reported in two case studies (Sandler et al. 2000; Rodakis 2015). However, high use of different antibiotics in children who subsequently developed autism was shown in a number of observational studies (Fallon 2005; Niehus and Lord 2006; Bittker and Bell 2018). Hypothetically, these observed effects could be attributable to microbiota alterations, triggering a disturbed immune response and the release of cytokines, thus, affecting the function of the central nervous system (de Theije et al. 2011). Recently, a number of observational studies have assessed a possible association of early-life antibiotic exposure and subsequent development of ASD. Here we aimed to systematically document available evidence on the association between early life antibiotic exposure and the prevalence of ASD later in in childhood.

Methods

Inclusion/Exclusion Criteria for the Review

Cohort studies, case–control studies, and cross-sectional studies were eligible for inclusion in this review. The included studies needed to investigate an association between pre- and/or postnatal antibiotic exposure and subsequent diagnosis of ASD. We included studies in which women during any trimester of pregnancy or infants and/or children underwent antibiotic treatment. We focused on early-life antibiotic exposure that preceded a diagnosis of ASD, which is usually established after the second year of life (Mandell et al. 2005). Studies on antibiotic exposure in breastfeeding mothers and subsequent risk of ASD in their children were not included. Since the hypothesized link between ASD and antibiotic use is based on the aforementioned microbiome-gut-brain axis mechanism, only studies which reported data on systemic and/or oral antibiotic therapy were taken into an account. Any antibiotic types and doses, as well as any treatment durations and indications were admissible, as long as the therapy fulfilled the aforementioned criteria. Studies that reported data on antibiotic use collectively (e.g., along with other medications) or that did not report numerical data were excluded. Studies in which the data on antibiotic use was collected only for the purpose of baseline characteristic of participants, with no analysis of the association between antibiotic use and ASD were also excluded.

Our outcome of interest was the diagnosis of ASD during childhood. Studies in which the diagnosis was made according to established criteria, such as those described in the Diagnostic and Statistical Manual of Mental Disorders-V (American Psychiatric Association 2013), as well as studies in which ASD was reported by parents, caregivers or doctors without any described specific criteria, were available for inclusion. Studies that reported data on ASD only collectively with other neurodevelopmental disorders (e.g., together with attention-deficit hyperactivity disorder), were excluded. Only studies that compared children with ASD up to 18 years of age to generally healthy children without this diagnosis were included.

Search Methods

A systematic literature search was performed on 28th of August 2018, with no language or publication date restrictions. The databases searched were PubMed, Embase, and PsycINFO. After drafting the first version of the manuscript, we ran a search update on the 11th of December 2018. The full search strategies for PubMed and Embase are presented in Online Resource 1. Additionally, reference lists of identified observational studies and relevant review articles were manually screened.

Study Selection

Three authors/reviewers (AH, BPG, JŁ) independently screened the title of each study identified with the search strategy as well as the abstracts of potentially relevant articles. Subsequently, the full text for each study potentially relevant after abstract screening, as well as that for studies of unclear relevance, was retrieved. Each author independently assessed the eligibility of the articles and, in cases of a disagreement, resolved differences by discussion.

Data Extraction and Risk of Bias Assessment

Data were extracted with the use of a standard data extraction form. The extracted data included study year, country, design, population, definition of exposure, definitions of cases and controls (for case–control studies), definition of outcome (for cohort studies), results, confounding factors, and data collection methods. We extracted and reported all the data using the same terminology as the authors of the original articles.

Risk of bias was assessed using the Newcastle–Ottawa Scale (NOS) (Wells et al. 2011), in which the reviewers assign stars in all predefined bias domains. In the comparability domain the reviewers have to assign 0, 1, or 2 stars on the basis of the number and types of confounding factors controlled for. Multiple important confounding factors may play a role in studies on the antibiotic-ASD association, including those related to different demographics, pregnancy-related complications, mothers' obstetrical histories, child characteristics at birth, infections, environmental exposures, and healthcare use (Lyall et al. 2017). The NOS requires the reviewers to assign stars depending on two chosen, most important factors. After the literature search and discussion, we found no rationale to decide whether any of