PRISMA 2020 Checklist

Table 1. Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	3 – 4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	4
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	4
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Supplementary Table 3
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	5
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Supplementary Table 3
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Supplementary Table 4, 5 and 6
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	NA
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	5
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	NA
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	5
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	NA
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Supplementary Table 4
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	NA
Reporting bias	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	5

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
assessment			
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	NA
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	6
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	5 – 6
Study characteristics	17	Cite each included study and present its characteristics.	7
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	7 and Supplementary Table 4, 5 and 6
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Table 4, 5 and 6
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	7
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Table 4, 5 and 6
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	7
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	NA
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	NA
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	NA
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	18 – 25
	23b	Discuss any limitations of the evidence included in the review.	26
	23c	Discuss any limitations of the review processes used.	26
	23d	Discuss implications of the results for practice, policy, and future research.	27
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	3
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	NA
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	29
Competing interests	26	Declare any competing interests of review authors.	29
Availability of	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included	29

Section and Topic	Item #	Checklist item	Location where item is reported
data, code and other materials		studies; data used for all analyses; analytic code; any other materials used in the review.	

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

Table 2. Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) 2020 for Abstract Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesise results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	No
Registration	12	Provide the register name and registration number.	

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. This work is licensed under CC BY 4.0. To view a copy of this license, visit https://creativecommons.org/licenses/by/4.0/

Table 3. Characteristics of The Included Studies

First author	Year	Country	Journal	Study Design	Study Period	Case (subtype) / Control	N	Age	Male (%)	Female (%)	Comorbidities (%)	NYHA (%)	Sequencing Method	Medications Consumed	Lifestyle
Yafarova A.A.	2024	Russia	International Journal of Molecular Sciences	Cross-sectional observational study	NA	HFrEF	43	71	NA	NA	HTN (86) AF (44.2) T2DM (18.6) Dyslipidemia (74.4) PMI (90.7)	I (14) II (72) III (14)	16s rRNA (V3-V4)	BB (79.1) Diuretics (72.1) RAAS inhibitor (95.3) CCBs (16.3) Antiplatelet agents (65.1) Anticoagulant (37.2) Statins (72.1) MRA (74.4) Antiarrythmics (7) PPI (37.2)	NA
						HC	53	54	NA	NA	HTN (58.5) AF (0) T2DM (0) Dyslipidemia (39.6) PMI (0)			BB (11.3) Diuretics (15.1) RAAS inhibitor (39.6) CCBs (9.4) Antiplatelet agents (7.5) Anticoagulant (5.7) Statins (20.8) MRA (5.7) Antiarrythmics (3.8) PPI (11.3)	NA
Yang C.	2024	China	Journal of Cardiovascular Translational Research	Cross-sectional observational study	Sep 2022 - Dec 2022	HFrEF + HFpEF + HFmrEF	62	75.74 ± 10.84	28 (90.4)	34 (9.6)	HTN (50) AF (37.1)	II (14) III (22) IV (26)	16s rRNA (V3-V4)	BB (41) ACEI/ARB/ARNI (22) MRA (42) SGLT2i (7)	Smoking (5) Drinking (12)
						HC	21	59.42 ± 10.97	10 (47.6)	11 (52.4)	HTN (19.1) AF (NR)			NA	NA
Ahmad A.F.	2023	Australia	American Journal of Physiology - Heart and Circulatory Physiology	Cross-sectional observational study	Mar 2020 - Sep 2021	HF (unspecified)	73	59.8 ± 12.4	61 (83.5)	12 (16.4)	HTN 31 (42.5) T2DM (27.4) Dyslipidemia (41.1)	NA	16s rRNA (V3-V4)	BB (67.1) ACEI (26.0) ARNI (56.1) Diuretics (68.5) HRT (2) Metformin (13.7) SGLT2i (8.2) Insulin (6.8) Gliclazide (1.3) Statins (57.5) Ezetimibe (5.5) MRA (56.1) Aspirin (46.6)	Current Smoker (9.6) Previous Smoker (42.5) Drinking (68.5)
						HC	59	56.0 ± 9.2	11 (18.6)	48 (81.4)	NA			NA	NA

Huang K.	2023	China	American Society for Microbiology	Cross-sectional observational study	Dec 2020 - Nov 2021	HF-NDS (unspecified)	36	65.56 ± 11.75	27 (75)	9 (25)	HTN (66.7) AF (36.1) CAD (33.3)	II (36.1) III (36.1) IV (27.8)	16S rRNA (V3-V4), Metagenome Sequencing	NA	Smoking (33.3) Drinking (25)
						HC	24	57.08 ± 9.34	12 (50)	12 (50)	HTN (45.8)			NA	Smoking (16.7) Drinking (4.2)
Zhang Z.	2023	China	Frontiers in Cardiovascular Medicine	Cross-sectional observational study	Jun 2021 - Jun 2022	HF (unspecified)	58	78.0 ± 3	NA	NA	HTN (58.6-65.5) AF (58.6-62.0) CVD (93.1-96.5)	III (50) IV (50)	16s rRNA (V3-V4)	BB (41.3-62) ACEI/ARB (31-44.8) Diuretics (100)	Smoking (17.2-31)
						HC	22	76.0 ± 3	NA	NA	HTN (59) AF (0) CVD (100)			BB (45.4) ACEI/ARB (45.4) Diuretics (13.6)	Smoking (18.1)
Peng J.	2023	China	Frontiers in Cellular and Infection Microbiology	Cross-sectional observational study	NA	HF (unspecified)	33	71.76 ± 7.93	24 (72.7)	9 (27.3)	NA	NA	16S rRNA (V3-V4)	BB (69.7) ACEI/ARB (75.8) Diuretic (60.6) Statin (81.8) CCB: 18.2	Smoking (42.4) Drinking (32.3)
						HC	15	67.67 ± 9.76	8 (53.3)	7 (46.7)	NA			BB (46.7) ACEI/ARB (13.3) Diuretic (NA) Statin (73.3) CCB (33.3)	Smoking (33.3) Drinking (13.3)
Wang Z.	2021	China	Mediators of Inflammation	Cross-sectional observational study	NA	HF (unspecified)	25	65 ± 3.17	14 (56)	11 (44)	NA	I (2) II (5) III (7) IV (11)	16S rDNA (V3/V4)	NA	Never Smoke (10) Previous Smoker (11) Current Smoker (4)
						HC	25	65 ± 3:07	13 (52)	12 (48)	NA			NA	Never Smoke (12) Previous Smoker (10) Current

															Smoker (3)
Beale A. L.	2021	Australia	Journal of the American Heart Association	Cohort observational study	Aug 2017 - Jan 2020	HFpEF	26	68 ± 7.5	6 (23)	20 (77)	NA	NA	16S rRNA (V3-V4)	NA	NA
						Metropolitan HC	39	58.3 ± 7.9	22 (56)	17 (44)	NA			NA	NA
						Regional HC	28	61 ± 6	9 (32)	19 (68)	NA			NA	NA
Sun W.	2022	China	Frontiers in Microbiology	Cross-sectional observational study	Apr 2020 - Aug 2020	HFrEF + HFpEF + HFmrEF	29	61 ± 12	24 (83)	5 (17)	HTN (48) AF (6.7) Diabetes (34)	III (10) IV (19)	16s rRNA (V3-V4)	BB (93) ACEI/ARB/ARNI (90) Diuretics (100) MRA (100) SGLT2i (72)	Smoking (41.4)
						HC	30	60 ± 10	10 (33)	20 (67)	HTN (37) AF (0) Diabetes (16.7)			NA	Smoking (20)
Emoto T.	2021	Japan	International Journal of Cardiology	Cohort observational study	NA	HFrEF + HFmrEF + HFpEF	22	74.5	14 (63.6)	8 (36.4)	HTN (95.5) AF (68.2) DM (36.4) Dyslipidemia (40.9)	I (13.6) II (68.2) III (18.2) IV (0)	16S rRNA (V3-V4), gene amplicon analysis	BB (90.9) ACEI/ARB (68.2) CCB (45.5) Aldosterone receptor antagonist (40.9) Diuretics (loop/thiazide) (95.5) PPI/H2 blocker (77.3)	Smoking (50)
						HC	11	72.5	6 (54.5)	5 (45.5)	HTN (81.8) AF (54.5) DM (45.5) Dyslipidemia (54.5)			BB (36.4) ACEI/ARB (45.5) CCB (27.3) Aldosterone receptor antagonist (0) Diuretics (loop/thiazide) (18.2) PPI/H2 blocker (81.8)	Smoking (27.3)

ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin II receptor blocker; ARNI: angiotensin receptor neprilysin inhibitor; AF: atrial fibrillation; BB: beta blocker; CCB: calcium channel blocker; DM: diabetes mellitus; H2: Histamine-2; HC: healthy control; HF: heart failure; HF-NDS: heart failure without depressive symptoms; HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction; HFmrEF: heart failure with mildly reduced ejection fraction; HRT: Hormone replacement therapy; HTN: hypertension; N: number of subjects; MRA: mineralocorticoid receptor antagonists; N/A: not applicable; NYHA: New York Heart Association Classification of Heart Failure; PMI: previous myocardial infaction; PPI: proton pump inhibitor; RAAS: renin-angiotensin-aldosterone system; SGLT2i: sodium-glucose cotransporter-2 inhibitor; T2DM: type II diabetes mellitus

Table 4. Risk of Bias Assessment (Newcastle-Ottawa Scale – Cross-sectional)

Study (Author, Year)	Selection (max 5★)	Comparability (max 2★)	Level of confounder adjustment	Outcome/Exposure (max 2★)	Total score	Risk of Bias
Yafarova et al., 2024	★★★★☆ (4) • Hospital-based sample, not population-based • Moderate sample size (n=189) • Exposure ascertainment valid (NGS, qPCR, lab tests) • Non-response not reported	☆☆ (0) • Adjusted confounders are not mentioned	None	★★ (2) • Independent blind assessment • Outcomes measured with validated methods • Appropriate statistical tests (FDR, GLM, PERMANOVA)	**6/9**	**Moderate**
Yang et al., 2024	★★★★☆ (4) • CHF cases clearly defined by clinical and nutritional risk (NRS2002) • Hospital-based sample, not population-based • Justified sample size • Validated screening tool (lab tests, PCR) • Non-response not reported	★★ (2) • Study investigates potential confounders (age, medications, gender, lifestyle)	Comprehensive	★★ (2) • Independent blind assessment • Stool analysis via 16S rRNA sequencing with standard pipeline • Multiple analytic methods (LEfSe, random forest, PICRUSt2) • Appropriate statistical tools (ROC, correlation analyses)	**8/9**	**Low**
Ahmad et al., 2020	★★★★☆ (4)	★★ (2) • Adjusted for major	Comprehensive	★★ (2)	**8/9**	**Low**

	• Hospital-based, not population-based • Justified sample size • Controls recruited from same setting, free of HF • Validated screening tool (lab tests, PCR) • Non-response not reported	confounders (age, gender, comorbidities, medications and diet)		• Independent blind assessment • Gut microbiome analyzed by shotgun metagenomics • Plasma metabolites quantified by validated LC–MS • Statistical analysis appropriate (multivariate regression, PERMANOVA)		
Huang et al.,2023	★★★★☆ (4) • HF patients from one hospital, HC from physical exam, not population-based butacceptable • 95 participants (adequate but moderate) • Not reported • Gut microbiota/ metabolites measured using validated 16S rRNA, metagenome	★★ (2) • Age, gender, BMI, smoking, alcohol partially controlled • Excluded participants with antibiotics/probiotics, major comorbidities	Comprehensive	★★ (2) • Depressive symptoms via PHQ-9 (validated), lab-based microbiota/metabolite analysis • Appropriate (Wilcoxon, LEfSe, random forest, ROC, multiple testing correction)	**8/9**	**Low**

	sequencing, GC-MS					
Zhang et al., 2022	★★★☆☆ (3) • Patients from one hospital, somewhat representative • 58 CHF + 22 controls, no sample size calculation • Not reported • Gut microbiota (16S rRNA) & PAGln (LC-MS/MS), validated methods	★★ (2) • Age, gender, and cardiovascular disease were controlled	Comprehensive	★★ (2) • Gut microbiota diversity & PAGln measured by validated methods • Appropriate (Chi-square, t-test, ANOVA, non-parametric tests, correlations), p-values reported	**7/9**	**Low**
Peng et al., 2023	★★★☆☆ (3) • Hospital patients + controls, somewhat representative • 54 participants, no justification • Not reported • 16S rRNA sequencing & LC-MS/MS metabolomics, validated	☆☆ (0) • Data not adjusted for all relevant confounders or risk factors provided	None	★★ (2) • Validated sequencing & metabolomics, objective but no blinding • Appropriate (Wilcoxon, LEfSe, correlations, multivariate), p-values/effect sizes reported	**5/9**	**Moderate**
Wang et al., 2021	★★★☆☆ (3) • Hospital-based, somewhat	☆☆ (0) • Data not adjusted for all relevant confounders or	None	★★ (2) • Validated methods, objective but no blinding	**5/9**	**Moderate**

	representative • 60 participants, no calculation • Not reported • Gut microbiota (16S rRNA) & serum metabolomics (LC-MS/MS), validated	risk factors provided		• Appropriate (Wilcoxon, LEfSe, correlations), well reported		
Sun et al., 2022	★★★★☆ (4) • CHF cases defined by NYHA III–IV • Hospital-based, not population-based • Justified sample size • Validated screening tool (lab tests, PCR) • Non-response not reported	★★ (2) • Study investigates potential confounders (age, medications, gender and lifestyle)	Comprehensive	★★ (2) • Independent blind assessment • Stool samples analyzed by 16S rRNA sequencing • Identical procedures for cases and controls • Validated laboratory methods	**8/9**	**Low**

Table 5. Risk of Bias Assessment (Newcastle-Ottawa Scale – Cohort)

Study (Author, Year)	Selection (max 4★)	Comparability (max 2★)	Level of confounder adjustment	Outcome/Exposure (max 3★)	Total score	Risk of Bias
Beale A. L. et al., 2021	★★★☆ (3) • HFpEF patients from hospital, somewhat representative • Healthy controls from	★★ (2) • Gut microbiota of patients with HFpEF are independent of BMI, age, hypertension, diet (including	Comprehensive	★☆☆ (1) • Microbiome sequencing validated • Follow-up: Not applicable • Adequacy of follow-up: Not applicable	**6/9**	**Moderate**

	same community • HFpEF diagnosis (clinical + echo) and microbiome sequencing, validated • Outcomes absent at start: Not applicable, no follow-up	fiber intake), and gender				
Emoto et al., 2021	★★★★ (4) • Somewhat representative of the average **o**ne hospital-based HF patient in the community • Non exposed cohort drawn from the same community as the exposed cohort • Laboratory records • Outcome of interest was **not present** at start of study	★★ (2) • Study controls for gut microbiome (age) • Study controls for additional factor (age, gender, and comorbidities)	Comprehensive	★☆☆ (1) • Independent blind assessment	**7/9**	**Low**

Table 6. Risk of bias assessment using Newcastle-Ottawa Scale (NOS)

Study	D1	D2	D3	Overall
Yafarova et al., 2024	+	X	+	-
Yang et al., 2024	+	+	+	+
Ahmad et al., 2020	+	+	+	+
Huang et al., 2023	+	+	+	+
Zhang et al., 2022	+	+	+	+
Peng et al., 2023	+	X	+	-
Wang et al., 2021	+	X	+	-
Beale et al., 2021	+	+	-	-
Sun et al., 2022	+	+	+	+
Emoto et al., 2021	+	+	-	+

D1: Bias due to Selection – Domain scoring: 0-1 (High), 2 (Some concerns), 3+ (Low)

D2: Bias due to Comparability – Domain scoring: 0 (High), 1 (Some concerns), 2+ (Low)

D3: Bias due to Outcome – Domain scoring: 0 (High), 1 (Some concerns), 2+ (Low)

Judgement	
+	Low
-	Some concerns
X	High