

**The Role of Microbial-Derived p-Cresol in Autism Spectrum Disorder: A
Systematic Review of the Gut-Brain Axis**

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Abstract:

Background

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Aims

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition increasingly associated with gut microbiota alterations. Among microbial metabolites, p-Cresol has emerged as a potential contributor to the pathophysiology of ASD. This systematic review aims to examine the evidence linking p-Cresol and its metabolites with ASD and explore their potential role within the gut–brain axis framework, a bidirectional communication system where gut microbiota influence brain function via immune, metabolic, and neural pathways (e.g., vagus nerve, microbial metabolites).

Methods

A comprehensive literature search was conducted in PubMed, Web of Science, Scopus, and Cochrane up to March 2025, following PRISMA 2020 guidelines. Studies were included if they quantified p-Cresol or its derivatives in biological samples from individuals with ASD. Data extraction and risk of bias assessment were performed independently by two reviewers. The protocol was prospectively registered in PROSPERO (CRD420251007080).

Results

Seventeen studies met the inclusion criteria. Most reported elevated urinary or fecal p-Cresol levels in individuals with ASD compared to controls, with consistent associations found between p-Cresol concentrations and (1); gastrointestinal symptoms, particularly constipation and diarrhea; (2) specific microbiota alterations including increased *Clostridium difficile* and *Desulfovibrio* abundance; and (3) behavioral manifestations and ASD severity. However, heterogeneity in study designs, small sample sizes, and variability in analytical techniques limit the generalizability of the results.

Conclusion

p-Cresol and its microbial precursors may contribute to ASD pathophysiology through gut–brain axis interactions. Although current evidence supports this association, further longitudinal and mechanistic studies are needed to confirm causality and evaluate p-Cresol as a biomarker or therapeutic target in ASD.

Keywords

Autism Spectrum Disorder; p-Cresol; Gut–brain axis; Microbiota-derived metabolites; Gastrointestinal symptoms

1. Introduction

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterized by persistent deficits in social communication and interaction, along with restricted and repetitive patterns of behavior (1). The prevalence of ASD has shown a marked increase in recent decades, with global estimates suggesting that approximately 1 in 100 children are affected (2). In the United States, data from the Centers for Disease Control and Prevention Autism and Developmental Disabilities Monitoring (ADDM) Network indicate a prevalence of 1 in 36 children aged 8 years (3). Although the exact etiology of ASD remains elusive, accumulating evidence points to a multifactorial origin involving genetic, environmental, and metabolic factors.

Recent research has highlighted the potential role of gut microbiota in modulating ASD symptomatology, reinforcing the concept of the microbiota-gut-brain axis (4,5). Dysbiosis—an imbalance in gut microbial composition—has been consistently observed in individuals with ASD, and alterations in microbial metabolites have been implicated in neurodevelopmental outcomes (6). Among these metabolites, p-Cresol (4-methylphenol) has garnered particular interest due to its potential neurotoxic effects and its association with gastrointestinal (GI) disturbances commonly reported in ASD patients (7).

p-Cresol is a microbial-derived phenolic compound produced primarily by gut bacteria, particularly species of *Clostridium*, through the fermentation of tyrosine and phenylalanine (8). Elevated levels of p-Cresol and its sulfate conjugate (p-Cresyl sulfate) have been detected in the urine of children with ASD, suggesting a potential biomarker for ASD-related dysbiosis (9). The mechanisms underlying the neurotoxic effects of p-Cresol remain under investigation, but studies suggest that it may disrupt dopaminergic signaling, impair neuronal differentiation, and contribute to oxidative stress in the central nervous system (10,11). It has been shown to inhibit dopamine beta-hydroxylase, leading to an accumulation of dopamine and reduced norepinephrine levels, which may contribute to ASD-related behavioral and sensory deficits (12). Moreover, p-Cresol has been linked to alterations in N-methyl-D-aspartate (NMDA) receptor function, synaptic plasticity, and neuroinflammatory responses (13,14). These findings suggest that p-Cresol-mediated neurotoxicity may be a key factor in ASD pathophysiology and provide a potential target for therapeutic interventions aimed at restoring gut microbiota homeostasis.

Given the growing recognition of the microbiota-gut-brain axis in ASD, therapeutic strategies targeting gut dysbiosis, such as prebiotics, probiotics, and dietary interventions, have been explored as potential avenues for symptom management (15,16). Future research should aim to elucidate the precise molecular pathways through which p-Cresol influences neurodevelopment and to evaluate the efficacy of microbiota-modulating therapies in ASD populations. Understanding these interactions may pave the way for novel diagnostic and therapeutic strategies, ultimately improving the quality of life for individuals with ASD and their families.

The aim of this systematic review is to comprehensively examine the existing literature on the relationship between p-Cresol and ASD, particularly its impact on neurodevelopmental and behavioral outcomes. This review is necessary due to the growing body of evidence implicating microbial metabolites in neurodevelopmental disorders, yet a clear consensus on the specific role of p-Cresol remains elusive. By systematically analyzing available studies, this work aims to identify gaps in current knowledge, highlight methodological limitations in previous research, and propose future directions that could contribute to the development of microbiota-targeted interventions for ASD management. Given the potential for gut microbiota modulation to serve as a therapeutic strategy, understanding the specific mechanisms of p-Cresol toxicity and its impact on ASD symptomatology is critical for advancing clinical and translational research in this field.

2. Material and Methods

2.1. Protocol and Registration

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. The protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD420251007080. The registration of this protocol ensures transparency and methodological rigor, allowing for reproducibility and minimizing the risk of bias in the selection and synthesis of studies.

2.2. Information Sources and Search Strategy

A systematic literature search was conducted in four electronic databases: PubMed, Scopus, Web of Science, and Cochrane. The search included studies published from

inception until March 2025, with no restrictions on language or publication date. The search strategy was designed to maximize sensitivity and included both controlled vocabulary (MeSH terms) and free-text keywords. The following three thematic groups of terms were used: (i) *Autism Spectrum Disorder (ASD)* and neurodevelopmental disorders, (ii) *p-Cresol and its microbial metabolites*, and (iii) *gut microbiota and dysbiosis*. Boolean operators were applied to optimize search precision. To minimize publication bias, we included non-English studies and screened reference lists of reviews to identify potentially eligible studies not captured by database searches.

A detailed outline of the exact search queries used for each database is provided in **Supplementary Table 1**. Additionally, to ensure the inclusion of all relevant studies, a manual review of reference lists from the selected articles and previously published systematic reviews was performed.

2.3. Selection Criteria

Studies were included in the systematic review if they met the following eligibility criteria. The population of interest consisted of children and adolescents aged 0–18 years diagnosed with ASD according to DSM-IV, DSM-5, or ICD-10 criteria. The primary exposure of interest was the measurement of p-Cresol and/or its metabolites (p-Cresyl sulfate, p-Cresyl glucuronide) in biological samples, including urine, feces, plasma, or serum.

The primary outcomes assessed were p-Cresol levels in ASD children compared to neurotypical controls (if available), associations between p-Cresol levels and ASD severity, and correlations between p-Cresol levels and gut microbiota composition. Secondary outcomes included GI symptoms associated with ASD, such as constipation, diarrhea, and bloating, as well as metabolic alterations linked to p-Cresol levels.

Observational studies with cross-sectional, cohort, and case-control designs, as well as non-randomized clinical trials, were considered for inclusion. Studies were excluded if they did not measure p-Cresol or its metabolites, focused exclusively on adult populations, or examined intervention-based treatments (such as probiotics, dietary modifications, or pharmacological treatments) without assessing p-Cresol levels. Additionally, narrative reviews, systematic reviews, meta-analyses, conference abstracts, case reports, book chapters, and opinion pieces were excluded from the final selection.

2.4. Study Selection Process

After conducting the search, all retrieved articles were imported into Mendeley (Windows 10 version; Elsevier, Amsterdam, The Netherlands) to remove duplicates. Two independent researchers (DV-M and MG-M) screened the titles and abstracts of the remaining studies to identify those that met the eligibility criteria. Full-text versions of the selected studies were then reviewed for final inclusion. In cases where disagreements arose, a consensus was reached through discussion between the two reviewers, and when necessary, a third researcher (AMG-M) was consulted to resolve discrepancies.

2.5. Data Collection Process and Data Items

Data extraction was performed independently by one researcher (DV-M) and was subsequently verified for accuracy by a second researcher (MG-M). If inconsistencies were identified, both investigators discussed the issue to reach a consensus, and if needed, a third researcher (AMG-M) resolved the discrepancy.

The extracted data included key characteristics of the studies, such as author(s), year of publication, country of study, and sample size. Additional information regarding the population characteristics was also collected, including mean age, age range, and number of participants diagnosed with ASD. Specific methodological details such as study design (cross-sectional, cohort, case-control, clinical trial), the biological sample used for p-Cresol measurement, and the analytical method employed were systematically recorded.

Particular attention was paid to studies that reported gut microbiota composition and GI in relation to p-Cresol levels. Additionally, data regarding the correlation between p-Cresol and ASD severity scores, assessed using validated clinical tools such as Autism Diagnostic Observation Schedule (ADOS), the Childhood Autism Rating Scale (CARS), and the Social Responsiveness Scale (SRS), were extracted when available. The complete list of excluded studies, along with the reasons for exclusion, is presented in **Supplementary Table 2**.

2.6. Outcome Measures

The primary outcome of interest was the quantification of p-Cresol levels in biological samples from children diagnosed with ASD. When possible, comparisons were made between ASD children and neurotypical controls to explore differences in microbial

metabolite concentrations. The relationship between p-Cresol levels and ASD symptom severity was evaluated based on standardized behavioral assessment tools.

The analysis also explored the role of gut microbiota composition in modulating p-Cresol levels was analyzed. Particular attention was given to the presence of *Clostridium spp.*, *Desulfovibrio*, *Firmicutes*, and *Bacteroides*, which have been previously implicated in the production of p-Cresol. Additionally, GI symptoms, including constipation, diarrhea, and bloating, were examined in relation to microbial metabolite profiles.

2.7. Study Quality Assessment

The risk of bias of the included studies was assessed using the NIH Study Quality Assessment Tools, developed by the National Heart, Lung, and Blood Institute (NHLBI) of the U.S. National Institutes of Health (17). Given the methodological heterogeneity among the studies, different versions of the tool were applied depending on the design.

For cross-sectional studies, the Observational Cohort and Cross-Sectional Studies tool was used. This instrument evaluates 14 methodological domains related to internal validity, including clarity of the research question, population definition, measurement of exposure and outcomes, adjustment for confounders, and statistical analysis. Item 6 was marked as Not Applicable in all cases due to the study design.

The corresponding version of the tool was used for case-control studies, focusing on the comparability of cases and controls, validity and consistency of case definitions, and the reliability and blinding of exposure measurements. Potential selection bias and the extent of confounding control were also assessed.

For the non-randomized clinical intervention study with a comparison group, the Controlled Intervention Studies tool was applied. This version evaluates allocation procedures, group comparability at baseline, blinding, adherence, and outcome reporting.

In the case of the pre–post intervention study without a control group, the Before–After Studies tool was used, emphasizing the clarity of the intervention, consistency of outcome measurements, timing, and follow-up.

All items were scored as "Yes", "No", "Cannot Determine", "Not Reported", or "Not Applicable" based on the explicit information provided in each study. Two independent reviewers assessed the risk of bias, and any disagreements were resolved through consensus or consultation with a third reviewer. Following NIH guidance, each study was

categorized as having Good, Fair, or Poor quality based on the number of positive ratings and the presence of major methodological flaws.

Two independent reviewers (DV-M and MG-M) conducted the quality assessment, and any discrepancies were resolved through discussion or consultation with a third researcher (AMG-M).

3.Results:

This systematic review includes 17 studies that investigate the relationship between p-Cresol and ASD (**Figure 1**). The selected studies span multiple countries, including Italy (18–26), the United States (27,28), China (29), France (30), Slovenia (31), Latvia (32), Georgia (33) and Australia (34), covering a diverse range of populations and methodologies. Most studies employ cross-sectional designs, with a few clinical trials assessing interventions aimed at modifying gut microbiota and its metabolic byproducts.

*****Figure 1 near here*****

3.1. Study Design and Sample Characteristics

Among the 17 included studies, 12 were cross-sectional (19,21–24,26,28–34), 2 were case-control studies (18,20), 1 was a non-randomized clinical trial (27), and 1 was a pre–post intervention study without a control group (25). The total sample size across the studies was considerable, with individual investigations ranging from 18 to 232 participants. The overall number of subjects was 1408. Participant ages varied widely, from 2 to 18 years, reflecting the heterogeneous nature of ASD across different developmental stages, with a mean age of 7.5 years.

***** Table 1 near here*****

3.2. Biological Sample Types and Analytical Methods

Most studies analyzed urinary p-Cresol (18–25,29,30,32,33), though several also measured its metabolites in plasma, feces, or both (22,26–28,34). The detection methods

varied, with high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) being the most employed techniques. These methods ensured a high degree of specificity and sensitivity in detecting p-Cresol and its conjugates, such as p-Cresyl sulfate and p-Cresyl glucuronide.

3.3. p-Cresol Levels in ASD and Control Groups

Multiple studies have reported higher urinary p-Cresol levels in ASD individuals compared to neurotypical controls. Altieri et al. (18), Gabriele et al. (19,20), Chen et al. (29), Daneberg et al. (32), Gevi et al. (21), Piras et al. (23), and Tevzadze et al. (33) all found significantly elevated p-Cresol concentrations in ASD children. Other studies focused on p-Cresyl sulfate, a conjugated metabolite of p-Cresol. Osredkar et al. (31), Mussap et al. (22), and Timperio et al. (24) found elevated p-Cresyl sulfate in ASD participants, with Osredkar et al. (31) specifically reporting a correlation between higher p-Cresyl sulfate levels and ASD severity.

However, not all studies found significant differences in p-Cresol levels. Wang et al. (34) and Needham et al. (28) did not observe major differences in fecal p-Cresol levels between ASD and control groups. Needham et al. (28) also analyzed other phenolic microbial metabolites, including 4-Ethylphenyl sulfate, but did not find a clear association between these metabolites and ASD-related symptoms.

A visual summary of the reported concentrations across different studies is presented in Supplementary *Figure S1*, which shows p-Cresol levels ($\mu\text{g/mL}$) in ASD and non-ASD groups using a bubble plot format to reflect variability in study size and findings.

3.4. Influence of Gastrointestinal Symptoms on p-Cresol Levels

Links between GI symptoms and p-Cresol levels were explored in multiple studies. Diémé et al. (30) reported that 50% of ASD children with GI symptoms exhibited higher p-Cresol sulfate levels, particularly in individuals experiencing diarrhea, constipation, and colitis. Gabriele et al. (19) found that ASD individuals with chronic constipation had elevated urinary p-Cresol levels, though the study did not establish a direct causative link between constipation and increased p-Cresol absorption.

The relationship between gut transit time and microbial metabolite accumulation was explored in Timperio et al. (24) and Mussap et al. (22). Both studies found an association between altered intestinal motility and higher *Clostridioides spp.* abundance, which has

been linked to increased p-Cresol production. Vernocchi et al. (26) identified a distinct gut microbiota composition in ASD individuals with constipation, diarrhea, and reflux, with alterations in volatile organic compounds (VOCs) related to microbial fermentation. Despite these associations, Needham et al. (28) and Wang et al. (34) found no direct correlation between p-Cresol levels and reported GI symptoms. Wang et al. (34) did, however, find increased fecal short-chain fatty acids (SCFAs) and ammonia in ASD individuals, suggesting broader gut microbial alterations that may not be limited to p-Cresol metabolism.

3.5. Gut Microbiota Composition and Its Relationship with p-Cresol in ASD

Microbiota composition and its role in p-Cresol production have been examined in detail. Mussap et al. (22), Piras et al. (23), and Gevi et al. (21) reported an overrepresentation of *Clostridium difficile* in ASD individuals with high urinary p-Cresol levels. Piras et al. (23) identified 55 additional bacterial strains contributing to gut dysbiosis in ASD individuals.

Timperio et al. (24) and Vernocchi et al. (26) identified *Clostridioides spp.*, *Bacteroides*, and *Desulfovibrio* as key contributors to altered metabolite production, including p-Cresol and other phenolic derivatives. Kang et al. (27) detected an increased abundance of *Desulfovibrio*, *Bifidobacterium*, and *Prevotella* in ASD individuals prior to microbiota therapy, suggesting a unique gut microbial composition in this population.

Osredkar et al. (31) found that p-Cresyl sulfate levels correlated with ASD severity, supporting the idea that microbial metabolites may contribute to neurodevelopmental symptoms. However, Needham et al. (28) did not find a consistent relationship between gut microbiota composition and p-Cresol levels.

3.6. Behavioral Outcomes and Neurodevelopmental Impact of p-Cresol

Several studies assessed the relationship between p-Cresol levels and neurodevelopmental outcomes in ASD. Gevi et al. (21) identified a link between elevated p-Cresol and altered dopamine metabolism, including reductions in noradrenaline and homovanillic acid (HVA) levels, which are involved in cognitive function, motivation, and attention regulation.

Osredkar et al. (31) found that p-Cresyl sulfate levels correlated with cognitive deficits, while Gabriele et al. (20) reported associations between higher p-Cresol concentrations

and compulsive, repetitive behaviors. Altieri et al. (18) observed that ASD children with high p-Cresol levels exhibited impaired verbal communication, lower cognitive function, and more pronounced stereotyped behaviors, particularly among those with intellectual disability or a history of regression.

However, two studies diverged from these patterns. Needham et al. (28) found no significant correlations between p-Cresol and standardized behavioral assessments, while Wang et al. (34), despite similarly finding no behavioral associations with p-Cresol, reported elevated SCFAs and ammonia in their ASD cohort. This indicates that in some ASD populations, gut microbial alterations may occur without measurable p-Cresol changes.

3.7. Interventions and p-Cresol Reduction

The impact of gut microbiota-targeted interventions on p-Cresol levels and ASD symptoms has been explored in both clinical and observational settings. Kang et al. (27) conducted an open-label microbiota transfer therapy (MTT) trial, finding that reducing p-Cresol sulfate levels was associated with improvements in GI function and ASD-related behaviors. Behavioral improvements included reduced irritability, better social communication, and fewer repetitive behaviors, while GI improvements included less constipation and bloating.

Turriziani et al. (25) implemented gut mobilization therapy using polyethylene glycol (PEG) in ASD children with chronic constipation. While the intervention led to decreased hyperactivity, anxiety, and stereotyped behaviors, p-Cresol reduction did not directly correlate with symptom improvements, suggesting that additional factors mediate the relationship between gut microbiota, microbial metabolites, and ASD symptoms.

Though probiotic-based interventions have been proposed as a method to alter p-Cresol-producing bacteria, none of the included studies directly measured their effect on p-Cresol levels. Needham et al. (28) emphasized that dietary patterns and gut microbial composition influence microbial metabolism, but no dietary interventions specifically targeting p-Cresol were identified in the analyzed studies.

3.8. Risk of bias

The risk of bias was assessed using the appropriate NIH Study Quality Assessment Tools based on the design of each study. Case-control studies were evaluated using the tool for

Case-Control Studies, the non-randomized clinical trial was assessed using the tool for Controlled Intervention Studies, the pre–post study without a control group was evaluated with the Before–After (Pre–Post) tool, and the remaining cross-sectional and cohort studies were assessed using the tool for Observational Cohort and Cross-Sectional Studies.

Among the case-control studies, Altieri et al. (18) showed a generally low risk of bias, with positive ratings across most domains. It clearly defined cases and controls, used validated outcome and exposure measures, and applied appropriate statistical analyses. However, the lack of sample size justification, absence of blinding of assessors, and the unreported response rate led to an overall judgment of fair quality. Gabriele et al. (20) received a similar evaluation, though it presented slightly more concerns regarding the clarity of reporting in some domains, resulting also in a fair quality rating (**Figure S2**).

The pre–post intervention study by Turriziani et al. (25) was also rated as fair quality. The study was well-structured, with clearly defined objectives, inclusion criteria, and intervention protocols. Outcome measures were reliable and consistently applied, and there were no losses to follow-up. However, important limitations included the small sample size, lack of assessor blinding, and the absence of multiple baseline or post-intervention measurements, which limited the ability to attribute observed changes solely to the intervention (**Figure S3**).

The non-randomized clinical trial by Kang et al. (27) showed strong internal consistency in terms of sample retention, protocol adherence, and statistical analysis. The outcomes were clearly defined and measured at multiple time points. Nonetheless, due to its open-label nature, the absence of randomization, lack of blinding, and the use of per-protocol rather than intention-to-treat analysis, the overall risk of bias was rated as fair (**Figure S4**).

For the remaining 13 studies with cross-sectional and cohort designs included in this review, five were rated as having overall “Good” quality, while eight were rated as “Fair.” The main reasons for the lower ratings were the lack of sample size justification (item D5) and the absence of statistical adjustment for confounding variables (item D14) (**Figure S5**).

All studies adequately met the criteria regarding a clearly stated research question, a well-defined study population, valid and reliable measurement of exposures and outcomes, and assessment of exposure levels.

However, only five studies (38%) performed statistical adjustments for potential confounding variables such as age, sex, or dietary factors (24,26,28,30,34). In most of the remaining studies, although relevant variables were collected, they were not incorporated into the statistical models, thereby increasing the potential risk of confounding bias.

4. Discussion

This systematic review highlights the potential role of p-Cresol in the pathophysiology of ASD through its interaction with the microbiota-gut-brain axis. Across the included studies, elevated urinary levels of p-Cresol and its sulfate conjugate were frequently observed in children with ASD compared to neurotypical controls (18–23,29,31). These elevations were often associated with increased symptom severity, including impairments in cognition, social interaction, and repetitive behaviors (28,34). These clinical observations are supported by mechanistic insights suggesting that p-Cresol may affect dopamine metabolism by inhibiting dopamine beta-hydroxylase, contributing to altered noradrenaline and HVA levels (21,22,35,36).

However, not all studies in the review confirmed this association. Daneberg et al. (32) and Needham et al. (28) did not observe significant differences in p-Cresol levels between ASD and control groups, nor consistent behavioral correlations. Wang et al. (34), while reporting increased levels of SCFAs and ammonia, did not find significant differences in fecal p-Cresol. These inconsistencies may reflect methodological and biological variations in analytical methods, sample matrices (urine vs. feces), dietary factors, and inter-individual microbiota composition. Moreover, factors such as intestinal permeability and genetic susceptibility may influence the systemic presence and effects of p-Cresol (37–39).

Notably, the biological matrix used for p-Cresol measurement appears to influence detection patterns. Urinary p-Cresol showed the most consistent elevations in ASD across studies (18,20,29), likely because it reflects systemic exposure. By contrast, fecal measurements demonstrated more variable results – while Kang et al. (27) and Vernocchi et al. (26) reported elevated fecal p-Cresol in ASD, Wang et al. (34) found no group differences despite detecting other microbial metabolites. Interestingly, plasma/serum

analyses revealed elevated p-Cresol sulfate in ASD (22,27), suggesting this conjugate may be a stable blood biomarker. Most compellingly, only Kang et al. (27) simultaneously measured fecal and plasma p-Cresol sulfate, finding that microbiota transplantation normalized both fecal and systemic levels while improving symptoms, a finding that points to promising directions for future biomarker validation.

A recurring finding in the reviewed studies was the presence of gut dysbiosis in ASD, particularly an increased Firmicutes/Bacteroidetes ratio and an overrepresentation of *Clostridium* spp., known producers of p-Cresol (11,22,27,32). However, as illustrated by Daneberg et al. (32), this microbial imbalance does not necessarily coincide with elevated p-Cresol levels, suggesting that microbiota composition alone is insufficient to explain its accumulation. This is further supported by Osredkar et al. (31), who found a positive correlation between circulating p-Cresyl sulfate and ASD severity, supporting the hypothesis that increased intestinal permeability may facilitate the systemic absorption of neuroactive microbial metabolites.

4.1. p-Cresol in ASD: Microbial Origins and Neurometabolic Impact

The clinical findings suggest that p-Cresol may serve as a potential biomarker for ASD, especially when associated with cognitive and behavioral symptoms (1,3,17). Its ability to alter dopamine and noradrenaline pathways (21,22,35,36) aligns with the neurochemical profiles observed in individuals with ASD (40), though inconsistencies across studies highlight the need for standardized protocols. Preclinical evidence strongly supports these clinical observations: p-Cresol exposure has been shown to inhibit dopamine beta-hydroxylase in mice (11), while germ-free animal studies suggests that microbiota-derived metabolites can modulate central neurotransmitter systems and behavior (41,42).

Across the reviewed literature, gut dysbiosis is consistent feature of ASD, with notable increases in p-Cresol-producing bacteria like *Clostridium difficile* and *Clostridioides* spp. (11,22,26,27,32). However, their presence alone does not predict p-Cresol levels, suggesting modulation by host factors such as intestinal permeability (“leaky gut”) (37–39), hepatic metabolism, and dietary intake. Notably, protein-rich diets (especially those high in tyrosine/phenylalanine) enhance p-Cresol production by providing bacterial substrates (43,44), while fiber supplementation reduces it in other populations (e.g.,

chronic kidney disease) by promoting competing microbial pathways (45–47), though ASD-specific dietary interventions remain unexplored beyond preliminary prebiotic studies (48).

The systemic effects of p-Cresol depend on its absorption and interaction with neural circuits. Circulating p-Cresyl sulfate correlates with ASD symptom severity (31) and microbiota transplants from ASD donors induce behavioral changes in mice independent of taxonomic composition (42). Additionally, Saito et al, (8) and Blachier et al. (49) also showed that dietary intake rich in tyrosine promotes p-Cresol production by *Clostridium* species, suggesting environmental factors play a key role in modulating its levels.

4.2. From Dysbiosis to Behavior: Microbiota-Derived p-Cresol and ASD Symptoms

The behavioral implications show variability, with several included studies reported associations between elevated p-Cresol levels and ASD-related behavioral phenotypes (18,23,31), while others found no such relationships (28,32,34). This inconsistency may arise not only from biological factors (e.g., host metabolic capacity, neurodevelopmental trajectories) but also from methodological differences across studies. Notably, the strength of associations varied depending on ASD assessment tools: studies using clinician-administered ADOS modules (18,27) reported stronger p-Cresol-behavior correlations than those relying on parent-reported scales like SRS (28) or broader instruments like CARS (20). Furthermore, the transition from DSM-IV to DSM-5 diagnostic criteria may have introduced population heterogeneity, particularly for milder phenotypes. Importantly, p-Cresol alone may not be sufficient to induce behavioral symptoms but may act as part of a larger network of microbial and immune interactions.

Experimental evidence strongly supports this multifactorial model. Bermudez-Martin et al. (11) demonstrated that mice chronically exposed to p-Cresol developed ASD-like behaviors accompanied by reduced dopaminergic activity in brain areas relevant to social behavior. Notably, transplantation of fecal microbiota from p-Cresol-treated mice into controls replicated these deficits, providing direct evidence for gut-brain axis involvement.

Moreover, p-Cresol has been implicated in oxidative stress and neuroinflammation. Flynn et al. (36) observed that p-Cresol induces mitochondrial dysfunction and reactive oxygen species accumulation in neuronal cultures, along with *in vivo* evidence of pro-

inflammatory cytokine increases and microglial activation following p-Cresol exposure (23).

4.3. Limitations and Future Directions

This review acknowledges several limitations. First, the exclusive reliance on observational studies prevents causal inferences, as residual confounding (e.g., by diet or medications) and reverse causality cannot be ruled out. Notably, while animal models suggest p-Cresol may induce ASD-like behaviors (11), human studies to date cannot determine whether these associations reflect causation, secondary effects of ASD pathophysiology, or shared underlying mechanisms. Second, the heterogeneity in study designs, sample sizes, biological matrices (urine, feces, plasma), ASD diagnostic tools and p-Cresol quantification methodologies restricts the comparability of the findings. Although we employed comprehensive search strategies to minimize publication bias (e.g., including non-English studies and screening reference lists), the narrative synthesis approach precluded formal statistical assessments (e.g., funnel plots or Egger's tests) that are only feasible in meta-analyses. Consequently, we cannot rule out potential underrepresentation of small-scale studies with null results. Third, the predominance of cross-sectional studies prevents causal inferences between p-Cresol levels and ASD symptomatology and fails to capture how p-Cresol fluctuations might correlate with functional outcomes over time. The lack of standardized cut-off values for p-Cresol in ASD further complicates its potential use as a biomarker, a limitation compounded by variable detection patterns across different sample types (e.g., consistent urinary vs. variable fecal p-Cresol levels).

To establish the temporal relationship between p-Cresol levels and ASD development, future research should prioritize longitudinal cohorts tracking p-Cresol from infancy in high-risk populations, intervention studies targeting p-Cresol reduction through dietary or microbial modulation, and Mendelian randomization approaches using genetic variants associated with p-Cresol production. These approaches would help establish whether p-Cresol alterations contribute to ASD development or represent secondary effects. Particular emphasis should be placed on studies that measure both p-Cresol dynamics and clinically meaningful outcomes (e.g., cognitive, social, and adaptive functioning) across developmental stages. Additionally, mechanistic investigations examining the interplay between p-Cresol and neurotransmitter systems could provide valuable insights into its role in ASD pathophysiology. This review highlights the need for more robust and

comprehensive research to better understand the complex relationship between p-Cresol, the gut microbiome, and autism spectrum disorder in children.

5. Conclusion

The findings of this systematic review underscore the potential role of p-Cresol in ASD pathophysiology, particularly through its influence on gut microbiota and neurodevelopmental processes. While elevated p-Cresol levels have been observed in ASD individuals, inconsistencies in the literature highlight the need for further research to clarify its role as a biomarker and therapeutic target. Understanding the precise mechanisms of p-Cresol neurotoxicity and its interactions with the gut microbiome may pave the way for novel microbiota-targeted interventions aimed at improving ASD-related symptoms and overall quality of life.

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Competing interests

All the authors disclose no conflicts of interest related to this work.

Author contributions

DV-M and AMG-M contributed to the conceptualization and methodology of the review. DV-M led the investigation, and was primarily responsible for writing the original draft and data visualization. AMG-M also provided supervision, validation and contributed to writing – review & editing. MG-M supported the investigation, and contributed to writing – review & editing. CL-A provided resources and participated in writing – review & editing. MM-R contributed to writing – review & editing, supervision and data visualization. PB-P supervised the project, managed administration, and also participated

in writing – review & editing. All authors have read and approved the final version of the manuscript.

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Figure caption:

Figure 1. Flow diagram for preferred reporting items for systematic reviews and meta-analyses (PRISMA).

Tables

Table 1. Characteristics of the studies

Study (Author, Year)	Country	Study Type	Total Sampl e (n)	Girl s (%)	Age (years)	AS D (n)	Biologic al Sample	p- Cresol/Metabolites Analyzed	Involved Bacteria	GI Symptoms	Behavioral Results	Conclusions
Altieri et al., 2011 (18)	Italy	Case-control	118	25.4	8.3	59	Urine	p-cresol and cotinine in urine via HPLC	NR	NR	↑ CARS scores, intellectual disability, regression history	Elevated urinary p-cresol in ASD; potential biomarker for severity.
Wang et al., 2012 (34)	Australia	Cross-sectional	54	33.3	10.9	23	Feces	p-Cresol, phenol, short-chain fatty acids (SCFA), ammonia	NR	Diarrhea/constipation (39% ASD)	No significant differences in p- cresol levels between ASD and controls	No ↑ p-cresol in ASD, but ↑ SCFA/ammonia suggests altered fermentation.
Gabriele et al. (a), 2014 (20)	France	Case-control	66	12.1	7.9	33	Urine	Total p-Cresol, p- Cresyl sulfate, p- Cresyl glucuronide	NR	NR	↑ Repetitive/stereotyp ed behaviors	Confirms p- cresol as biomarker in young ASD children.
Gabriele et al. (b), 2015 (19)	Italy	Cross-sectional	53	17.0	8.1	53	Urine	Total p-Cresol in urine via HPLC	<i>Clostridium spp.</i> (No correlation found)	Chronic constipation	NR	p-Cresol linked to constipation but not to <i>Clostridium</i> or antibiotics.
Diémé et al., 2015 (30)	France	Cross-sectional	62	14.5	8.6	30	Urine	p-Cresol sulfate, indoxyl sulfate, phenylacetylglutamine	NR	50% of ASD children had GI symptoms (diarrhea, constipation, colitis)	NR	Metabolic alterations in amino acids and microbiota- derived metabolites, including p- cresol sulfate, were detected. Gut dysbiosis is suggested to be related to ASD.
Tevzadze et al., 2017 (33)	Georgia	Cross-sectional	42	42.9	1-4	14	Urine	p-Cresol via HPLC	NR	NR	ASD children showed elevated p- cresol levels compared to controls	Urinary p-cresol ↑ in ASD vs. controls; may play a pathogenic role.

Chen et al., 2019 (29)	China	Cross-sectional	220	21.8	5.7	156	Urine	p-cresol measured along with 76 other organic acids via GC/MS	NR	NR	No specific individual behaviors reported, but associated with metabolic alterations in ASD.	p-Cresol ↑ in ASD; supports its role as a biomarker.
Gevi et al., 2020 (21)	Italy	Cross-sectional	80	22.5	5.0	40	Urine	p-Cresol, neurotransmitters, and related metabolites	<i>Clostridium difficile</i>	NR	Alterations in dopamine, homovanillic acid (HVA), noradrenaline, and adrenaline deficit	p-Cresol may disrupt catecholamine pathways, exacerbating ASD symptoms.
Kang et al., 2020 (27)	USA	Non-randomized clinical trial	38	NR	7-16	18	Feces and plasma	p-cresol sulfate (plasma and feces)	<i>Desulfovibrio</i> , <i>Bifidobacterium</i> , <i>Prevotella</i>	Constipation, diarrhea	Behavioral improvement post-FMT	FMT reduced p-cresol sulfate and improved GI/behavioral symptoms.
Needham et al., 2021 (28)	USA	Cross-sectional	231	NR	3-12	130	Plasma and feces	Phenolic metabolites such as 4-Ethylphenyl sulfate (4-EPS), cresol, and other phenolic derivatives via UHPLC-MS/MS	Not specifically analyzed, but an altered gut microbiota is mentioned	GI symptoms evaluated through questionnaires. No clear correlations found between metabolites and GI symptoms	Association of lipid, amino acid, and phenolic metabolite levels with behavioral scores (ADOS)	Metabolic alterations in ASD were found, including changes in oxidative stress, mitochondrial dysfunction, and elevated levels of microbiota-derived phenolic metabolites.
Daneberga et al., 2022 (32)	Latvia	Cross-sectional	44	NR	2-12	44	Urine	p-Cresol and 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPPHA) measured via GC-MS	Higher relative abundance of Firmicutes; decreased Bacteroidetes/Firmicutes ratio in HPPHA samples	NR	NR	HPPHA (Clostridium marker) not confirmed; p-cresol detected but not significant.
Piras et al., 2022 (23)	Italy	Cross-sectional	27	27.8	8.3	13	Urine	p-Cresol and phenolic derivatives via mass spectrometry	<i>Clostridium difficile</i> , 55 other bacterial strains	Gut dysbiosis associated with bacterial overgrowth	NR	Bacterial overgrowth (e.g., <i>Clostridium</i>) may drive p-cresol production in ASD.
Timperio et al., 2022 (24)	Italy	Cross-sectional	28	21.4	13.0	14	Urine	p-Cresol and 4-ethylphenyl sulfate	<i>Clostridioides spp.</i>	Chronic constipation in	Alterations in purine, tryptophan,	Urinary metabolic

										28.6% of ASD children	and phenylalanine metabolism	profiles of ASD children differ from their typically developing siblings, with increases in p-cresol and 4-ethylphenyl sulfate.
Turriziani et al., 2022 (25)	Italy	Pre-post intervention study without control group	21	14.3	4.6	21	Urine	Total p-Cresol in urine via HPLC	NR	Chronic constipation	Significant reduction in hyperactivity, anxiety, socio-communication deficits, and stereotyped behaviors after intestinal mobilization	Intestinal mobilization improves behavioral symptoms in ASD children with chronic constipation, although urinary p-cresol variation showed no significant correlation with these changes.
Osredkar et al., 2023 (31)	Slovenia	Cross-sectional	191	22.0	9.7	143	Urine	p-Cresyl sulfate (pCS) and indoxyl sulfate (IS) via LC-MS/MS	NR	NR	pCS correlated with autism severity and cognitive deficits	Elevated pCS and IS levels in ASD children suggest their possible role in the disorder's pathophysiology.
Vernocchi et al., 2023 (26)	Italy	Cross-sectional	76	25.0	3-15	41	Feces	p-Cresol and other volatile compounds (VOCs)	Bacteroides, Clostridium, Desulfovibrio	Constipation, diarrhea, abdominal pain, reflux	Impairment in behavioral symptoms and ASD severity	Fecal volatilome (incl. p-cresol) as diagnostic tool for ASD subtypes.

ADOS, Autism Diagnostic Observation Schedule; ASD, Autism Spectrum Disorder; CARS, Childhood Autism Rating Scale; FMT, Fecal Microbiota Transplantation; GC-MS, Gas Chromatography-Mass Spectrometry; GI, Gastrointestinal; HPLC, High-Performance Liquid Chromatography; HPHPA, 3-(3-Hydroxyphenyl)-3-Hydroxypropionic Acid; IS, Indoxyl Sulfate; LC-MS/MS, Liquid Chromatography-Tandem Mass Spectrometry; NR, Not Reported; pCS, p-Cresyl Sulfate; SCFA, Short-Chain Fatty Acids; UHPLC-MS/MS, Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry; VOCs, Volatile Organic Compounds.

Supplementary material

Figure caption

Figure S1. Comparative p-Cresol Concentrations in ASD vs. Non-ASD Groups.

Figure S2. Risk of bias for case-control designs included in this review.

Figure S3. Risk of bias for studies with no control group included in this review.

Figure S4. Risk of bias for Controlled Intervention Studies included in this review.

Figure S5. Risk of bias for cross-sectional and cohort designs included in this review.

Tables:

Table S1. Search strategy

Table S2. Excluded studies and reasons for exclusion