an incidence- and pathogen-based approach for estimating the burden of communicable diseases expressed in Disability Adjusted Life Years (DALYs) [11]. Relevant health outcomes of communicable diseases are represented by outcome trees which map the weighted progressions of diseases over time by ordering the conditional probabilities of associated health outcomes [11]. Outcome trees take into account probabilities and duration of health outcomes. The burden of healthcare-associated infections (HAIs) was not yet addressed by ECDC for two reasons: (i) Patients with HAIs differ from the general population in terms of comorbidities [7] and may be different regarding other factors as for example certain risk behaviour and social determinants; (ii) HAIs cannot be allocated to a specific pathogen, and thus the pathogen-based approach is not applicable.

Figure 1

Selection process for (panel A) systematic review of systematic reviews and (panel B) primary studies on neurological sequelae of neonatal sepsis, umbrella review on neurological sequelae of healthcare-associated sepsis in very-low-birthweight infants, date of search 25 September 2013



DIMDI: Deutsches Institut für Medizinische Dokumentation und Information.

Statements about the burden of communicable diseases must be based on evidence-based medicine principles as in other fields of medicine. Systematic reviews have become the gold standard of assessing the evidence in medicine but they are time consuming and expensive. Systematic reviews of systematic reviews (so-called 'umbrella reviews') offer a time-saving alternative to identify and exploit the current state of evidence in a field [12,13]. The aim of the study was to identify the relevant sources for the construction of an evidence-based outcome tree for neurological sequelae due to healthcare-associated neonatal sepsis in VLBW infants by systematically identifying and analysing existing systematic reviews that addressed neurodevelopmental impairment during infancy. From a clinical perspective, we aimed at investigating the extent by which sepsis in VLBW infants causes neurological impairments. Ultimately, the resulting outcome tree will constitute the basis for the estimation of the burden of hospital-acquired neonatal sepsis expressed in disability-adjusted life years (DALYs), within the general framework of the ECDC Burden of Communicable Diseases in Europe project (BCoDE).

Methods

We followed the approach described by Whitlock et al. [14] and Robinson et al. [15] to identify and exploit existing systematic reviews by re-analysing their data. Our study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) [16].

Identification of studies

In a first step, we performed a systematic review of systematic reviews (i.e. an umbrella review) on the association between neonatal sepsis and neurodevelopment in later life. To identify relevant systematic reviews we performed a systematic literature search using MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL), without language restrictions (Box 1). All systematic reviews published from 1 January 2000 until 25 September 2013 were eligible if meeting predefined inclusion criteria (see below).

A further search for planned, ongoing and published systematic reviews was performed in the Prospective International Register of Systematic Reviews (PROSPERO).

In a second step, we performed an umbrella review on the positive predictive value of neurodevelopmental impairment for later cognitive function. To identify appropriate systematic reviews, we performed a systematic literature search (date of last search: 2 July 2014) using MEDLINE, EMBASE and the Cochrane Central Register of Controlled Trials (CENTRAL) (Box 2).

Electronic search was complemented by manually checking the reference lists of identified reviews and studies.

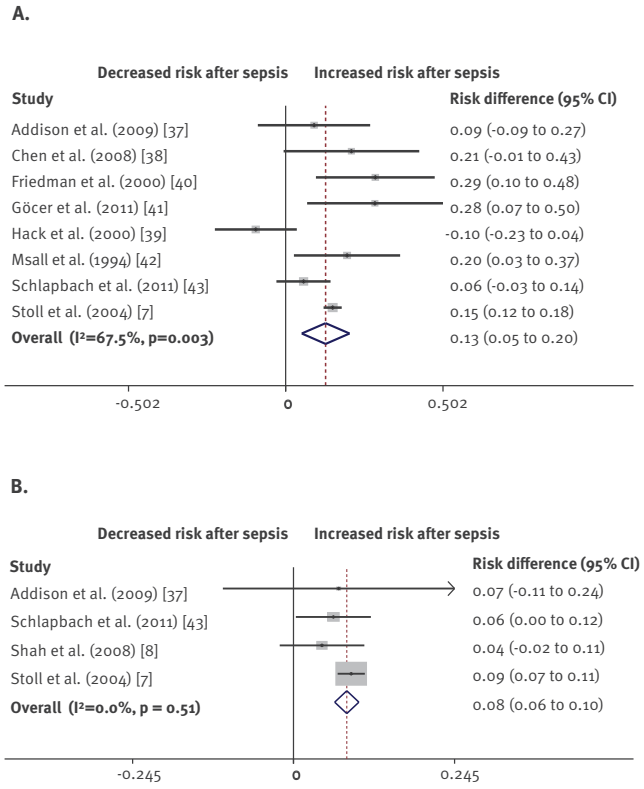
Study eligibility

In a first step, we searched for systematic reviews on the association between neonatal sepsis and neurodevelopment in later life. These systematic reviews had to capture primary studies which fulfilled the following inclusion criteria: (i) study population had to be neonates; (ii) the exposure had to be sepsis acquired in a healthcare setting; (iii) the comparator (or control) had to be participants without sepsis; (iv) the studies included had to be cohort studies or clinical trials; (v) the studies had to investigate at least one neurodevelopmental outcome during follow-up, and (vi) the studies had to be conducted in a healthcare setting within an upper-middle- or high-income country [17]. An expert panel discussed and agreed on the addressed outcomes to be relevant (for names and affiliations of the members of the expert panel, see Acknowledgements).

In a second step, the systematic reviews were searched for positive predictive values of neurodevelopmental impairment for later cognitive function. To be eligible,

Figure 2

Forest plot of risk differences of (panel A) impaired neurodevelopment (mental development index < 70) and (panel B) cerebral palsy in neonates with sepsis compared with those without sepsis, umbrella review on neurological sequelae of healthcare-associated sepsis in very-low-birthweight infants, date of search 25 September 2013



CI: confidence interval; MDI: Mental Developmental Index.

Studies are ordered alphabetically by first author. The pooled risk differences (overall; diamonds) were calculated by means of a random-effects model. Ninety-five percent CIs are shown in parentheses and as horizontal bars.

Neurodevelopmental impairment was defined as having a MDI<70 [18].

a systematic review had to fulfil the following inclusion criteria: (i) participants of the included studies had to be infants (aged 1–6 years) at first examination; (ii) neurodevelopmental impairment had to be measured by the Bayley Scales of infant development [18] at first examination; (iii) cognitive function (intelligence quotient) had to be measured by a standardised test (e.g. Wechsler Intelligence Scale [19]) at second (follow-up) examination; (iv) a positive predictive value or data allowing its calculation had to be reported in the review or in the studies analysed in the review.

Data extraction

Two independent reviewers (SH and TH) screened the systematic reviews, located the primary studies analysed in the reviews and extracted the following data: citation, study period, study design, demographics, sex, ethnicity, definition of sepsis, definition of outcome, length of follow-up, number of exposed and non-exposed with outcome, test used for first and second examination, positive predictive value and prevalence of condition. Discrepancies between the reviewers were solved by discussion until a consensus was reached.

Risk of bias assessment

The assessment of multiple systematic reviews (AMSTAR) tool was used to determine the methodological quality of the systematic reviews included [20]. Risk of bias in the included cohort studies was assessed using the Newcastle Ottawa Scale [21]. Following the suggestions of the Cochrane Collaboration, we assessed risk of bias separately for each outcome in each study [22]. For the studies reporting positive predictive values, the Scottish Intercollegiate Guidelines Network (SIGN) checklist for diagnostic accuracy studies was used to assess risk of bias [23]. The results of the risk of bias assessments were expressed in terms of 'high risk of bias', 'low risk of bias' and 'unclear risk of bias'. All risk of bias assessments were conducted by two independent investigators (SH and TH).

Assessment of the quality of the body of evidence

We adapted the methodology of the Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group to assess the quality of the body of evidence [24,25]. The GRADE methodology was initially developed to assess intervention studies. According to GRADE, the quality of evidence indicates the extent to which one can be confident that the estimate of effect is correct. Taking into account the entire body of evidence on one outcome, four levels of evidence quality are applied: +, very low; + +, low; + + +, moderate; and + + + +, high. Adapting the original GRADE approach and considering the proposal by Huguet et al. [26], all bodies of evidence were initially graded as high quality of evidence. Considering the following criteria led to decreasing evidence quality: (i) risk of bias, (ii) inconsistency, (iii) indirectness, (iv) imprecision and (v) publication bias (for details on the criteria, see [24].

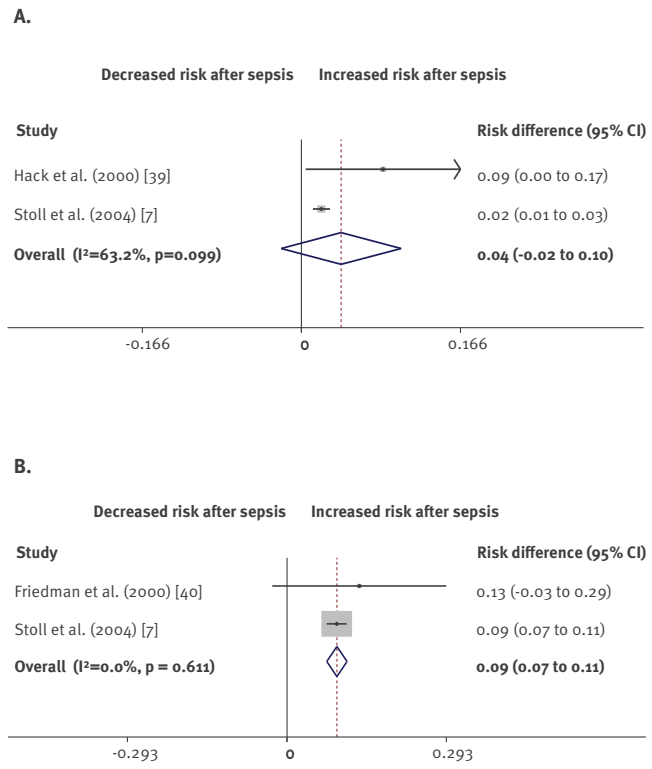
Data synthesis

Extracted study characteristics and data were summarised in tables, together with risk of bias assessments. For data synthesis, the following two effect measures were used:

- Risk differences were applied to calculate attributable risk to sepsis exposure as follows: Using data of the individual studies we subtracted the absolute risk of developing sequelae in controls from the risk of developing sequelae in cases (infected minus uninfected and corresponding 95% confidence intervals).

Figure 3

Forest plot of risk differences of (panel A) hearing impairment and (panel B) vision impairment in neonates with sepsis, compared with neonates without sepsis, umbrella review on neurological sequelae of healthcare-associated sepsis in very-low-birthweight infants, date of search 25 September 2013]



CIs: confidence intervals.

Studies are ordered alphabetically by first author. The pooled risk differences (overall; diamonds) were calculated by means of a random-effects model. Ninety-five percent CIs are shown in parentheses and as horizontal bars.

- Positive predictive values were used to estimate the probability that an individual with an adverse neurodevelopmental outcome during infancy continues to suffer from impairment during later life. Positive predictive values were either taken directly from the publications or calculated using the reported data as follows: number of true positives divided by the sum of true positives and false positives.

Risk differences were pooled across the studies, using the 'metan command' in Stata (Stata 12, Stata Corp, TX, US). In the presence of heterogeneity, a random-effects model was used. Otherwise, study data were combined using a fixed-effects model. I^2, a direct measure of inconsistency of study results in a meta-analysis, was used to quantify the extent of heterogeneity. I^2 ranges from 0% to 100%, with 0% indicating no inconsistency. Because the numbers of studies were too small (<10), publication bias was not investigated. Positive predictive values were not pooled but rather presented as a range of values to account for heterogeneity [27].

Development of the outcome tree

The results of the systematic reviews were used to construct an outcome tree, based on the methodology described by Kretzschmar et al. [11]. An outcome tree maps the weighted progression of a disease over time by ordering the conditional probabilities of associated health outcomes. Blocks indicate health outcomes. Arrows indicate transition between outcomes (e.g. the transition from neurodevelopmental impairment to permanent cognitive impairment). Attributable risks (i.e. risk differences) that derived from the systematic review were attached to the respective blocks and arrows.

Results

Neurodevelopmental sequelae of neonatal sepsis

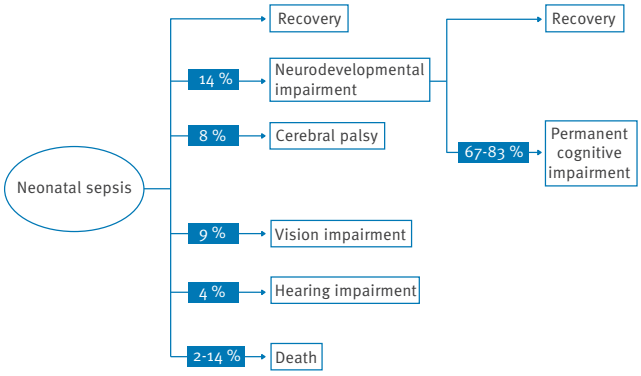
Our search identified a total of 207 titles (Figure 1A). After eliminating duplicates and screening of titles and abstracts four publications were left for full text evaluation. Of these, only one systematic review fulfilled the inclusion criteria and thus, was eligible for further analysis [3], whereas the remaining three publications were not eligible [28-30]. This systematic review was of acceptable methodological quality (AMSTAR summary score: 7/11). The review included 17 original studies reporting data on neurodevelopmental sequelae of neonatal sepsis in VLBW infants. We screened the abstracts and full texts of these studies and identified nine of them to be eligible (see Figure 1B), whereas seven did not fulfil the inclusion criteria [6,31-36] and one citation could not be located in data banks or libraries.

All nine included studies [7,8,37-43] were cohort studies. Details are shown in Table 1. The studies included a total of 5,620 neonates born between 1983 and 2007 and were conducted in six upper-middle and high-income countries. Three studies provided data on infants with extremely low birth weight (ELBW; <1,000g) [7,40,44]. A further three studies reported on neonates with VLBW [37,38,41], whereas the remaining three studies based their inclusion criteria on gestational age [8,42,43]. Eight studies provided a definition of neonatal sepsis that was based on clinical and/or laboratory parameters. One study did not provide a definition [41]. One study reported on invasive *Candida* spp. infections only [40]. Duration of follow-up varied between 12 and 52 months.

From the reported outcomes we considered the following five outcomes as clinically relevant: neurodevelopmental impairment, cerebral palsy, vision impairment, hearing impairment and death. Neurodevelopmental impairment was defined as having a Mental Developmental Index (MDI) <70 [18]. For vision and hearing impairment, varying definitions were used in the studies. According to the Newcastle-Ottawa Scale [21], three studies had high risk of bias, while the remaining six showed a low risk of bias (Table 1).

Figure 4

Evidence-based outcome tree for neurological sequelae of neonatal sepsis, umbrella review on neurological sequelae of healthcare-associated sepsis in very-low-birthweight infants, date of search 2 July 2014



Outcomes (e.g. neurodevelopmental impairment) are shown in blocks. Arrows represent transitions between outcomes. Percentages (%) attached to arrows correspond to transitional probabilities between outcomes.

Figure 2A shows the results of the meta-analysis of risk differences for neurodevelopmental impairment in infants with neonatal sepsis, as compared with those without sepsis. Eight studies reported risk estimates. We calculated a statistically significant pooled risk difference of 13% (95% CI: 5–20), with a large and significant between-study heterogeneity. This heterogeneity was mainly due to the study by Hack et al. [44]. Excluding this study lowered heterogeneity (I^2 =32.6%; p=0.18) but had only a small impact on the pooled risk difference (15%; 95%CI: 9–20%). Since the study by Friedman et al. [40] was the only study in which the exposure was non-bacterial (*Candida* spp.), further sensitivity analysis was performed with six studies [7,37,38,41-44]. The pooled risk difference (14%; 95%CI: 9—–19%) did not differ largely from the estimate of the complete dataset, but showed lower heterogeneity (I^2=26.1%; p=0.24).

Figure 2B displays the single study estimates and the pooled risk difference for the outcome cerebral palsy, which was reported by four studies. Infants who experienced neonatal sepsis had an 8% (95% CI: 6–10) higher risk of developing cerebral palsy than those who did not. Study results were highly homogenous (I^2=0%).

Only two studies reported data on hearing impairment (Figure 3A) and vision impairment (Figure 3B) following neonatal sepsis. While there was a significant effect on vision impairment (9%; 95% CI: 7–11), the risk difference for hearing impairment was smaller and not significant (4%; 95% CI: -2 to 10).

Two studies analysed mortality in association with neonatal sepsis. From Friedman et al. [40] we could calculate a risk difference of 2% for invasive *Candida* spp. infection (95% CI: -13 to 17), while Msall et al. [42] provided data to calculate a risk difference of 14% for bacterial sepsis (95% CI: -5–to 33). Meta-analysis was not conducted because exposure and results were too heterogeneous.

Neither grouping of primary studies by birth weight, nor by publication date had an influence on risk differences of neurodevelopmental impairment or cerebral palsy. The number of studies was too small to allow stratified meta-analysis.

To systematically assess the quality of evidence for each outcome we applied the GRADE methodology. For neurodevelopmental impairment, the quality of the evidence had to be graded down by three levels: (i) for serious risk of bias, (ii) for serious inconsistency due to widely differing point estimates of the single studies, and (iii) for serious imprecision due to a wide CI around the pooled estimate. Therefore, evidence quality was only 'very low' for this outcome. Regarding cerebral palsy, very serious risk of bias (grading down two levels) led to an evidence quality of 'low' for this outcome. For the outcome vision impairment, evidence quality was graded down to 'moderate' due to serious risk of bias. Accounting for serious risk of bias and serious imprecision, evidence quality was graded down to 'low' for the outcome hearing impairment.

Predictive value of early neurodevelopmental impairment for later cognitive function

Our search identified three potentially eligible reviews. After title and abstract screening, only one publication remained for full text screening [45]. This systematic review fulfilled our inclusion criteria and was therefore used as a database for further analysis.

The review was of acceptable methodological quality (AMSTAR summary score: 7/11). It contained a total of 18 publications that reported data on the relation between MDI scores during the first three years of life and cognitive function measured later in life in VLBW infants. After abstract and full text screening, four studies were eligible for further analysis [39,46-48], whereas the remaining 14 did not meet the inclusion criteria [49-62].

All included studies were cohort studies. Details are shown in Table 2. Studies accumulated a total of 472 infants of either VLBW (n=2 studies) or ELBW (n=2 studies) who were born between 1977 and 2004 in three different high-income countries. All four studies used the Bayley Scale of infant development to assess the proportion of infants with neurodevelopmental impairment (i.e. MDI<70) at 12 to 24 months, and re-evaluated the study sample at 3.4 to 8.6 years of age, using three different test batteries. According to the SIGN50 checklist [23], all four studies had a low risk of bias.

Table 1

Characteristics of included studies on neurodevelopment after neonatal sepsis, umbrella review on neurological sequelae of healthcare-associated sepsis in very-low-birthweight infants, date of search 25 September 2013

Reference	Location	Birth year(s)	Population	Definition of sepsis	Duration of follow-up	n[a]	Risk of bias[b]
Addison et al. (2009) [37]	US	1999–2001	VLBW	Positive blood culture; at least 7 days of antibiotics	12–18 months	65	High
Chen et al. (2008) [38]	Taiwan	1998–2005	VLBW	Culture-proven sepsis and unstable vital signs	18–39 months	122	Low
Friedman et al. (2000) [40]	Canada	1988–1996	ELBW	Positive-culture *Candida* spp. or supportive brain autopsy	24 months	299	Low
Göcer et al. (2011) [41]	Turkey	2002	VLBW	Not defined	33–45 months	117	High
Hack et al. (2000) [44]	US	1992–1995	ELBW	Positive blood culture and clinical signs	20 months	221	Low
Msall et al. (1994) [42]	US	1983–1986	23–28 weeks GA	Positive blood culture and 14–21 days antibiotics	52 months	149	Low
Schlapbach et al. (2011) [65]	Switzerland	2000–2007	24–28 weeks GA	Positive blood culture and clinical signs and antibiotics for ≥ 5 days	18–24 months	372	Low
Shah et al. (2008) [8]	Australia	2001–2003	<30 weeks GA	Positive blood culture and biomarker and antibiotics ≥ 5 days	24 months	192	High
Stoll et al. (2004) [7]	US	1993–2001	ELBW	Positive blood culture and antibiotics ≥ 5 days	18–22 months	4083	Low

ELBW: extremely low birth weight; GA: gestational age; US: United States; VLBW: very low birth weight.

[a] Study size

[b] According to Newcastle-Ottawa scale [21]

Table 2

Characteristics of included studies on predictive value of early neurodevelopmental impairment for later cognitive function, umbrella review on neurological sequelae of healthcare-associated sepsis in very-low-birthweight infants, date of search 2 July 2014

Reference	Location	Birth year(s)	Population	Test at first examination	Test at second examination	Age at first test	Age at second test	n[a]	PPV (95% CI)	Prevalence of MDI<70	Risk of bias[b]
Munck et al. (2012) [47]	Finland	2001–2004	VLBW	Bayley Scale vers. Two (MDI<70)	Wechsler Scale (FSIQ<70)	2 years (corrected)	5 years	124	83% (36–100)	4%	Low
Hack et al. (2005) [39]	US	1992–1995	ELBW	Bayley Scale vers. Two (MDI<70)	Kaufman Assessment Battery for Children, Mental Processing Composite (MPC<70)	20 months	8.6 years	200	37% (27–49)	15%	Low
Kitchen et al. (1987) [46]	Australia	1977–1980	ELBW	Bayley Scale (MDI<70)	Wechsler Preschool and Primary Scale (FSIQ<71)	2 years (corrected)	5.5 years (corrected)	54	67% (22–96)	7%	Low
Ross et al. (1985) [48]	US	1978–1979	VLBW	Bayley Scale (MDI<70)	Stanford-Binet Intelligence Scale (IQ<70)	12 months	3.4 years	94	75% (35–97)	6%	Low

CI: confidence interval; ELBW: extremely low birth weight; FSIQ: full-scale intelligence quotient; IQ: intelligence quotient; MDI: mental development index; MPC: mental processing composite; PPV: positive predictive value; US: United States; VLBW: very low birth weight.

[a] Study size.

[b] According to SIGN50 checklist [23].

Box 1

Search strategy for umbrella review on the association between neonatal sepsis and neurodevelopment in later life, date of search 25 September 2013

We used the following search strategy:

#1 "outcome"

#2 "follow-up"

#3 "sequel*"

#4 "consequence"

#5 "death"

#6 "cerebral palsy"

#7 "retinopathy"

#8 "necrotizing enterocolitis"

#9 "bronchopulmonary dysplasia"

#10 "neurodevelopmental impairment"

#11 "periventricular leukomalacia"

#12 "intraventricular haemorrhage"

#13 neonat*"

#14 "newborn"

#15 "sepsis"

#16 (#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12) AND (#13 OR #14) AND #15

Filters: Publication date from 2000/01/01; Humans; "Medline" OR "systematic review" OR "meta-analysis" OR "intervention"

Box 2

Search strategy for umbrella review on the positive predictive value of neurodevelopmental impairment for later cognitive function, date of search 2 July 2014

The search strategy was the following:

#1 "predictive value"

#2 "Bayley scale*"

#3 "systematic review"

#4 "meta-analysis"

#5 #3 OR #4

#1 AND #2 AND #5 (no filters)

The positive predictive value, i.e. the probability of having a positive test result at the second examination when the first test result was positive, varied between 37% (95% CI: 27–49) and 83% (95% CI: 36–100). Heterogeneity between estimates was mainly due to the study by Hack et al. [39], which was the only study that did not use an externally validated test battery to assess IQ at follow-up. Excluding their estimate from the study pool resulted in positive predictive values between 67% (95% CI: 22–96) and 83% (95% CI: 36–100).

Outcome tree for neurological sequelae of neonatal sepsis

The results of the systematic review were used to develop the outcome tree (Figure 4). Risk differences obtained from meta-analyses were used to estimate the transitional probabilities for acquiring neurodevelopmental impairment, cerebral palsy, vision impairment, hearing impairment and death after having experienced sepsis during neonatal life. Furthermore, we used the positive predictive values identified in the second systematic review to estimate the probability of having a permanently impaired cognitive function after early neurodevelopmental impairment.

Discussion

We developed an outcome tree for neurological sequelae of neonatal sepsis in VLBW infants using the methods of evidence-based medicine. Our study shows that 4–14% of neurological sequelae in ELBW and VLBW are attributable to neonatal sepsis. Although this may be lower than anticipated in this high-risk group, about three-quarters of infants with early neurodevelopmental impairment suffer from persistent cognitive impairment later in life. Evidence quality was low to very low, mainly due to high risk of bias in the single studies as well as imprecision of estimates.

Due to the inclusion criteria of the systematic review, primary studies which used different definitions of neonatal sepsis were analysed together. While some authors defined sepsis as culture-proven sepsis plus clinical signs [38,44], others applied a definition that included antibiotic treatment in addition to positive blood culture [37,42,43]. Moreover, one study did not provide any definition of neonatal sepsis [41]. Definition issues also applied for outcomes. Vision impairment was defined differently in the primary studies: while both studies defined vision impairment as uni- or bilateral blindness, one study also allowed the need for corrective lenses [44]. To estimate the impact of definition of exposure and/or outcome on the associations of interest, a larger number of carefully conducted prospective studies with subgroup analyses of sufficient power would be needed.

To assess attributable risk, we used the original data of the studies included in the systematic reviews to calculate risk differences. This approach may not adjust for potential confounders, which might bias the relation between exposure and outcome. In nearly all analysed studies the infants with neonatal sepsis differed in a number of important prognostic variables from controls such as gestational age, birth weight and co-morbidities. While the original studies did adjust for such variables by applying multivariate analysis, we could not do the same because (i) potential confounders were

not uniform, (ii) some studies adjusted for variables which are not confounders for our purpose, and (iii) we have not had access to the original database with the individual data to do so. We therefore did not follow this approach any further, but considered the problem of confounding in the risk of bias assessments. The study by Hack et al. [44] on ELBW infants born between 1992 and 1996 illustrates that confounding may be outcome specific and leads to surprising results. In this particular study, neonatal exposure to sepsis was associated with hearing impairment, but also with a lower likelihood of neurodevelopmental impairment. This may best be explained by confounding due to postnatal use of corticosteroids administered in that time period in neonates without symptoms of infection to prevent chronic lung disease. Among others, Yeh et al. were able to show the strong side effects of this therapeutic strategy on neurodevelopment when they evaluated long-term neurodevelopmental outcomes of children who had participated in a randomised controlled trial on the effects of dexamethasone therapy [63]. As for the other studies, it was surprising to find that risk differences for neurodevelopmental impairment and cerebral palsy following neonatal sepsis were similar across different settings. Grouping of studies by birth weight or year of publication did not reveal trends for risk differences. More studies would be needed to analyse whether they are independent of birth weight, gestational age, setting and time. It may be concluded, however, that the attributable risks can be used as endpoints for studies evaluating the effectiveness of specific sepsis therapy. Further, it may be hypothesised that sepsis therapy has not, over the years, improved to a similar extent as overall neonatal intensive care.

For meta-analysis, we pooled the risk differences from the individual studies to arrive at a single measure of attributable risk for each outcome. Statistical pooling of risk differences has been reported to cause problems with consistency, with relative risk estimates (including odds ratios) being more consistent than risk differences [64]. For comparison, we pooled the estimates of the calculated relative risks (data available upon request from the authors). Since this analysis did not detect less inconsistency we concluded pooling of risk differences to be an adequate approach.

Our study has several strengths. We based our analyses on a comprehensive systematic review of systematic reviews. By using an outcome-focused approach, we were able to perform a detailed assessment of risk of bias and evidence quality, thereby emphasising the limitations of the current evidence base.

Limitations of our study mainly arise from the limitations in the systematic reviews and primary studies included. In particular, risk of bias and imprecision of the reported estimates might limit the scientific and clinical value of the data summarised here. The search by Alshaikh et al. [3] was last performed June 2012. We did not conduct a more recent search for primary studies. Thus we may not exclude the possibility that more recent studies could have influenced our findings. Our results may be further improved by using the primary datasets of the included cohort studies and then adjusting for confounders compiled throughout all studies, such as sex, birth weight and gestational age.

In conclusion, this systematic review of systematic reviews shows that VLBW infants with sepsis during neonatal life have an increased risk of developing permanent neurological impairment during later life. The magnitude of this effect varies by outcome, while evidence quality was low to very low. To improve the evidence base, carefully planned and conducted prospective studies are needed.

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Conflict of Interest

None declared

Authors' contributions

Sebastian Haller, Thomas Harder conceptualised the study, performed systematic reviews, extracted the data, performed the statistical analysis and drafted the manuscript. Philipp Deindl gave scientific advice, reviewed the data and revised the manuscript. Alessandro Cassini conceptualised the study, reviewed the data and revised the manuscript. Carl Suetens gave scientific advice, reviewed the data and revised the manuscript. Walter Zingg gave scientific advice, reviewed the data and revised the manuscript. Muna Abu Sin reviewed the data, contributed to analysis and interpretation of the data and revised the manuscript. Edward Velasco reviewed the data and contributed to analysis and interpretation of the data. Bettina Weiss reviewed the data, contributed to analysis and interpretation of the data and revised the manuscript. Tanja Ducomble reviewed the data and contributed to analysis and interpretation of the data. Madlen Sixtensson reviewed the data and contributed to analysis and interpretation of the data. Tim Eckmanns conceptualised the study, coordinated the study, reviewed the data and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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WHO recommendations on the composition of the 2016/17 influenza virus vaccines in the northern hemisphere

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On 25 February 2016, the World Health Organization (WHO) published recommendations on the composition of the trivalent and quadrivalent vaccines for the 2016/17 northern hemisphere influenza season [1]. The recommendations for the influenza A(H1N1)pdm09 strain remained the same as in the previous year while the recommended strains for influenza A(H3N2) and B viruses have changed from those recommended in 2015/16. WHO recommends that trivalent influenza vaccines should contain the following:

- an A/California/7/2009 (H1N1)pdm09-like virus;
- an A/Hong Kong/4801/2014 (H3N2)-like virus;
- a B/Brisbane/60/2008-like virus.

For the quadrivalent influenza vaccines which contain two influenza B viruses, the WHO recommends that the above three viruses plus a B/Phuket/3073/2013-like virus be used.

As in previous years, national or regional authorities approve the composition and formulation of vaccines used in each country and are responsible for making recommendations regarding the use of the vaccine.

For more information, read here

References

1. World Health Organization (WHO). Recommended composition of influenza virus vaccines for use in the 2016-2017 northern hemisphere influenza season. Geneva: WHO. Feb 2016. Available from:http://www.who.int/influenza/vaccines/virus/recommendations/201502_recommendation.pdf?ua=1

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