Microbiome and Sudden Death

Subjects: Microbiology

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Sudden unexpected death (SUD) is one of the most important and worthy investigation case profiles in emergency medicine and forensic pathology. Sudden unexpected deaths in adults (SUDA) are frequently caused by cardiac events, while infections usually cause those in infants younger than one year (SUDI), and to a lesser extent, in children older than one year (SUDC). Several studies demonstrate that the microbiome influences host immunity, alters susceptibility to viral respiratory infections, and has a vital role in various health, disease, and death outcomes.

Keywords: forensic sciences ; microbiome ; sudden death

1. Introduction

A post-mortem examination has different purposes. Firstly, to avoid the burial of those who simply appear dead, and then to prevent violent deaths from being hidden, and ultimately to advance in the discovery of different causes of death, still unknown, that allow the advancement of forensic pathology. Thus, there are different deaths in police custody, with different causes of death ranging from sudden natural death to suicide, undiagnosed head trauma and poisoning [1].

Death is termed sudden when it occurs within 24 h of the beginning of symptoms in a non-violent and unexplained manner and without signs of disease [2]. In emergency medicine and forensic pathology, sudden unexpected death (SUD) is one of the most important and deserving investigation case profiles [3][4]. When it occurs in adults (SUDA), it is often due to cardiac events, with an annual incidence worldwide estimated to range between 4 and 5 million cases per year [5]. Sudden unexpected death in infants (SUDI) refers to occurrences in which a child under the age of one year dies abruptly and unexpectedly [6], generally due to infections [7]. However, there are times when a new-born, under the age of one, dies suddenly and unexplainedly and no reason is determined despite a full inquiry, including a review of clinical history, the study of the death scene, and a complete autopsy. This is called Sudden Infant Death Syndrome (SIDS) [8][9][10][11]. As a result, SUDI is a catch-all term for SIDS, other unexplained baby fatalities, including new-born suffocation [12]. On the other hand, other authors define sudden unexplained death in childhood (SUDC), such as the sudden death of a child aged 12 months or more that remains unexplained after an exhaustive investigation of the case, including the performance of the complete autopsy, the examination of the scene of death and the review of the clinical history, presenting its highest incidence in children between 1 and 4 years of age [13].

The San Diego classification establishes different categories of SIDS. It includes the category IA, those infant deaths in subjects older than 21 days and less than nine months of age, with a standard medical history, full-term pregnancy, and absence of similar deaths in siblings, close relatives, and other children cared for by the same person. In addition, these are deaths in which an investigation of the circumstances surrounding them has been carried out, and an accidental death has been ruled out. On the other hand, in the autopsy, fatal pathological findings are absent as well as explained trauma and “thymic stress”, with negative results for toxicological, microbiological, radiological, biochemical studies in the vitreous humour and metabolic screening. When infant death includes the above criteria, the investigation of the possible places and circumstances involved in the death have not been carried out. Neither have any toxicological, microbiological, radiological, biochemical, or metabolic screening been classified in the category IB. Third, category II includes infant deaths that meet category I criteria, except for some of the following: age outside the category I range; similar deaths in siblings, close relatives, or children cared for by the same person; neonatal or perinatal conditions that have resolved at the time of death; possible suffocation or suffocation; abnormal growth and development observed at autopsy without contribution to cause of death; and inflammatory changes or abnormalities insufficient to cause death. The last category involves sudden indeterminate infant death, which includes those deaths that do not meet any of the criteria established to be included in the previous categories but where there is no alternative diagnosis of a natural or violent cause of death, including those cases in which no autopsy has been performed [14].

Different hypotheses have been established about the cause of SIDS [15][16][17][18][19], but there is an accord among many authors who consider that SIDS is a multifactorial disease, establishing that its occurrence needs a heritably vulnerable

baby in a critical period of growth and age, and an environmental trigger, that is, the presence of a vulnerability and an exogenous stressor to increase susceptibility [18][20][21][22].

In both forensic medicine and paediatrics, determining the cause of SIDS is critical [23] as it is the leading cause of death in infants [21][24][25][26] and is responsible for approximately 40–50% of infant mortality in developed countries, presenting a maximum incidence between the first month and one year of life [27]. Different hypotheses have been established about the cause of SIDS [15][16][17][18][19], but there is an accord among many authors who consider that SIDS is a multifactorial disease [18][20][21][22]. SIDS affects families from all socioeconomic, ethnic, and racial backgrounds, with the risk or probability being higher in mothers who receive insufficient prenatal care, mothers who smoke during pregnancy, male babies, preterm or low-weight new-borns, and babies who sleep on their stomachs or with their heads covered, among others [8][10][26][28][29][30][31][32][33]. These risk factors have significant effects on blood pressure and heart rate, both on its control and its excitation during sleep [34].

The microbiota contributes to multiple physiological processes of the host, including immunity. It plays an essential role in human health [35] because it associates gut microbiota alterations during neonatal life with paediatric disorders and the onset of disease in old age [36]. The microbiota can metabolize both dietary and host-derived metabolites through a series of biochemical reactions that enhance the genome-encoded metabolic capacities of the host and has an important role in many aspects of health and disease [37]. Several studies suggest the microbiome affects host immunity and modifies susceptibility to viral respiratory infections [37][38][39][40][41][42].

Coronary heart disease (CHD), on the other hand, is one of the leading causes of sudden mortality in adults. Gut bacteria have long been suspected of playing a role in the development of CHD by influencing multiple signalling pathways in the host, including lipid metabolism and inflammation [43].

2. Microbiome Analysis in Post-Mortem Forensic Studies of Sudden Death

2.1. Sudden Unexpected Death in Adults

Tuomisto et al. [44] proposed an age-dependent association between coronary atherosclerosis and gut bacteria as a possible cause of sudden death in adults. They searched at 67 males (ages 44 to 95) who died outside the hospital, with the entire middle torso and bowel, no signs of bacterial infections or drug addiction, and no visible wounds or necrosis. They also collected faeces samples from seven healthy volunteers to compare to the faeces samples of the deceased study participants. The relative ratios of faecal *Lactobacillus* spp., *Bifidobacterium* spp., *Clostridium coccoides* group, and *Bacteroides* spp. were unaffected by age and did not differ between autopsy patients and healthy volunteers served as a control.

The ratios of the *Clostridium leptum* group, Enterobactericeae, and *Streptococcus* spp. rose with age, while the ratios of the Clostridium leptum group, Enterobactericeae, and *Streptococcus* spp. decreased. With increasing age, the percentages of *Streptococcus* spp. DNA findings reduced, and the percentages of Enterobacteriaceae DNA findings increased in coronary plaques. They predicted that as the number of harmful bacteria in the stomach grows, so does the likelihood of translocation and that these infections can then enter the circulation and end up in coronary plaques.

2.2. Sudden Unexplained Death in Childhood

Two articles study the microbiota in children older than one year (**Table 1**).

Table 3. Microbiome analysis in human forensic studies of sudden death [a].

References	n	Age (Range) *	Sex (M/F)	Clinical Variables	Population Analyzed	Type of Sample	Microbiota Detected
Sudden Unexpected Death in Adults (SUDA)							

References	n	Age (Range) *	Sex (M/F)	Clinical Variables	Population Analyzed	Type of Sample	Microbiota Detected
Tuomisto et al. [44]	67	18–95	M	No signs of bacterial infections or drug addiction.	Finland	Feces and coronary plaques	*Bacteroides spp., Bifidobacterium spp., Clostridium leptum group, Clostridium coccoides group, Enterobacteriaceae, Streptococcus spp., and Lactobacillus spp.*
Sudden Unexplained Death in Childhood (SUDC)							
Prtak et al. [45]	116	0–24	n.i.	n.i.	United Kingdom	Blood cardiac, cerebrospinal fluid (CSF), bronchial swab, lung swab, lung tissue, nasopharyngeal aspirate	*Streptococcus pneumoniae, Haemophilus sp., S. aureus, Escherichia coli, Beta-haemolytic streptococcus group A, Beta-haemolytic streptococcus group B, Haemolyticstreptococcus, Moraxella sp., Leuconostoc sp., Pseudomonas sp., Bordetella pertussis, Mycobacterium bovis (BCG), Neisseria meningitidis, Clostridium septicum, Ureaplasma and Candida sp.*
Burger et al. [46]	82	0–13	M/F	Bed-sharing (65%); smoke parents (29%); prematurity (27%); alcohol parents and prone position (24%)	South Africa	Lung tissue	*Adenovirus, Cytomegalovirus, Respiratory syncytial virus.*
Sudden Unexpected Death in Infants (SUDI)							
Weber et al. [47]	507	0–12	n.i.	n.i.	United Kingdom	Cardiac blood, cerebrospinal fluid (CSF), lung and spleen	*Staphylococcus aureus, Escherichia coli, Beta-haemolytic streptococcus group A Beta-hemolytic streptococcus group B, Streptococcus pneumoniae, Neisseria meningitidis*
Weber et al. [48]	490	0–12	n.i.	n.i.	United Kingdom	Lung tissue	Adenovirus, Influenza Virus, Parainfluenza Virus, Respiratory Syncytial Virus, Measles Virus, Cytomegalovirus, and Enterovirus
Weber et al. [49]	507	0–12	n.i.	n.i.	United Kingdom	n.i.	*Staphylococcus aureus toxins*
Vargas et al. [50]	128	0–12	n.i.	n.i.	Chile	Lung tissue	*Pneumocystis jirovecii*
Yagmur et al. [51]	39	0–12	M/F	n.i.	Turkey	Blood, cerebrospinal fluid (CSF), lung, spleen, stool, and tracheal swab.	*Cytomegalovirus*

References	n	Age (Range) *	Sex (M/F)	Clinical Variables	Population Analyzed	Type of Sample	Microbiota Detected
Sudden Infant Death Syndrome (SIDS)							
Álvarez-Lafuente et al., [52]	11	1–5	n.i.	No previous infections	Spain	Lung, brain, kidney, and spleen tissues	Herpesvirus-6, Epstein-Bar virus, and Cytomegalovirus
Stray Pedersen et al. [53]	160	0–12	M/F	n.i.	Norway	Fecal, cerebrospinal fluid, and gastric antrum tissue	*Helicobacter pilory*
Pearce et al. [54]	231	n.i.	n.i.	n.i.	Australia	Fecal	*Different serotypes of Escherichia coli*
Highet and Goldwater [55]	57	0–12	M/F	Anybody used antibiotics before death.	Australia	Intestine	*Staphylococcus aureus*
Highet et al. [56]	52	3–52	M/F	Anybody used antibiotics before death.	Australia	Intestine	*Clostridium perfringens, Clostridium difficile, Clostridium innocuum, Bacteroides thetaiotamicron, and Staphylococcus aureus*
Gaaloul et al. [57]	39	3–9	M/F	Mild fever and insomnia for a few days before death.	Tunisia	Heart and pericardial fluids	Enterovirus CV-B3
Leong et al. [58]	44	0–12	M/F	n.i.	Australia	Fecal	Bacteria to the orders Clostridiales, Bacteroidales, Lactobacillales, Enterobacteriales, Bifidobacteriales

[a] n.i., no indicated; M/F, Male/Female. * age in months for all references, except for Tuomisto et al. [59], age in years, and Highet et al. [54] age in weeks.

Prtak et al. [45] looked at the role of bacteriology and virology in 51 cases of SIDS, 32 cases of sudden death in a previously healthy child where the cause of death was discovered at post-mortem, 17 cases of sudden death in a child with a chronic but stable condition, and 16 cases of sudden unexpected death where the cause of death was an illness. They found a potentially pathogenic organism in 41.2% of SIDS compared to 29% of those with a chronic condition because that infection can be an essential contributor to SIDS.

Burger et al. [46] analysed the lung tissue of 48 male and 34 female cases. The risk factor most frequently reported by the SUDI cases was bed-sharing (65%), followed by minor clinical symptoms before death and smoking parents (29% each), prematurity (27%), and finally, alcoholic parents and sleeping in the prone position (24% each). More positive results for single viruses (adenovirus, cytomegalovirus, or respiratory syncytial virus) were obtained than cytomegalovirus and respiratory syncytial virus combined (31 versus 2). This study suggests that many cases classified as SIDS could be caused by viruses and highlights the importance of laboratory tests.

2.3. Sudden Unexpected Death in Infancy

Several articles that analyse the relationship between microorganisms and SUDI cases are analysed below (**Table 1**).

Different studies by Weber et al. [47][48][49] reviewed cases of unexplained SUDI, non-infective explained sudden infant death and explained SUDI due to bacterial infection.

On the one hand, the authors found significantly more bacteriological isolates of *Staphylococcus aureus*, *Escherichia coli*, groups A and B beta-hemolytic streptococcus, *Streptococcus pneumoniae*, and *Neisseria meningitidis* from infants whose death unexplained than from those whose death was explained by non-infective causes (*Staphylococcus aureus*: 19/211, 9%; difference 7.1%, 95% CI 2.2–10.8, $p = 0.005$; *Escherichia coli*: 3/211, 1%, difference 4.3%, 1.5–5.9, $p = 0.003$) [47].

On the other hand, they found no significant differences in the frequency of virus detection in virological tests between sudden unexplained deaths and sudden deaths due to non-infective causes [48]. Another later study by Weber et al. [49] showed significantly more isolated *S. aureus* in the unexplained SUDI group than in the non-infectious SUDI group (21%; difference 19.0%, 95% CI 5.4% to 29.3%, $p = 0.006$).

Another study [50] studied the prevalence of *Pneumocystis* in SUDI, proving it was not different between infants with unexplained and infants with explained deaths. For that reason, they suggest that *Pneumocystis* is not sufficient to cause SUDI.

Finally, the study of Yagmur et al. [51] investigated cytomegalovirus as a possible cause of deaths classified as SUDI, using the RT-PCR method. Out of 39 post-mortem SUDI patients, they discovered cytomegalovirus DNA in 19 (49%) and additional bacterial and viral infectious agents in 23 (60%). It should be pointed out here that the finding of 19 out of 39 SUDI patients being positive for CMV does not mean a strong case for its involvement as the prevalence of the virus in the population is very high [59].

2.4. Sudden Infant Death Syndrome

Many theories, including microbiological and immunological, have been proposed to explain this illness [60]. There is controversy among researchers when it comes to indicating the moment in which microbiota colonization of the intestine begins, and there are those who point out the presence of bacteria in the placenta, umbilical cord, and amniotic fluid in healthy term pregnancies [61][62][63]; while other researchers argue against intestinal colonization beginning in the maternal uterus [64][65][66]. In addition, the colonization and maturation of the gut microbiota could be influenced by different perinatal conditions, the mother's diet, age, and metabolic status, family genetics, lifestyle, environment, exposure to antibiotics, and other possible causes [67][68][69][70][71]. Because of that reason, more studies about the gut infant microbiota are necessary [68].

Differences have also been found in the gut microbiota of breastfed infants and their bottle-fed counterparts [72] because breastfeeding has a protective effect against SIDS and the critical role it already plays on cellular and humoral immunity [73].

Diet, bacterial infections, drugs, surgeries, and other factors alter the gut microbial community after the first three years of life. Then, as people get older, the variety of their microbiota decreases concerning young people. Age-related changes in the gut microbiota have been proposed as a critical determinant of age-related disease conditions [44].

Álvarez-Lafuente et al. [52] compared the prevalence and viral loads of the human herpesvirus-6, Epstein–Barr virus, and cytomegalovirus between a group of eleven consecutive cases of SIDS and a control group of sudden deaths of previously healthy children. The DNA prevalence of herpes viruses was 72.7% (8/11), while this prevalence among the controls was 22.2% (2/9); this difference was statistically significant between cases ($p = 0.042$) and tissues ($p = 0.048$). They support the hypothesis that some herpesviruses infections, particularly those caused by Epstein–Barr virus and herpesvirus-6, could be related to some instances of SIDS.

Other authors [53] associated the *Helicobacter pylori* antigen with SIDS. They observed a statistically significant difference in the detection of *H. pylori;* 31% (21/67) of SIDS cases were antigen positive compared with 1.5% (1/68) of live controls ($p < 0.001$).

The study of Pearce et al. [54] compared the diversity of Escherichia coli serotypes detected in the intestinal contents of SIDS victims to babies who died of other causes and healthy babies. According to the authors, specific *E. coli* serotypes, particularly those associated with extraintestinal infections, were more frequent in SIDS than healthy infants used as controls ($p = 0.0002$).

Highet and Goldwater [55] studied the presence of *S.aureus* and its enterotoxins in the intestinal tract. They found a statistically significant increase in both *S. aureus* species and enterotoxin genes in the SIDS group than in the comparison infants. Due to this, the notion that SIDS new-borns have a predisposition or innate susceptibility to *S. aureus* infection cannot be ruled out.

Staphylococcus aureus was also isolated from sterile environments (58%). For these reasons, the authors concluded that while it remains to be seen whether the differences between the microbiomes of SIDS victims and healthy babies are critical differences that can lead to death or not, they should be taken into account because they may increase susceptibility to infection and, as a result, SIDS.

Finally, the microbiome composition was studied in 44 SIDS cases and 44 healthy new-borns, with no significant differences in age, sex, or feeding method between the two study groups. There was no substantial change in microbial diversity between SIDS cases and controls, according to the researchers. They also ran tests to look for previously linked SIDS infections (*Clostridium difficile, Escherichia coli,* and *Staphylococcus aureus*) but found no significant differences between SIDS and healthy cases. However, there was a positive association between the species richness of the samples tested and age [58].

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