In addition to the direct ecological effects of antibiotics, residuals in the environment contribute significantly to the development and spread of antibiotic resistance, a critical issue that traditional ecological risk assessment frameworks, such as the persistence, bioaccumulation, and toxicity (P–B–T) criteria, fail to address comprehensively. We propose a novel 3M (microflora–microcosm–modeling) framework designed specifically to assess the risk of antibiotics in promoting bacterial resistance within environmental bacterial communities. Our research shows that aquatic microflora in natural water environments exhibit unexpectedly high levels of antibiotic resistance, often comparable to levels found in clinical settings, even though the ambient concentrations of antibiotics are orders of magnitude lower. This finding underscores the importance of considering bacterial resistance as a central indicator in antibiotic risk assessments. Building upon previous studies of microflora in controlled laboratory media, we developed an integrated microflora-based microcosm model, which is combined with advanced ecological and pharmacodynamic modeling to create the innovative 3M framework. This new framework provides more realistic risk thresholds than traditional ecological risk assessment criteria, offering a more accurate reflection of environmental risks. When applied to the Yangtze River, Asia’s largest river, the 3M framework identified moderate to high antibiotic resistance risks at 21.7%, 30.6%, and 47.3% of sampling sites in the upper, middle, and lower reaches, respectively. This study presents an adaptable, evidence-based complement to traditional ecological risk assessments, aligning with evolving regulatory requirements for environmental antibiotic risk management.