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专论与综述

供水系统非结核分枝杆菌生长因素及控制措施研究进展

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摘　要：近年来，非结核分枝杆菌感染在世界范围内日益普遍，严重威胁公众健康。供水系统是非结核分枝杆菌重要环境来源和主要传播途径，但目前对供水系统非结核分枝杆菌生长因素及控制措施的认识仍有较多不足。本文介绍了供水系统非结核分枝杆菌的生长传播特征，探讨了多个工程环境因素(如消毒剂、有机碳、管材和温度)和生物因子(如生物膜、阿米巴原虫和细菌)对非结核分枝杆菌丰度和物种多样性特征的影响，分析了供水全流程不同阶段控制措施对非结核分枝杆菌的控制效用，提出了深化认识供水系统非结核分枝杆菌的研究需求。

关键词：供水系统；非结核分枝杆菌；工程环境因素；生物因子；控制措施

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Growth factors and control measures of nontuberculous mycobacteria in drinking water distribution systems: a review

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Abstract: The prevalence of nontuberculous mycobacteria (NTM) infection has been increasing worldwide in recent years, posing a threat to public health. Drinking water distribution systems (DWDSs) represent an important reservoir and a primary transmission route for NTM. However, the growth factors and control measures of NTM remain elusive in DWDSs. This review summarizes the growth and transmission features of NTM and analyses the influence of environmental and engineering factors (disinfectant, organic carbon, pipe material, and temperature) and biotic factors (biofilm, amoeba, and bacteria) on the abundance and diversity of NTM. Further, we summarize the control approaches towards NTM and their effectiveness in DWDSs from source to taps and identify the research gaps and further research needs.

Keywords: drinking water distribution systems (DWDSs); nontuberculous mycobacteria (NTM); engineering environmental factors; biotic factors; control measures

非结核分枝杆菌(nontuberculous mycobacteria, NTM)是除结核分枝杆菌(*Mycobacterium tuberculosis*)和麻风分枝杆菌(*Mycobacterium leprae*)外分枝杆菌属细菌的统称。NTM 约含 200 个物种[1]，近 1/3 为条件致病菌[2]，易对免疫低下人群(老年人、术后患者和艾滋病患者等)构成严重健康威胁。根据生长速率的差异，NTM 可分为快速生长型分枝杆菌(rapidly growing mycobacteria, RGM)和缓慢生长型分枝杆菌(slowly growing mycobacteria, SGM)[3]，SGM 生长速率较慢可能与其缺失氨基酸转运和代谢等相关基因有关[4]。典型的 RGM 包括脓肿分枝杆菌复合群(*Mycobacterium abscessus* complex)、偶发分枝杆菌(*Mycobacterium fortuitum*)和龟分枝杆菌(*Mycobacterium chelonae*)等病原体，SGM 则包括堪萨斯分枝杆菌(*Mycobacterium kansasii*)、鸟分枝杆菌(*Mycobacterium avium*)和胞内分枝杆菌(*Mycobacterium intracellulare*)等[5]。近年来，NTM 感染率在世界范围内快速上升[6-8]。我国虽无 NTM 流行病学的精准统计数据，但全国历次结核病流行病学调查显示，疑似肺结核患者样本中 NTM 分离率从 1979 年的 4.3%增至 2000 年的 11.1%和 2010 年的 22.9%[5]，提示 NTM 感染的日益普遍。

NTM 为寡营养微生物，细胞表面疏水性强，易形成生物膜，具有消毒剂抗性和一定的高温耐受性，在供水系统中广泛存在[1]。供水系统中致病性 NTM 可通过呼吸吸入(淋浴、吞咽过程产生的气溶胶)、皮肤接触等方式感染人体[9]。证据显示，NTM 感染率的升高与集中

式供水系统的普及和现代生活对淋浴的偏好存在密切关联[10-11]。2020 年，中华医学会结核分会最新发布的《非结核分枝杆菌病诊断与治疗指南》明确指出，“应密切关注城市饮用水中 NTM 污染问题，预防 NTM 从环境传播到人”[5]。供水系统作为 NTM 的重要环境来源和主要传播途径需引起足够的重视。

1 NTM 在供水系统的检出情况

NTM 是供水系统的土著微生物，在水厂、管网和用户端等均有检出[12-14]。部分地区龙头水 NTM 的检出率甚至高达 94.4%[15]。供水系统中检出频率高的多为致病性 RGM 和 SGM[16]，如鸟分枝杆菌、堪萨斯分枝杆菌、龟分枝杆菌和偶发分枝杆菌，可能与供水系统消毒剂所形成的胁迫选择压力有关[17]。

NTM 在供水系统具有极高的再生长潜势。多项研究发现管网和用户端 NTM 数量及相对丰度远高于给水厂出水[18-19]。例如，Falkinham 等对美国 8 个供水管网的研究发现，管网水 NTM 平均数量是给水厂出水的 25 000 倍[14]。与其他异养菌相比，NTM 适应饮用水寡营养环境且具有消毒剂抗性，在管网和用户端因具有竞争优势而被富集[20]。

NTM 临床菌株具有明显的地域性分布特征[21]。例如，我国南方地区 RGM 感染率较北方高，沿海地区 NTM 感染菌株多样性随纬度的降低而升高[22]。然而，目前尚不清楚供水系统 NTM 物种水平的时空分布特征，有必要进一步研究并解析其与临床菌株分布特点的关联性。

2 供水系统中影响 NTM 的工程环境因素和生物因子

工程环境因素如消毒剂、可同化有机碳(assimilable organic carbon, AOC)、管材和温度等，以及生物因子如生物膜、自由生活阿米巴原虫(free-living amoebae, FLA)和细菌等均会影响供水系统 NTM 丰度、物种多样性和致病风险(图 1)。

2.1 工程环境因素

2.1.1 消毒剂

氯、氯胺和二氧化氯是给水厂常用的消毒剂，其强氧化性可灭活 NTM，但效用存在差异。在 pH 值为 7.0、温度为 25 °C的水体中，5 种鸟分枝杆菌菌株灭活率达 99.9%所需氯、二氧化氯和一氯胺剂量分别为 51−204、2−11 和 91−1 710 mg/L (暴露 1 min)[23]。pH 和温度可影响消毒剂灭活效果。pH (6.0−9.0)降低，氯和氯胺对鸟分枝杆菌灭活效果升高[24-25]，而二氧化氯基本不变[26]。温度升高(5−30 °C)，三者的灭活效果均升高[24-26]。灭活反应动力学研究发现三者活化能大小关系为氯>二氧化氯>一氯胺[24-26]，说明升高相同温度条件下 3 种消毒剂对鸟分枝杆菌灭活效用的增强程度存在差异，这可能与消毒剂自身性质有关。

2.1.2 AOC

AOC 是指可被微生物同化的易溶性小分子有机碳(如糖类、有机酸和氨基酸)，是饮用水生物稳定性的重要指标之一[27]。NTM 能在低碳环境(50 μg/L AOC)下生长[28]，这一特性使其在供水系统中与其他异养菌的竞争中占据优势。AOC 对 NTM 生长的影响尚无定论。对芬兰和美国 2 个供水系统的研究显示，NTM 数量与 AOC 呈正相关[14,29]；而对荷兰 8 个供水系统的研究却未发现二者的关联[30]。究其原因，这可能与供水系统 AOC 含量差异及消毒剂的使用与否有关。上述研究中，荷兰的饮用水 AOC 含量为 3.1−28.2 μg/L，远低于芬兰(17−234 μg/L)和美国(38−350 μg/L)，可能未达到 AOC 对 NTM 数量产生明显影响的浓度范围；其次，荷兰的供水

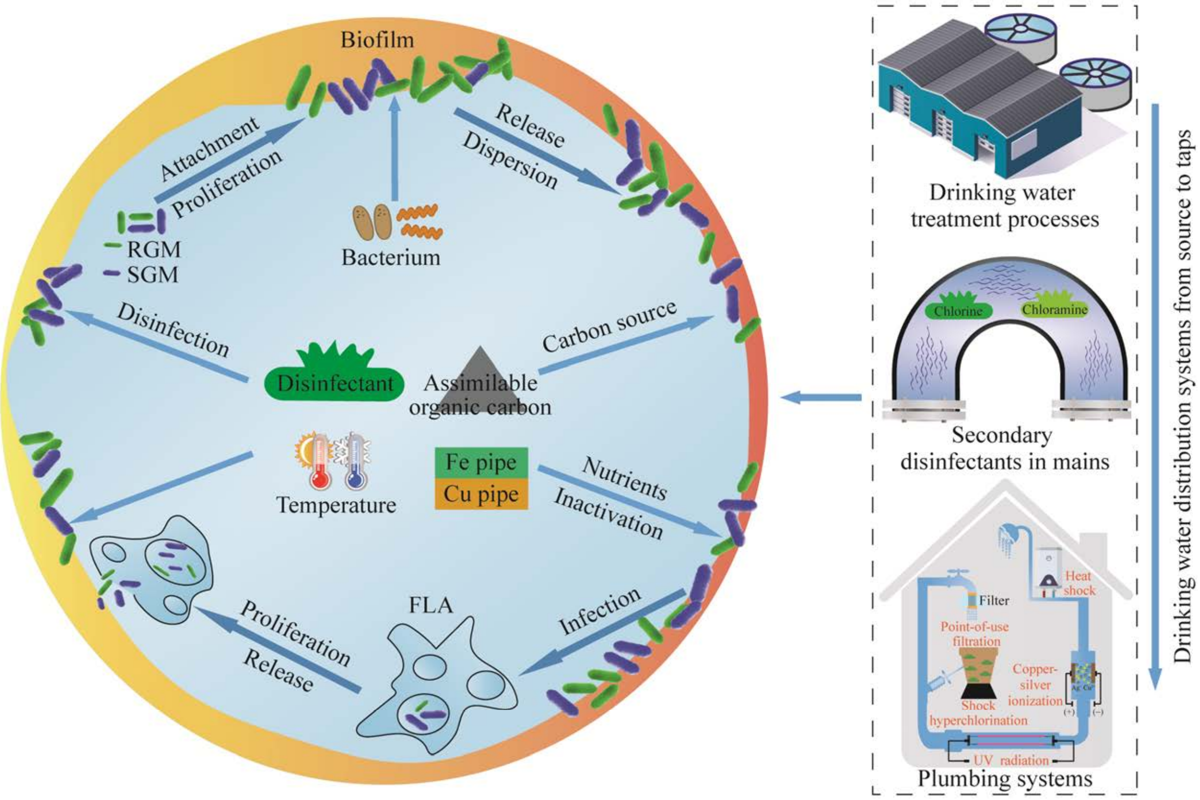

图 1　供水系统 NTM 生长影响因素及全流程控制措施

Figure 1　Factors affecting NTM growth in drinking water distribution systems and “source-to-tap” control strategies.

系统未使用消毒剂，芬兰和美国供水系统消毒剂的使用可能富集了 NTM，削弱了其他异养菌与 NTM 的竞争作用，有利于剂量-效应影响趋势的呈现[14,29-31]。由于 AOC 组成成分复杂，尚不清楚 NTM 对不同 AOC 组分的具体响应规律。

2.1.3　管材

供水系统的管材多样，除主干管以球墨铸铁管为主外，入户管常见管材包括塑料管、不锈钢管和铜管等。由于表面粗糙程度、化学活性及组成成分等差异，不同管材会影响微生物定殖。多个研究比较 NTM 在不同管材表面定殖的难易程度，发现 NTM 在铜管表面形成的生物膜密度低于塑料管材，这可能与铜具有抗菌性能抑制 NTM 定殖有关[32-33]。然而，不同物种 NTM 对铜的敏感性存在差异，Mullis 等的研究发现胞内分枝杆菌在铜片上的生物膜密度分别为鸟分枝杆菌和脓肿分枝杆菌(*Mycobacterium abscessus*)的 16 倍和 19 倍；另外，铜是 NTM 生长必需的微量营养元素，研究发现铜片上胞内分枝杆菌的生物膜密度是聚氯乙烯(polyvinyl chloride)的 3−11 倍[34]。类似地，球墨铸铁管渗出的铁是分枝杆菌代谢酶必不可少的结构和催化辅助因子[35]，铁离子浓度从 0.02 μg/mL 升至 0.10 μg/mL，培养基上耻垢分枝杆菌的干重和活细胞数量分别增长 3.5 倍和 3.2 倍[36]。

2.1.4　温度

一般而言，水温升高有利于 NTM 增殖。Li 等调查上海 10 所住宅的管网水和龙头水，发现 NTM 丰度与温度呈强正相关性[37]；荷兰 8 个供水系统的龙头水调查结果同样显示 NTM 的基

因拷贝数与季节性温度波动具有相关性[30]。

另外，NTM 具有高温耐受性，能在热水系统定殖[20]。有研究在温度高达 55 ℃的热水中分离出蟾分枝杆菌[38]。不同物种 NTM 的高温耐受性存在差异。55 ℃条件下，蟾分枝杆菌的 10 倍减少时间(decimal reduction time)为 6 h，鸟分枝杆菌、草分枝杆菌(*Mycobacterium phlei*)和瘰疬分枝杆菌为 1 h，堪萨斯分枝杆菌仅为 6 min[39]。超过 55 ℃时，NTM 对高温的耐受性急剧下降，蟾分枝杆菌的 10 倍减少时间在 70 ℃时仅为几秒钟[39]。

2.2 生物因子

2.2.1 生物膜

NTM 具有疏水性细胞壁，有利于其快速附着在管壁表面并形成生物膜。NTM 可在饮用水生物膜中高度富集，相对丰度较在水相中高 100 倍[40]。NTM 也是饮用水生物膜中的关键优势物种，在管网和淋浴头生物膜中的相对丰度可分别高达 59%和 99%[41-42]。生物膜有助于 NTM 抵抗消毒剂，灭活附着态 NTM 所需氯剂量为悬浮态细胞的 5 倍左右[43]。消毒剂渗透生物膜基质并扩散至细胞的能力是决定其效果的限制因素，生物膜外部的细胞及基质中有机物均能消耗消毒剂，从而保护内部细胞[43]。此外，从生物膜释放的 NTM 较始终处于悬浮态的细胞消毒剂抗性更强，生物膜释放的鸟分枝杆菌和胞内分枝杆菌灭活率达 99.9%所需氯消毒剂的剂量为悬浮态的 1.7−2.4 倍和 1.2−1.3 倍[44]。

2.2.2 FLA

供水系统中的 FLA 可作为 NTM 的宿主。2014 年，Dealfont 等首次在实际市政管网中发现棘阿米巴(*Acanthamoeba*)和 *Vermamoeba* 携带龟分枝杆菌和拉特分枝杆菌(*Mycobacterium llatzerense*)等 NTM 的直接证据[45]。实验室研究表明，FLA 有助于 NTM 抵御消毒剂等环境胁迫压力，提高其存活能力；在一氯胺剂量为 500 mg/L (暴露 1 min)时，与卡氏棘阿米巴(*Acanthamoeba castellanii*)共培养的鸟分枝杆菌的存活率为单独培养的 37 倍[46]。除为 NTM 提供物理屏障外，FLA 与 NTM 的相互作用有利于筛选出消毒剂抗性更高的 NTM[47-48]。当环境条件变得有利时，NTM 可通过裂解 FLA 或形成囊泡的方式释放[49]。FLA 释放的 NTM 往往具有较强的毒性。有实验研究发现，卡氏棘阿米巴释放的鸟分枝杆菌侵入人体巨噬细胞的效率是单独培养的 5 倍[50-51]。

2.2.3 细菌

供水系统中微生物组成复杂多样，已有超过 30 个细菌菌门被报道[37,52-55]。微生物之间存在竞争、拮抗和共寄生等复杂的相互作用。目前，已发现甲基杆菌属(*Methylobacterium*)、硝化细菌等微生物能抑制或促进 NTM 在供水系统中的定殖和生长。Feazel 等[40]和 Falkinham 等[56]通过对水龙头和淋浴头的调查发现，甲基杆菌与 NTM 鲜有出现在同一生物膜样品中，提出了甲基杆菌属可能对 NTM 存在抑制作用。Muñoz 等进一步研究发现甲基杆菌对鸟分枝杆菌附着的抑制作用与甲基杆菌的细胞活性无关[57]。此外，部分研究指出，硝化细菌等自养型微生物可在饮用水环境中固定无机碳，为 NTM 等异养菌提供有机碳源[58]。氯胺消毒系统中硝化细菌的滋生可引发硝化反应加速氯胺降解，促进包括 NTM 在内的微生物滋长[59-60]。

3 供水系统 NTM 的控制措施及效用

水厂给水处理工艺和管网残余消毒剂可实现饮用水中微生物总量和传统粪源致病菌控制，对 NTM 也具有一定的去除和抑制效果。

针对部分 NTM 能逃逸给水处理工艺并在管网和用户端生长的现象，铜银电离系统、紫外辐射、高氯冲击、点过滤和热控制等技术可用于用户端 NTM 的灭活和控制(图 1)。

3.1 水厂

给水处理工艺对 NTM 具有一定的去除效果。King 等对比了美国 25 个给水厂的原水和出厂水，发现出厂水中鸟分枝杆菌、胞内分枝杆菌的基因拷贝数较原水下降了 1 个数量级[61]。Wang 等考察了给水处理工艺不同阶段对分枝杆菌属的去除效率，发现生物氧化、生物过滤和沉淀单元对减少其基因拷贝数的效果均有限；砂滤使基因拷贝数下降了近 3 个数量级[62]。然而，King 等的调查也发现 NTM 在原水和出厂水中的检出率相当(24%−25%)，而且出厂水中仍可分离培养出多种 NTM[61]，说明部分 NTM 能逃逸给水处理工艺进入管网。

3.2 管网

氯、氯胺等饮用水二级消毒剂(secondary disinfectant)可抑制管网微生物生长，但 NTM 由于其消毒剂抗性反而被定向富集。氯胺对 NTM 的控制效果较氯差，多个研究发现以氯胺为二级消毒剂的管网中 NTM 的检出率或丰度更高。当消毒剂从氯转换为一氯胺时 NTM 的检出率增加了 1.2 倍[63]。Waak 等对比美国(氯胺消毒管网)、挪威(无消毒剂管网)的 2 个实际管网发现，氯胺消毒管网生物膜中总细菌更少但 NTM 密度却显著更高[11]。研究表明，氯胺氧化性较弱，虽能穿透生物膜却无法使细菌立即丧失活性[64-65]，而硝化反应加速氯胺降解可能促进包括 NTM 在内管网微生物的增加[55,59]。

此外，二级消毒剂的使用有利于消毒剂抗性更强的物种富集，抑制 NTM 的多样性。基于 *hsp65* 基因测序的研究显示，氯胺消毒剂管网生物膜中 NTM 几乎为戈登分枝杆菌，而无消毒剂管网生物膜中 NTM 物种多样性程度高[11]。另一基于 *hsp65* 基因测序的研究也显示，氯胺消毒剂管网水相中腓特烈斯堡分枝杆菌(*Mycobacterium frederiksbergense*)占分枝杆菌属的 78.9%−98.9%[66]。

3.3 用户端

3.3.1 铜银电离系统

铜银电离系统可通过电离释放具有抗菌性能的铜银离子。铜离子能破坏细胞壁的渗透性，有利于银离子进入细菌内部并干扰蛋白质和酶的合成[67]。目前，铜银电离系统常用于控制医院供水系统的军团菌(*Legionella*)，推荐的铜银离子浓度分别为 0.2−0.4、0.02−0.08 mg/L[68]。分枝杆菌对铜银离子的敏感性比军团菌低，100 倍的铜银离子剂量才足以灭活相当数量的鸟分枝杆菌[69]。Rhoads 等研究热水循环系统发现，0.3 mg/L Cu^{2+}可使水相中鸟分枝杆菌平均浓度下降了 80.5%[70]。此外，理化因素(磷酸根离子、pH 等)可通过降低铜银离子的生物可获得性影响其抗菌性能[71-73]。

3.3.2 紫外辐射

紫外辐射灭活 NTM 的机理分两方面：一是通过形成嘧啶二聚体破坏 DNA 结构；二是通过光解生成活性氧破坏细胞膜[74]。在 pH 为 7.4 的缓冲溶液中(室温)，紫外对鸟分枝杆菌灭活率达 90%、99.99%所需剂量分别为 6、20−22 mJ/cm^2[75]。不同 NTM 物种对紫外的耐受性不同，偶发分枝杆菌灭活率达 99.9%所需剂量是鸟分枝杆菌、胞内分枝杆菌和慢生黄分枝杆菌(*Mycobacterium lentiflavum*)的 2.5 倍[76]。水相中悬浮物(如颗粒物和胶体)、无机污染物(如铁、亚硫酸盐和亚硝酸盐)和溶解有机物(如腐殖酸、芳香化合物)可通过散射或吸收紫外辐射的方式降低紫外灭活效果[77]。另外，紫外灭菌还面临着 DNA 修复和细菌突变等问题[28,77]。

3.3.3 高氯冲击

高氯冲击是指向饮用水中短时注入高浓度的氯以灭活微生物，常作为用户端病原菌暴发后的临时补救措施。一家养老院实施高氯冲击24 h后，快速生长型NTM下降了近2个数量级[78]。然而，该法面临着病原体重新定殖的风险[78]，为实现长效控制，高氯冲击需多次实施。由于不同物种 NTM 的抗氯性存在差异[78-79]，多次高氯冲击可能富集消毒剂耐受性强的物种，其长效性尚待评估。

3.3.4 点过滤

点过滤是指在水龙头和淋浴头等特定位点安装滤膜用于移除水相中 NTM，其原理是基于滤膜孔隙尺寸的选择透过性。分枝杆菌通常长 1.5−4.0 μm、宽 0.3−0.5 μm[80]，常用的微滤、超滤、纳滤和反渗透膜均可截留 NTM。目前，点过滤常用于高危患者聚集的场景(肿瘤病房、骨髓移植病房和 ICU 等)。有研究发现，在医院水龙头安装 0.2 μm 的滤膜，7 d 后分枝杆菌的丰度相比对照组下降了 99%[81]，但滤膜长期使用后会富集营养物质并形成生物膜，增加 NTM 等病原体在滤膜上再生的可能性[82]。因此，定期更换滤膜是该方法控制 NTM 的关键。

3.3.5 热控制

热控制是指利用热水器提高用户端水温(如出口水温高于 55 ℃)实现对 NTM 控制的方法，已有多个研究论证了该法控制 NTM 的具体效果[38,83]。例如，Sebakova 等发现将医院热水温度提高至 50 ℃以上，NTM 的检出率下降了近 72%[38]。然而，热控制可富集某些耐热 NTM 菌株[35]，也存在高能耗、用户的意外烫伤等弊端[82]。另外，管网末端因不良设计或无人使用等原因往往难以达到预定高温，可能导致 NTM 的灭活不充分和重新定殖[84]。

4 结语

供水系统是 NTM 的重要环境来源和主要传播途径。本文综述了供水系统 NTM 的污染特征，探讨了工程环境因素和生物因子对它的影响，阐明了供水全流程不同阶段 NTM 的控制措施和效用。然而，目前对供水系统 NTM 污染特征、风险评估及控制等方面的认识仍存在较多局限，亟须展开更深入的研究。

4.1 污染特征识别

供水系统 NTM 污染特征识别对风险评估和控制至关重要。目前，国内外对供水系统 NTM 污染特征的识别通常利用培养法揭示 NTM 优势物种的检出情况[85-86]，或采用 qPCR 对分枝杆菌属及少数临床常见菌种进行定量[15,37,87]，或利用扩增子测序检出分枝杆菌属的丰度[66,88-89]。然而，以上方法均无法全面识别供水系统 NTM 物种水平的污染特征。鉴于不同物种 NTM 的生长特点、致病性和临床表现存在巨大差异，有必要对供水系统 NTM 进行物种水平污染特征的精准识别，为准确评估供水系统 NTM 致病风险提供基础数据。

4.2 NTM 风险评估

NTM 经饮用水从环境传播到人，可通过呼吸吸入(淋浴、吞咽过程产生的气溶胶)、皮肤接触等方式感染人体。目前，虽有少量研究报道了鸟分枝杆菌复合群(*M. avium* complex)的剂量反应模型，但这些模型主要建立在动物模型的基础上[90-93]。Hamilton 等结合剂量反应模型提出了微生物定量风险评估(quantitative microbial risk assessment)的框架[94]，然而尚缺乏许多关键参数，如暴露模型、NTM 群落特征和易感人群等，因而无法准确评估供水系统 NTM 风险。因此，有必要完善微生物定量风险评估框架来评估不同用水场景、不同污染特征、不同

风险人群和不同评估终点下 NTM 的风险，为供水系统安全预警和风险管理提供科学依据。

4.3 NTM 控制措施

由于 NTM 能逃逸给水消毒工艺并在管网中再生长，强化用户端 NTM 控制对保护免疫低下人群具有现实意义。目前，虽有部分研究采用高氯冲击、点过滤和热控制等技术对医院给水管网等特殊环境中 NTM 进行控制，但仍缺乏对这些措施长效性的实际评估。此外，铜银电离系统和紫外辐射在实际供水系统中对 NTM 控制的实践仍十分稀少。进一步对用户端 NTM 控制技术进行对比研究和优化，构建行之有效的 NTM 控制方案，可为降低 NTM 经饮用水从环境传播到人的风险、保障易感人群生命健康提供技术支撑。

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