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Review 综述

新生隐球菌的病原学特性及耐药机制研究进展

苏妍谕[1,2,3]，孙天舒[4]，李颖星[5]，李懿[1,2]，奕巧莲[1,2]，徐英春[1,2*]

1 中国医学科学院北京协和医院检验科，北京
2 中国医学科学院北京协和医院，疑难重症及罕见病全国重点实验室，北京
3 中国医学科学院北京协和医学院研究生院，北京
4 中国医学科学院北京协和医院，临床医学研究所，转化医学国家重大科技基础设施，临床生物样本中心，北京
5 中国医学科学院北京协和医院，临床医学研究所，转化医学国家重大科技基础设施，细胞、组织与生物医学工程平台，北京

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摘　要：新生隐球菌(*Cryptococcus neoformans*)是一种常见机会致病菌，因其嗜中枢性，常引发隐球菌性脑膜炎。新生隐球菌的侵袭能力与多种因素密切相关，包括荚膜多糖、黑色素、水解酶等毒力因子，以及对宿主体内环境的适应性。在诊断方面，尽管传统方法如真菌培养和印度墨汁染色仍在使用，但其局限性显而易见。相较之下，分子检测、影像技术和生物芯片等新兴手段显著提升了隐球菌感染的诊断准确性和灵敏度。在临床治疗方面，两性霉素 B 和氟康唑作为一线药物被广泛应用，但唑类药物的耐药问题日益严峻，导致临床治疗失败率升高。这种耐药性主要归因于靶点基因突变、外排泵表达上调及基因组倍性改变。针对其毒力因子和耐药机制的深入研究，促进了新型抗真菌疗法的探索，包括旧药新用、新药研发以及创新给药策略。本文结合最新研究，综述了新生隐球菌的毒力因子、诊断技术进展、耐药机制及新疗法的研究动态，旨在为隐球菌病的临床诊疗提供借鉴与启示。

关键词：新生隐球菌；毒力因子；诊断方法；耐药机制

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*Corresponding author. Tel: +86-10-69159766, E-mail: xycpumch@139.com
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Research progress in pathological properties and antifungal resistance mechanisms of *Cryptococcus neoformans*

SU Yanyu[1,2,3], SUN Tianshu[4], LI Yingxing[5], LI Yi[1,2], YI Qiaolian[1,2], XU Yingchun[1,2*]

1 Department of Laboratory Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China

2 State Key Laboratory of Complex, Severe, and Rare Diseases, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China

3 Graduate School, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China

4 Clinical Biobank, National Facility for Translational Medicine, Institute of Clinical Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

5 Biomedical Engineering Facility of National Facility for Translational Medicine, Institute of Clinical Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Abstract: *Cryptococcus neoformans* is a common opportunistic pathogen, exhibiting pronounced neurotropism that often results in cryptococcal meningitis. Its invasive ability is closely associated with multiple factors, including capsular polysaccharides, melanin, hydrolases, and adaptability to the host environment. Conventional diagnostic methods such as fungal culture and India ink staining, though still in use, have notable limitations, whereas emerging techniques like molecular diagnostics, imaging technologies, and biochips have significantly enhanced the diagnostic accuracy and sensitivity. In clinical treatment, amphotericin B and fluconazole are widely used as first-line antifungals, while the resistance to azoles is a growing problem and results in an elevated rate of clinical treatment failure. This is mainly attributed to target alterations, upregulation of efflux pump expression and genomic ploidy changes. Recent studies on virulence factors and resistance mechanisms have driven the development of novel antifungal therapies, including drug repurposing, novel drug development, and innovative drug delivery strategies. This article reviews the latest research in the virulence factors, diagnostic techniques, antifungal resistance mechanisms, and therapeutic development of *C. neoformans*, providing insights into the clinical management of cryptococcosis.

Keywords: *Cryptococcus neoformans*; virulence factors; diagnostic methods; resistance mechanisms

隐球菌病是一种常见的、临床致命的传染病，新生隐球菌(*Cryptococcus neoformans*)是引起该病最常见的病原体，占致病菌株的 90% 以上；继 1946 年首次被报道以来，新生隐球菌感染目前在全球范围内广泛流行[1-3]。新生隐球菌主要感染免疫功能低下的患者，并可与患者体内的其他病原体相互作用，加重感染[4-9]。在自然环境中，该病原体常存在于树木和鸟类(如鸽类)，但也有研究报道它们可在水生环境中生存和传播[10-12] 。接触鸽类或吸入含鸽粪的空气是免疫功能低下人群的关键致病因素，使用糖皮质激素对隐球菌病的负面预后影响同样被证

实[13]。此外，家庭宠物也可能成为潜在感染源，一项来自日本的研究发现，家猫可作为新生隐球菌的携带者[14]；另一项来自土耳其的研究表明，家犬可以感染隐球菌病[15]。这些都可作为导致人类隐球菌病的潜在危险因素。

新生隐球菌最常见的宿主为鸽。鸽粪中的隐球菌孢子播散至空气，被免疫力低下人群吸入后易发生肺部感染，同时新生隐球菌在人体内的播散可引起隐球菌性脑膜炎、骨髓炎[4,6-7,16-18]。在水环境、土壤、树木等自然环境中也有新生隐球菌的检出，提示其具有广泛的潜在传播源[10-12](图 1)。

新生隐球菌主要通过孢子进行呼吸道传播，具有嗜中枢性和强大的侵袭性。它可经呼吸道进入肺泡间质并进一步穿越肺泡毛细血管屏障进入血液，随后可迅速突破多个宿主组织[19]。“特洛伊木马”是最为著名的一种关于新生隐球菌通过血脑屏障的机制，即病原体利用宿主吞噬细胞的携带作用实现免疫逃逸并通过血脑屏障[20]。一些酶类，如一种将宿主花生四烯酸代谢为白三烯的核心酶 5-脂氧合酶(5-lipoxygenase, 5-LO)，以及脲酶和磷脂酶，也在促进新生隐球菌通过血脑屏障中发挥作用[21-22]。除中枢神经系统和呼吸系统的感染外，骨髓或浅表皮肤的感染也有报道[23-28]。此外，Andrade 等[29]研究表明该病原体可能对宿主外周血单个核细胞的 DNA 造成损伤，进一步扩大其致病范围。值得注意的是，新生隐球菌在宿主体内可以呈现多种细胞大小和形态，包括典型酵母细胞(5−7 μm)、增大细胞[也称泰坦细胞(Titan cells)，直径约 10 μm]以及较小的细胞类型(<5 μm)，如微细胞、滴细胞、种子细胞和泰坦细胞；这种形态异质性受到人血浆、营养剥夺、缺氧和低 pH 等多种因素的调控，并在新生隐球菌的感染、播散和宿主环境适应性中发挥重要作用[30-34]。

虽然已有研究尝试用减毒毒株、热灭活株

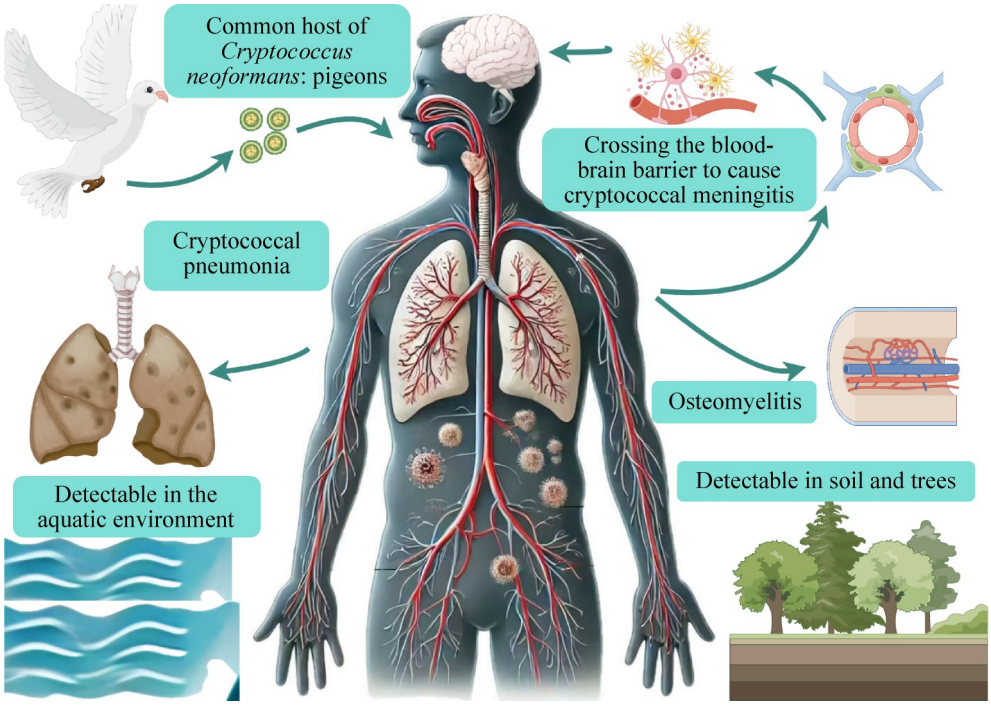

图1　新生隐球菌的感染途径及在自然界的分布情况[4,6-7,10-12,16-18]。本图由Figdraw (2.0)绘制。

Figure 1　Infection routes of *Cryptococcus neoformans* and its distribution in nature[4,6-7,10-12,16-18]. This image was drawn by Figdraw (2.0).

或合成复合物作为被动免疫疗法预防感染，但目前尚无明确有效的疫苗来预防隐球菌病[35-38]。因此，新生隐球菌感染的预防和治疗主要依赖于唑类、两性霉素 B (amphotericin B, AmB)和 5-氟胞嘧啶(5-fluorocytosine, 5-FC)。唑类是治疗隐球菌病的一线药物，但近年来新生隐球菌的唑类耐药率正在增加[39-42]。随着 AmB、5-FC 应用的普及以及新型药物的不断发展，隐球菌引发的感染已在一定程度上得到控制。然而，由于隐球菌病非典型的临床特征和较差的临床预后，它仍对公共卫生构成重大威胁。本文基于现有研究成果，系统综述了新生隐球菌的毒力因子、诊断学进展、常见耐药机制以及新型疗法开发进展，以期为隐球菌病的诊断和治疗的进一步发展提供参考。

1 毒力因子

新生隐球菌倾向于感染免疫功能低下的患者[4-8]，其侵袭性可归因于多种毒力因子(表 1)，包括多糖荚膜、黑色素和水解酶等，这些毒力因子之间还可发挥协同作用。

1.1 多糖荚膜

多糖荚膜是新生隐球菌最具代表性的毒力因子，在病原体的生存和播散中发挥重要作用，其主要由葡糖醛酸氧甘露聚糖(glucuronoxylomannan, GXM)和葡糖醛酸甘露聚糖(glucuronoxylomannogalactan, GXMGal)组成[43-50]。GXM 通过抑制多形核白细胞穿过血脑屏障，减弱宿主炎症反应[51]；而 GXMGal 有助于提高真菌在高温环境中的耐受性，增强其在不良环境中的生存能力[52]。此外，这 2 种多糖均可诱导巨噬细胞发生形态变化，包括早期的自噬反应以及通过 Fas/FasL 相互作用诱导的晚期凋亡[53]。

进一步研究表明，多糖荚膜在毒力形成中具有核心作用。例如，Qu 等[54]的研究发现，高亲和性磷酸盐转运相关基因缺失株的毒力显著下降，这与缺失株磷酸盐摄取能力的降低以及多糖荚膜的变薄和脱落密切相关。此外，多糖荚膜还通过降低吞噬细胞活性等机制，成功实现免疫逃逸并促进病原体在宿主体内的传播[55-56]。

1.2 黑色素

黑色素是在新生隐球菌感染病理过程中一个重要的毒力因子[57-58]。它能够增强真菌抵御免疫反应和环境压力的能力，保护真菌免受细胞破碎或萎缩的影响[59-60]。在分子层面，黑色素还能与电子传递链和细胞内的铁平衡相互作用[61]。

黑色素的合成受环境因素影响。Kumari 等[62]研究发现，黑色素含量与 pH 值和温度的变化相关，pH 值为 8.5 和温度为 30 ℃时最有利于黑色素的生成。此外黑色素与其他毒力因子存在相互作用。Baker 等[63]的研究表明，黑色素的

表1 新生隐球菌的毒力因子及相关机制

Table 1 Virulence factors of *Cryptococcus neoformans* and related mechanisms

Virulence factors	Mechanisms	Related genes	References
Polysaccharide capsule	The thick layer covering the surface of fungal cells is associated with high-temperature stress tolerance and immune evasion	*CAS*, *Pho*, *CAP*	[43-56]
Melanin	Enhancement of *C. neoformans* immune escape and tolerance to stressful environments	*MET3*, *cir1*, *hapX*, *LAC1*, *LAC2*, *CAT1*, *CAT3*, *MRJ1*,	[57-63]
Hydrolysis enzyme	Urease hydrolyzes urea to release ammonia, which elevates environmental pH and promotes melanization of surrounding cells. Phospholipase hydrolyzes phospholipid junctions in cell membranes, which play an important role in the survival and spread of *Cryptococcus neoformans*	*PLB1*, *RAC1*, *URE1*, *LAC1*	[64-71]

增加会螯合钙离子，从而限制钙在多糖亚基之间形成组装外囊所需的二价桥，导致多糖流失，使得黑色素化细胞的荚膜明显比非黑色素化细胞的荚膜薄，这种变化对宿主的免疫反应产生深远的负面影响。这些研究为基于调控环境及互作影响黑色素毒力来开发新型抗菌药物提供了方向。

1.3 水解酶

新生隐球菌能够分泌多种水解酶，其中脲酶和磷脂酶最具代表性，它们在毒力中发挥重要作用。脲酶通过水解尿素释放氨，提高局部环境的 pH 值，进而促进黑色素的生成，这一过程将多种毒力因子联结在一起，共同发挥毒性作用[64]。磷脂酶作为一种常见的真菌胞外酶，能够水解细胞膜上的磷脂，生成脂肪酸、游离脂肪酸、磷脂酸和溶血磷脂等代谢产物。在新生隐球菌中，磷脂酶不仅对其在巨噬细胞和中枢神经系统中的生存至关重要，还与新生隐球菌从肺部播散至其他部位的能力密切相关[65-67]。通过敲除脲酶和磷脂酶编码基因并比较敲除株与野生株在小鼠体内的毒力，可以证实它们在毒力中的重要性[68-69]。此外，在血源性传播过程中，脲酶和磷脂酶均能促进新生隐球菌穿越血脑屏障，侵入中枢神经系统[70-71]。

2 宿主体内生存

对于病原体来说，致病首先需要适应宿主体内的环境，通过调节自身从周围环境中获取必要的物质。此外，它们还必须设法摆脱宿主免疫系统的“清剿”，最终发挥其致病作用(表 2)。

2.1 适应宿主体内环境

2.1.1 耐热性

新生隐球菌的最适生长温度为 25−30 °C，但它能够在人体体温(37 ℃)下存活并保持致病性，这种耐热性依赖于多种基因及细胞通路的协同作用。研究表明，其适应热应激的机制涉及多个方面。例如，通过对 Hog1/p38 通路的翻译重编程，新生隐球菌能够有效应对高温条件，这一机制由 Goich 等[72]揭示；组蛋白去乙酰化酶复合物 Set3C 的亚基 Set302 也在热适应中发挥重要作用，因为缺失 Set302 不仅显著降低了新生隐球菌在 39 ℃下的生长能力，还削弱了其在 50 ℃下的瞬时存活率[73]。此外，通过敲除 *TVF1* 基因并进行动物实验，证实 *TVF1* 基因是新生隐球菌在体外及宿主环境中应对热应激的必需因子[74]。同时，*Dnj1* 基因的功能被阐明为调控温度胁迫下内质网平衡的关键因素[75]。另一项研究则揭示，未分类蛋白 Csn1 不仅提高了新生隐球菌的耐热性，还增强了其耐盐能力，显示出在多种应激条件下的重要保护作用[76]。这些发现共同揭示了新生隐球菌复杂而精密的热适应机制。

表2 新生隐球菌宿主内生存机制

Table 2 Intra-host survival mechanisms of *Cryptococcus neoformans*

Mechanisms	Related genes	References
Adaptation to host environment		
Temperature	Hog1/p38 pathway, *Set3*, *TVF1*, *Aaps*, *dnj1*, *CSN1201*	[72-76]
Gas condition	Target of rapamycin (TOR) pathway	[77-78]
Nutrition metabolism	*CTR4*, *CGP1*, *Aaps*, *Cuf1*, *Ctr1*, *Ctr4*	[79-86]
High-salt	*CSN1201*	[76]
Lipid homeostasis	*Opi3*	[87]
Immune evasion		
Intra-phagocyte survival	*Csn1201*	[76,88-95]
Anti-oxidation	*Sod*, *Ccp1*	[95-98]

2.1.2 二氧化碳耐受

对于宿主体内 CO_2 环境的适应性同样至关重要。新生隐球菌具有在不同浓度 CO_2 环境中调节 CO_2 适应性的能力。Chadwick 等[77]对不同耐受 CO_2 水平的新生隐球菌进行了杂交和小鼠感染实验，结果表明即使是敏感菌株也会在感染后更加适应宿主体内的 CO_2 浓度并发挥其致病性。然而，TOR 通路和细胞膜脂质的重塑可能是 CO_2 适应的关键因素[78]。这些发现突出了适应性对于新生隐球菌的重要意义，揭示了病原体对宿主环境的不同适应机制，为抗真菌药物的开发提供了新的途径。

2.1.3 营养代谢策略

新生隐球菌也有能力改变其能量利用策略，以在不同的环境中生存。在宿主体内常见的营养限制环境中，新生隐球菌可通过上调编码铜转运蛋白和微管相关蛋白的 *CTR4* 和 *CGP1* 基因，促进在氮限制下的生长[79]，也可通过氨基酸渗透酶(*Aaps*)表达将不同氨基酸及其共轭物作为糖类限制下的碳源[80]。

铜作为一种先天免疫反应的组成部分，可以促进新生隐球菌细胞内的活性氧(reactive oxygen species, ROS)产生[81]，也可上调蛋白酶体活性、调节与蛋白质翻译和泛素介导的蛋白质降解相关的蛋白质表达，在机体拮抗新生隐球菌中发挥重要作用[82]。新生隐球菌同样进化出了在铜胁迫条件下维持细胞稳态的代谢机制，例如通过 Cuf1 调节铜代谢、通过细胞壁成分几丁质壳聚糖等调节铜的结合和摄取，以及在人体内高铜、低铜的生态位分别通过 *Ctr1* 和 *Ctr4* 动态调节铜的摄取和降解，以实现细胞铜稳态的维持[83-85]。同时，在肺部侵袭时，新生隐球菌为适应宿主调控的铜的动态分布环境，发展出在宿主体内铜压力下的独特代谢特性，如变更对不同碳源的代谢优先度，以实现铜毒性的拮抗以及压力条件下生长的维持[86]。这种代谢的动态博弈，也为针对新生隐球菌代谢的药物研发提供了新思路。

2.1.4 脂质稳态

脂质稳态对于真菌细胞中多种代谢的维持以及细胞膜、内质网结构的稳态至关重要，因此可能会影响致病菌入侵宿主并致病的能力。为了研究新生隐球菌是否有能力维持脂质平衡，从而维持对宿主的侵袭性和致病性，Lee 等[87]使用了一种缺乏脂质平衡蛋白的新生隐球菌突变体，该突变体缺乏编码亚甲基-脂肪酰-磷脂合成酶的 *OPI3* 基因，并证明这种表型可以通过外源胆碱、磷脂酰胆碱、山梨糖醇和聚乙二醇来挽救，从而保护内质网的功能，而缺失 Opi3 蛋白并不会影响其在肺泡巨噬细胞中的存活，也不会影响其在小鼠体内的致病能力。总之，这项工作确定了脂质平衡对新生隐球菌毒力因子的贡献，并表明宿主胆碱足以支持疾病期间的增殖。

2.2 免疫逃逸

内在免疫反应是抗真菌的第一道防线。巨噬细胞表面的补体和甘露糖受体可介导对新生隐球菌的吞噬作用[88]。中性粒细胞也能捕获和降解病原体，这主要与髓过氧化物酶对真菌产生的强氧化应激有关[89]。自然杀伤细胞(natural killer cell, NK cell)和树突状细胞(dendritic cell, DC cell)也参与了新生隐球菌的内化和破坏[90]。获得性免疫系统的 T 细胞和 B 细胞则通过产生细胞因子和抗体来发挥抗菌作用[91-92]。然而，新生隐球菌也进化出了独特的机制来逃避甚至利用这些免疫反应。例如，除了介导吞噬作用外，新生隐球菌与巨噬细胞的互作还可能导致新生隐球菌附着于巨噬细胞，甚至诱导巨噬细胞发生类自噬的形态变化或凋亡[53,93]。

新生隐球菌的多糖荚膜和形态学变化在免疫逃逸中发挥重要作用[55-56,94]。它甚至可以利用吞噬作用实现跨血脑屏障传播，例如通过“特洛伊木马”机制[20]，或通过各种策略增强其在巨噬细胞中的生存能力。Black 等[95]发现，新生隐球菌通过调节抗氧化剂谷胱甘肽来调节促进黑色素和泰坦细胞生成的氧化还原活动，从而增强

自身在巨噬细胞中的环境适应能力、生存能力和毒力。此外，上文提到的耐热蛋白 Csn1201 不仅有助于病原体在巨噬细胞中存活，还能调节适应性免疫反应，促进新生隐球菌的肺外传播[76]。由于吞噬细胞的杀菌作用在很大程度上依赖于氧化应激，抗氧化系统与新生隐球菌的免疫耐受密切相关，例如超氧化物歧化酶(superoxide dismutase, SOD)可将超氧自由基转化为过氧化氢和氧气，而细胞色素 c 过氧化物酶(cytochrome c peroxidase, Ccp1)可将过氧化氢转化为水[95-97]。新生隐球菌还具有在超高 ROS 环境下生存的特殊策略。Kelley 等[98]证实，在 ROS 条件下，新生隐球菌的 tRNA 修饰和转录本受到的影响较小，表明由于 mRNA 翻译/蛋白质合成受密码子偏差的影响较小，新生隐球菌能够迅速适应氧化环境。这些机制有助于新生隐球菌在宿主体内进一步传播。

3 临床诊断学

目前，对隐球菌感染的诊断主要依赖于实验室检测。常见的检测方法包括印度墨汁染色(India ink staining, IIS)、真菌培养、血清学检测、组织病理学方法和成像技术(表 3)。同时，新的诊断技术也在不断被开发。

3.1 印度墨汁染色

印度墨汁染色是新生隐球菌感染的快速诊断方法之一。其操作简单，光镜下可见细胞周围呈半透明厚荚膜，提示新生隐球菌的存在。然而，IIS 在不同人群中的敏感度和阳性率不稳定可能导致诊断偏误[99]。此外，印度墨汁染色法不能直接反映真菌负荷和药物治疗的疗效，因此存在一定的局限性。

3.2 真菌培养

真菌培养是诊断真菌病原体的金标准。新生隐球菌常用的培养基包括沙保葡萄糖培养基和酵母胨葡萄糖(yeast extract peptone dextrose, YPD)培养基，在 25 ℃和 37 ℃下培养 2−4 d 即可观察到菌落生长[1,6]。此外，可根据培养基上不同的生长形态进行区分鉴别，例如格特隐球菌在卡纳瓦宁-甘氨酸-溴百里酚蓝(canavanine glycine bromothymol blue, CGB)培养基上会产生蓝色色素，而新生隐球菌则无变化[100]。然而，

表3 隐球菌感染的临床诊断技术

Table 3 Clinical diagnostic techniques for *Cryptococcus neoformans* infections

Techniques	Clinical features	Applications	References
India ink staining	Faster but lower specificity	No specific restrictions	[99]
Fungal culture	Gold diagnostic standard	No specific restrictions	[100-102]
Tissue biopsy	Invasive procedures are limited when they are severe and associated with risk of infection	Respiratory infections, spondylitis, surface infections, etc.	[16,103-106]
Immunological tests	Detection of pathogen antigens, antibodies, cytokines, and inflammatory proteins may help with monitoring and prognosis	No specific restrictions	[107-113]
Molecular tests	Fast, sensitive, and effective in differentiating cross-infection pathogens	No specific restrictions	[17,114-119]
Magnetic resonance imaging	Reflection of fungal load	Central nervous system infections	[120-121]
In vivo confocal microscopy	Diagnosis and infection monitoring	Corneal infections	[122]
Electric microfluidic biochip	Effective differentiation between *C. neoformans* and the rare *C. gattii*, with a differentiation rate close to 100%	No specific restrictions	[123]

2014 年的一份病例报告显示，汉逊德巴利酵母(*Debaryomyces hansenii*，前称 *Candida famata*)菌株在 CGB 培养基上也可呈现蓝色[101]，这表明除真菌培养外，还需采用其他技术，如脲酶水解试验，进行辅助区分[102]。

3.3 组织活检

组织活检常用于呼吸道感染、脊柱炎和体表感染的诊断[16,103-105]。组织活检样本的常规染色方法包括苏木精-伊红(hematoxylin-eosin, H&E)染色法和 Gomori 甲酚胺银染色法，后者可用于检测带荚膜的隐球菌[106]。然而，由于取样的侵入性，该方法难以用于中枢神经系统感染，且在病情严重时可能无法进行，还存在感染风险。

3.4 免疫学诊断

检测患者体液中的隐球菌抗原主要依赖于酶联免疫吸附试验(enzyme-linked immunosorbent assay, ELISA)和流式细胞术。例如，ELISA 可用于检测新生隐球菌多糖抗原，定量流式细胞术和免疫荧光显微镜可用于定量检测巨噬细胞的吞噬作用，碘化丙啶染色法可用于检测隐球菌多倍体和分析细胞周期[107-110]。然而，抗原检测存在“后区”现象，抗原过量时可能导致假阴性结果，因此需要进行稀释或其他检测[111]。除了病原体抗原和抗体外，检测脑脊液中的细胞因子和炎症蛋白对患者的监测和预后也很有用。Okafor 等[112]定量检测了 337 名隐球菌脑膜炎患者脑脊液中的细胞因子和趋化因子，并结合 14 d 的存活率进行分析，发现细胞毒性相关的 IL-12、TNF-α、颗粒酶 B 和 IP-10 水平升高可降低急性期 14 d 的死亡风险，这表明炎症蛋白和细胞因子检测在隐球菌病病程监测中具有重要作用。此外，早期诊断对于隐球菌疾病的治疗和预后至关重要。Pruksaphon 等[113]开发了一种基于 MAb18B7 单抗的夹心免疫层析检测法，该方法对新生隐球菌 GXM 抗原具有高度特异性，有望降低隐球菌脑膜炎的死亡率和发病率。

3.5 分子试验

目前用于新生隐球菌感染分子诊断的技术主要包括聚合酶链式反应(polymerase chain reaction, PCR)、重组酶聚合酶扩增(recombinase polymerase amplification, RPA)、簇状规律性间隔短回文重复序列(clustered regularly interspaced short palindromic repeats, CRISPR)以及元基因组下一代测序(metagenomic next-generation sequencing, mNGS)(表 4)。

脑脊液 PCR 有助于中枢神经系统病原体的感染检测。检测方法包括通过 *ITS* 和 *rRNA* 基因检测隐球菌属真菌，以及通过检测物种内特异基因(如 *QSP* 基因、*cyt b* 基因)实现物种间区分[114-117]，从而实现病原体的定性检测；定量 PCR 技术还可用于感染负荷的定量分析[115]。然而，传统 PCR 检测难以实现床旁检测。基于传统 PCR 开发的新型多重 PCR 试剂盒(如 QIAstat-Dx ME 试剂盒)在隐球菌脑膜炎的诊断中具有高效、快速、准确的特点[114]。此外，Liu 等[118]开发了一种基于 RPA 和 CRISPR 的新型诊断疗法，

表4 隐球菌感染的分子诊断技术

Table 4 Molecular diagnostic techniques of *Cryptococcus* infections

Diagnostic technique	Target genes	Evaluation	References
PCR	*QSP1*, *28S rRNA*, *18S rRNA*, *ITS*, *cyt b*	Qualitative and quantitative, but difficult to achieve immediate detection; multiplex PCR technology can detect a variety of pathogens	[114-117]
RPA-Cas 12a	*ITS*	Efficient, fast, accurate, no cross-reactivity	[118]
mNGS	*ITS*, *18S rRNA*	Improved efficiency in the diagnosis of multi-pathogen co-infections	[17,119]

通过 RPA-Cas12a 荧光检测法和 RPA-Cas12a 免疫层析检测法实现了高效、快速、准确的诊断。

在某些合并感染或感染部位不常见的情况下，单一病原体的检测变得更加复杂和困难。mNGS 显著提高了病原体诊断的效率。例如，在 2 名隐球菌性骨髓炎合并踝关节感染或前列腺感染的患者中，活检和病理组织测序证实了新生隐球菌的存在，这表明 mNGS 在诊断新生隐球菌感染方面具有临床可行性和相较于其他诊断技术的优越性[17,119]。

3.6 磁共振成像 (magnetic resonance imaging, MRI)

由于新生隐球菌的嗜中枢性，脑部成像技术也可在隐球菌病的诊断中发挥重要作用。早在 1995 年，Kondo 等[120]就发现磁共振成像有助于隐球菌脑膜脑炎假性囊肿的早期诊断。Musetta 等[121]通过比较不同分离株引起的单个病灶，发现了定量 MRI 技术在评估新生隐球菌感染负荷中的潜力。此外，MRI 技术还可用于评估脑隐球菌病患者病变中与真菌负荷相关的基于图像的生物物理特性，以确定个体化治疗方法。

3.7 其他实验室诊断技术

在一些罕见的病例中，如角膜感染，可结合体内共聚焦显微镜 (*in vivo* confocal microscopy, IVCM)进行诊断和监测。在一名患有角膜隐球菌病的 66 岁男性中，IVCM 检查发现患者角膜上皮和表面基质中存在许多圆形或类圆形病原体，且每个病原体中央都有一个高反射体，证实了新生隐球菌感染；患者在接受唑类和 AmB 联合治疗后成功康复[122]。

为实现高效、准确的鉴别诊断，Kong 等[123]开发了一种可在 20 min 内检出隐球菌，并以接近 100% 的区分率有效区分新生隐球菌和格特隐球菌的电动力微流控生物芯片(图 2)。该装置集电穿孔裂解细胞和电化学检测分析核酸于一体，结合纳米复合材料和特异性探针技术，实现了对新生隐球菌 60 pg/mL 的检测限以及对格特隐球菌 100 pg/mL 的检测限[123]。这项研究表明，集成微流控生物芯片在检测效率和灵敏度方面具有广泛的应用潜力，可用于隐球菌诊断和疾病预防。

该生物芯片包含 2 个区域：用于细胞破裂的电裂解区和用于核酸检测的电化学检测区，

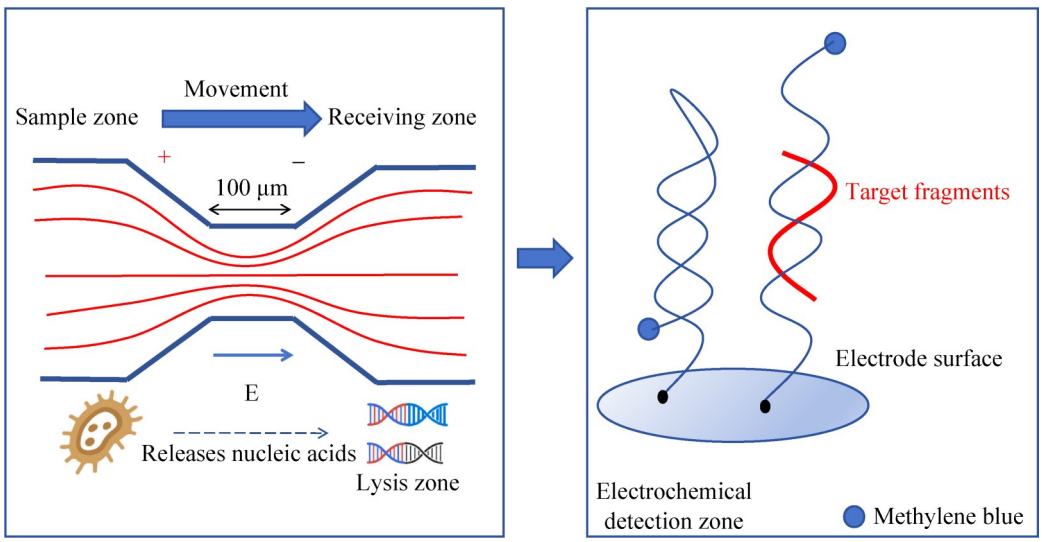

图2 用于新生隐球菌检测的电动力微流控生物芯片。根据文献[123]改编。

Figure 2 Electric microfluidic biochip for *Cryptococcus neoformans* detection. Adapted from literature [123].

两者通过蛇形通道连接；电裂解区由正负 2 个电极组成，形成多个电穿孔裂解区段，裂解后的样品将进入电化学检测区；电化学检测单元固定末端带有亚甲基蓝的自组装发夹探针；在没有目标物的情况下，亚甲基蓝分子靠近电极，由于与电极表面的相互作用，产生较高的电流信号；当目标物存在时，会与探针杂交，导致“发夹”打开并将亚甲基蓝分子末端移离电极，导致电流信号降低；通过电流信号的大小可以确定检测结果[123]。

4 常见的抗真菌耐药性机制

目前，治疗新生隐球菌感染的药物主要有 AmB、5-FC 和唑类 3 类。AmB 以膜麦角固醇为靶点，通过导致孔隙形成、通透性改变和 ROS 积累，最终引起真菌细胞死亡[124-126]。唑类药物作用于 14-α-去甲基酶，抑制麦角甾醇的生物合成，导致细胞膜渗透性和代谢状态改变，从而抑制细胞生长或导致细胞死亡[127-128]。5-FC 通过胞嘧啶渗透酶进入细胞，并在尿嘧啶磷酸核糖转移酶的作用下转化为 5-氟尿嘧啶(5-fluorouracil, 5-FU)，抑制 DNA 和 RNA 的合成[129-130]。由于破坏细胞壁的完整性有助于细胞吸收，AmB 和唑类还可与 5-FC 协同作用[131] (图 3)。

然而，近年来由于新生隐球菌对抗真菌药物(尤其是唑类)耐药率的上升，隐球菌病的防治变得更加困难。相关耐药机制见表 5。对新生隐球菌耐药性机制的探索有助于开发新药和优化临床疗法，从而改善公众健康。

4.1 靶点变更

14-α-去甲基酶是唑类药物的靶点，在耐药性中发挥关键作用。其编码基因 *ERG11* 的突变可导致酶的结构和活性发生变化，从而降低药物与靶点的结合能力，降低药物敏感性[132]。Bosco-Borgeat 等[133]研究了从隐球菌脑膜炎患者体内分离出的耐药菌株，发现耐药菌株中 14-α-去甲基化酶发生 G484S (丝氨酸取代甘氨酸)突变，从而对氟康唑产生耐药性。此外，不同物种之间的唑类敏感性差异也可归因于 *ERG11* 基

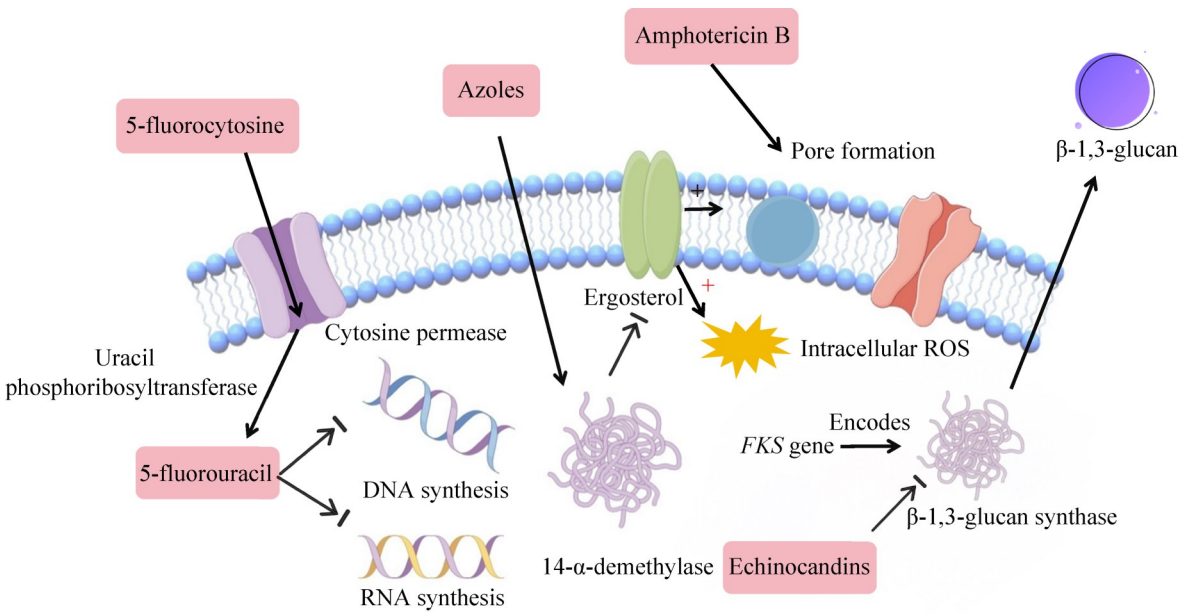

图3 唑类、两性霉素B和5-氟胞嘧啶的作用机制[124-131]。本图由Figdraw (2.0)绘制。

Figure 3 Mechanisms of azoles, amphotericin B (AMB) and 5-FC[124-131]. This image was drawn by Figdraw (2.0).

表5 新生隐球菌的耐药机制及相关基因

Table 5 Resistance mechanisms of *Cryptococcus neoformans* and related genes

Mechanisms	Explanation	Related genes	References
Target alteration	Lower binding effectivity of drug-target combination	*ERG11*	[132-135]
Upregulation of efflux pumps	Increased drug effluent	*Afr3*	[136-138]
Aneuploidy	Gene copy number variations	*AFR1*, *GEA2*, *ERG11*	[30,142-144,148-149]
Alteration of ergosterol	Altered cell membrane permeability and drug-target binding efficiency	*ERG2*	[135,137,145]

因或 14-α-去甲基酶的结构差异，例如格特隐球菌的氟康唑最小抑菌浓度(minimum inhibitory concentration, MIC)值高于新生隐球菌[134]。类似地，麦角甾醇分子作为 AmB 的作用靶点，其在细胞膜中的含量变化是产生 AmB 耐药的关键机制之一[135]。

4.2 转运蛋白功能的上调

Sanguinetti 等[136]早期研究发现，编码 ATP 结合盒(ATP binding cassette, ABC)转运体的基因参与了氟康唑的主动外排，导致新生隐球菌产生耐药性，并在增强隐球菌毒力方面发挥作用。Yoo 等[137]研究了麦角甾醇合成、ABC 转运体和线粒体代谢在调节年龄依赖性唑类耐受性中的作用，发现老化的新生隐球菌细胞会增加麦角甾醇的产生合成，上调 ABC 转运体，并表现出与代谢活性增加一致的转录和表型特征，从而导致氟康唑耐受性。此外，Oliveira 等[138]描述了一种新型 ABC 转运体 *Afr3*，该转运体在慢性感染过程中积累的新生隐球菌细胞中过度表达，通过促进药物外排增强对唑类的耐药性。

4.3 非整倍体

非整倍体指染色体数目异常，可能导致特定基因的拷贝数变化[139-140]。新生隐球菌特有的“泰坦化”是非整倍体的一种表现形式，已被证实与耐药性密切相关[141]。Gerstein 等[30]发现，在隐球菌病治疗过程中，多倍体泰坦细胞产生的子细胞对氟康唑的耐药性更强，且单个泰坦母细胞能够产生多种类型的非整倍体子细胞，有助于后代在不同环境压力下的存活。Stone 等[142]对临床新生隐球菌菌株进行基因组分析，发现在异质性耐药菌株和复发感染分离株中，非整倍体现象较为常见，其中以 1 号染色体断裂为主。这是因为 1 号染色体上存在氟康唑的药物靶点 *ERG11* 和编码药物外排泵的 *AFR1*。由此可见，非整倍体在真菌的环境适应和耐药性中发挥着重要作用[139-140]。

抗真菌药物暴露可诱导新生隐球菌的基因组变化，导致不同程度的耐药性。Yang 等[143]发现，短时间(48 h)暴露于亚抑制浓度的氟康唑可导致新生隐球菌获得不同的非整倍体染色体，并产生对氟康唑的异质性耐药性以及对 AmB 和 5-FC 的交叉耐药性。由此可见，接触一类药物可促进对更多抗真菌药物的耐药性。

此外，非典型抗真菌药物或非药物因子也会诱导新生隐球菌的耐药性甚至交叉耐药性。Zhang 等[141]发现，内质网应激诱导剂布雷非德菌素 A (brefeldin A, BFA)可导致新生隐球菌的非整倍体：1 号染色体的断裂导致了对氟康唑和 5-FC 的交叉耐药性，以及对 AmB 的超敏反应；1 号染色体上的 *AFR1* 和 3 号染色体上的 *GEA2* 过度表达反映了染色体断裂导致的耐药性。Bosch 等[144]证实，真菌自然栖息地中的环境压力(如氮限制)会增加新生隐球菌对 AmB 和氟康唑的耐药性，并增强对氟康唑的异质性耐药性。尽管非整倍体的不稳定性使得这种耐药性并不稳定，但这些研究仍突出了外部诱导因素和基因组不

稳定性在新生隐球菌耐药性中的重要作用。

4.4 麦角甾醇变化

麦角甾醇是维持隐球菌细胞膜结构和功能的关键成分，参与调节膜的通透性和流动性。麦角甾醇同时也是 AmB 的作用靶点，细胞膜中麦角甾醇含量的降低可能导致药物与靶点的结合率降低，从而增加耐药性[135]。麦角甾醇含量的变化也与唑类耐药密切相关。Altamirano 等[145]研究了异质性耐药的机制，发现新生隐球菌细胞对氟康唑的异质性耐药与菌落大小和细胞膜中麦角甾醇的含量有关。Yoo 等[137]通过研究年龄依赖性唑类耐受性，发现老化的新生隐球菌细胞会增加麦角甾醇的合成，从而导致氟康唑耐受性。此外，在麦角甾醇含量变化的情况下，新生隐球菌可能通过代谢调节来维持细胞膜的功能[146-147]。这种代谢重塑可能间接导致耐药性的增强。

5 隐球菌病的新疗法

目前，针对新生隐球菌感染开发的新型疗法层出不穷。已有多项研究开发了非典型抗隐球菌药物，并验证了其抗菌效果。钙通道阻滞剂维拉帕米不仅对包括新生隐球菌在内的多种真菌具有显著的抗菌活性，还能与 AmB、棘白菌素及 HIV 蛋白酶抑制剂协同增强疗效[150-152]；从传统民间药物“喙尾琵甲(*Blaps rhynchopetera*)”中分离出的抗菌肽 Blap-6，因其优异的抗新生隐球菌特性，成为一种备受关注的潜在治疗方案[153]；同时，用于治疗补体相关疾病的 C5 补体靶向药物 Eculizumab 和抗蠕虫药物芬苯达唑也展示了良好的新生隐球菌抑制效果[154-155]；盐酸度洛西汀则表现出抑制隐球菌浮游细胞以及影响免疫功能低下人群生物膜形成的双重作用[156]。

开发全新机制的抗新生隐球菌药物也是研究热点。研究发现，异巴比松可通过抑制三羧酸循环的关键酶和降低线粒体膜电位，达到抗新生隐球菌的效果[157]；宿主防御肽模拟物 brilacidin 能够显著降低新生隐球菌在巨噬细胞中的存活率，并展现出与卡泊芬净及 AmB 的协同作用[158]；针对 β-1,3-葡聚糖合成酶的驼科单域纳米抗体，在预防和治疗隐球菌感染中展现了良好效果，揭示了纳米抗体靶向真菌酶的潜在应用前景[159]；此外，吲哚乙炔通过干扰细胞膜稳定性及相关通路中的基因表达，对隐球菌具有显著的抑制作用[160]；双硫仑则通过干扰复制、代谢、膜转运和酶活性等多种生物途径，有效抑制了隐球菌的活性[161]；醋酸格拉替雷因其能够促进细胞聚集和多糖分泌，展现出独特的抗真菌机制[162]。

在药物递送与疗效优化方面，研究也取得了新的突破。例如，一种吸入级联靶向递送平台通过将 AmB 封装于功能化聚合物颗粒中，利用巨噬细胞的高效吞噬作用实现细胞内精准靶向，从而显著提高治疗效果并降低肾毒性[163]。此外，药物在感染者体内的药代动力学也会受到偶然因素的影响。例如，Palmucci 等[164]报道生酮饮食可通过优化药物的药代动力学，显著提升唑类的抗真菌疗效，为临床治疗提供了新的策略。

6 总结与展望

新生隐球菌相关的侵袭性真菌感染严重威胁人类健康。近年来，新生隐球菌感染的发病率和耐药株分离率呈上升趋势，给真菌防控系统敲响了警钟，针对其致病机制、分子检测和防控策略的深入研究显得尤为重要。

新生隐球菌致病机制的复杂性为其研究带来了机遇与挑战。未来的研究重点应放在毒力因子的动态调控及其在宿主环境中的作用机制上。例如，新生隐球菌的形态异质性如何帮助其逃避宿主免疫、跨越血脑屏障以及适应不同器官的微环境，亟待通过基因组学、转录组学和蛋白质组学的联合分析予以揭示。这些研究不仅将帮助我们理解隐球菌的生存策略，还将

为新的治疗靶点提供依据。

在分子检测方面，未来的发展方向在于实现快速、高效且便捷的诊断。基于 CRISPR-Cas 技术的新型检测技术以及微流控生物芯片等技术的应用，显著提升了隐球菌感染的早期诊断能力。同时，可考虑在诊断中整合多组学分析技术，以进一步揭示感染过程中的生物标志物，为个性化治疗方案提供支持。

在防控策略方面，开发安全有效的疫苗是解决隐球菌病的关键一步。虽然目前尚无广泛应用的疫苗，但通过整合蛋白质组学和免疫学技术，筛选高度保守且能诱导保护性免疫反应的抗原靶点，将为疫苗研发提供新思路。此外，针对耐药机制的全新抗真菌药物研发仍是研究热点。同时，创新药物递送策略，如靶向纳米递送系统和吸入式递送平台，也将在提升药物疗效和减少毒副作用方面发挥重要作用。

最后，应加强基础研究与临床实践的联动，联合流行病学、分子生物学、免疫学和生物信息学等领域的力量，从整体上提升隐球菌病的防治能力。这不仅将推动新生隐球菌相关领域的科学进步，还将为应对未来的真菌感染流行趋势奠定坚实基础。通过持续的创新与努力，隐球菌病的诊断、治疗和预防必将迎来更加光明的前景。

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