**Objectives:**

Onychomycosis, a fungal nail infection affecting about 4% of the general population, is primarily caused by dermatophytes, though non-dermatophyte molds (NDMs) and yeasts can be involved. The diagnostic gold standard combines direct microscopy and fungal culture. While microscopy is a rapid and specific method for detecting fungal elements, culture allows for species identification but lacks sensitivity and is time-consuming. This study evaluates the applicability of molecular mycology techniques, specifically PCR/sequencing and DNA microarray analysis, as diagnostic tools to complement fungal culture.

**Material & Methods:**

In this retrospective study, 817 microscopy-positive nail samples from patients with suspected onychomycosis, for which conventional culture was negative for dermatophytes or failed to yield conclusive results, were analyzed using molecular mycology, i.e. PCR/sequencing (D2 LSU rDNA) and DNA microarray analysis (EUROArray Dermatomycosis).

**Results:**

Among the 262 samples with NDM/yeast-positive cultures, a dermatophyte was detected in 18 (6.9%) and 146 (55.7%) of the samples using PCR/sequencing and DNA microarray, respectively. Species identification by culture was confirmed by PCR/sequencing in 94 (35.9%) samples and by DNA microarray in 74 (26.3%) samples. Among the 555 nail samples that had produced contaminated or negative cultures, PCR/sequencing identified fungi in 230 (41%) samples, including 97 dermatophytes, while the DNA microarray detected fungi in 411 (74.1%) samples, inclusive of 341 dermatophytes.

**Conclusions:**

Molecular mycology methods, particularly the EUROArray Dermatomycosis, improve the detection of onychomycosis pathogens in nail samples compared to fungal culture. Incorporating molecular techniques into routine practice provides increased sensitivity and reduces the time required for diagnosis and treatment.