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Case Report of Nasal Rhinosporidiosis in South Africa

Appendix

Methods

Microscopy

Routine protocols were used for tissue processed for light microscopy. For the scanning electron microscopy (SEM) illustrated below, small pieces of tissue no larger than 4 mm^3 were fixed in 2.5% glutaraldehyde (EM grade) in 0.1 M sodium cacodylate buffer (pH 7.01, osmotically adjusted with the addition of 0.01 M calcium chloride, 0.1 M sucrose and 0.01 magnesium chloride). After four days, the tissue blocks were rinsed three times in fresh buffer, post-fixed for 2 hours in 1% osmium tetroxide in buffer, repeatedly rinsed in fresh buffer, and dehydrated in a graded ethanol series at hourly intervals. Once in absolute ethanol, the tissues were stored until usage of an SEM was available. Some of the dehydrated tissue pieces were cut with a razorblade under liquid nitrogen (a 'primitive' cryo technique), then all tissue was chemically dried with hexamethyldisiloxane. Critical point drying, which is preferable for fungal samples (*1*) was not available. Dried specimens were mounted on stubs with carbon adhesive, sputter coated with platinum, and viewed on a Zeiss Supra 55 VP Field Emission Scanning Electron Microscope at 2 KV.

Molecular

The following primers were used for the *LSU* PCR:

LROR: 5' ACCCGCTGAACTTAAGC 3'

LR5: 5' TCCTGAGGGAAACTTCG 3'

And for the *ITS* PCR:

ITS1: 5' TCCGTAGGTGAACCTGCGG 3'

ITS4: 5’ TCCTCCGCTTATTGATATGC 3’

ITS1: 5’ TCCGTAGGTGAACCTGCGG 3’

ITS2: 5’ GCTGCGTTCTTCATCGATGC 3’

And for the human beta globin PCR (human specimens):

G1: 5’ GAA GAG CCA AGG ACA GGT AC 3’

G2: 5’ CAA CTT CAT CCA CGT TCA CC 3’

The PCR method of White et al. (*2*) was followed. Sequencing was performed by the NICD core facility, and the analyses were done using NCBI (*3*).

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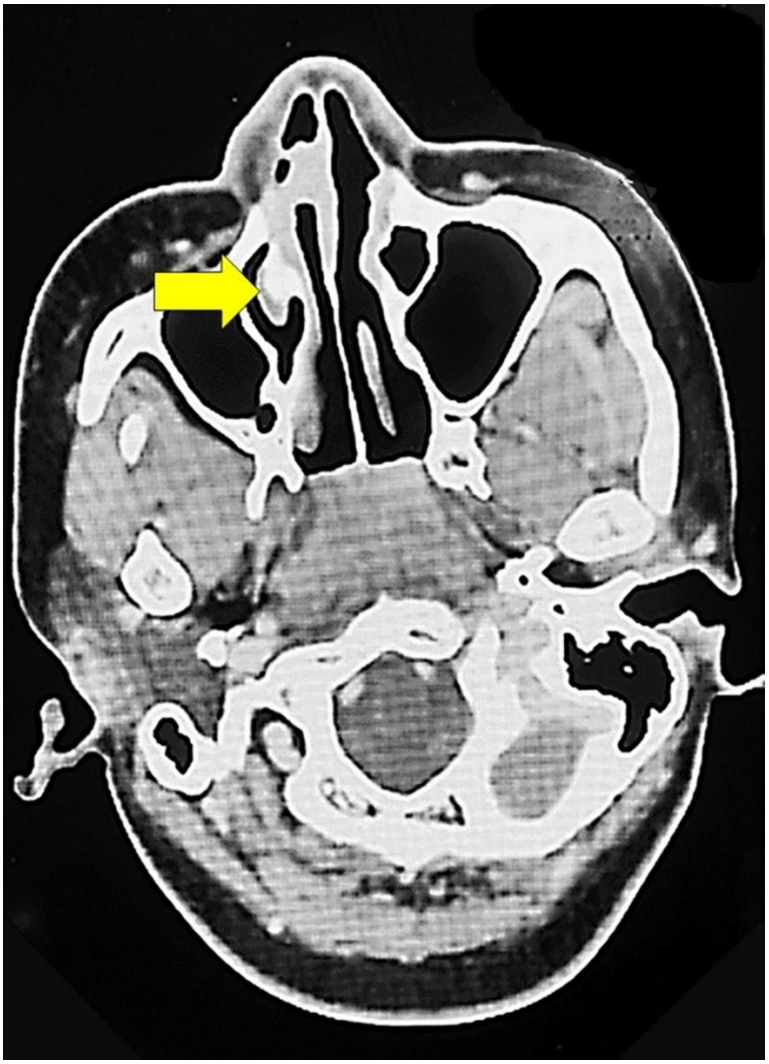
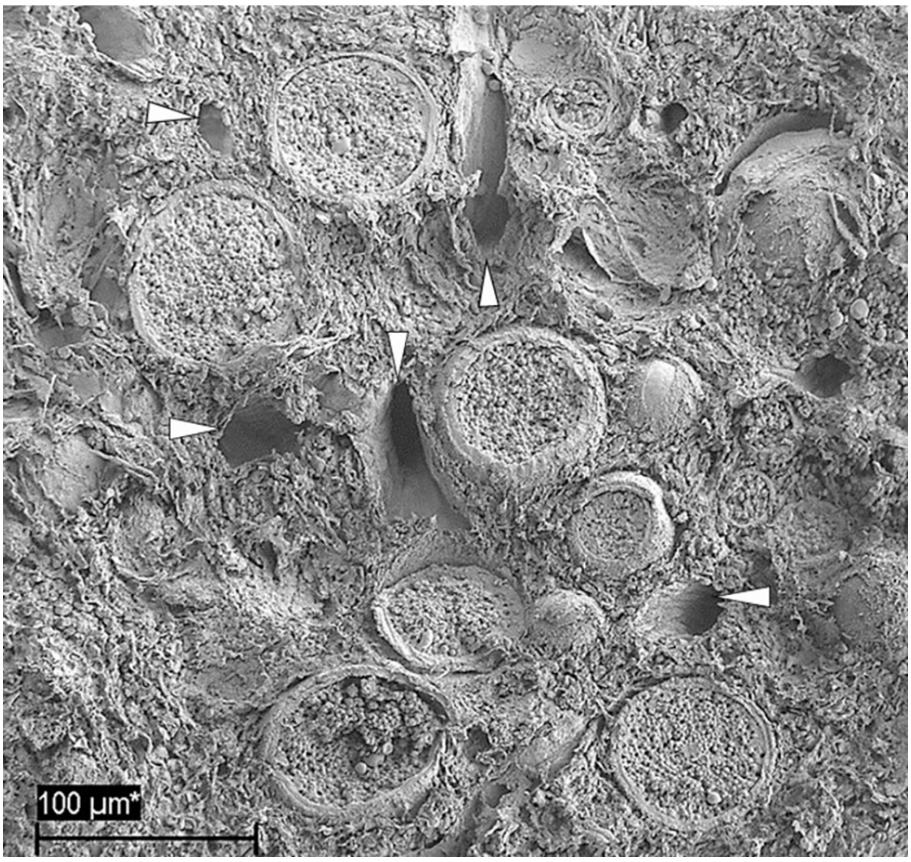
Appendix Table. Countries reporting autochthonous cases of rhinosporidiosis

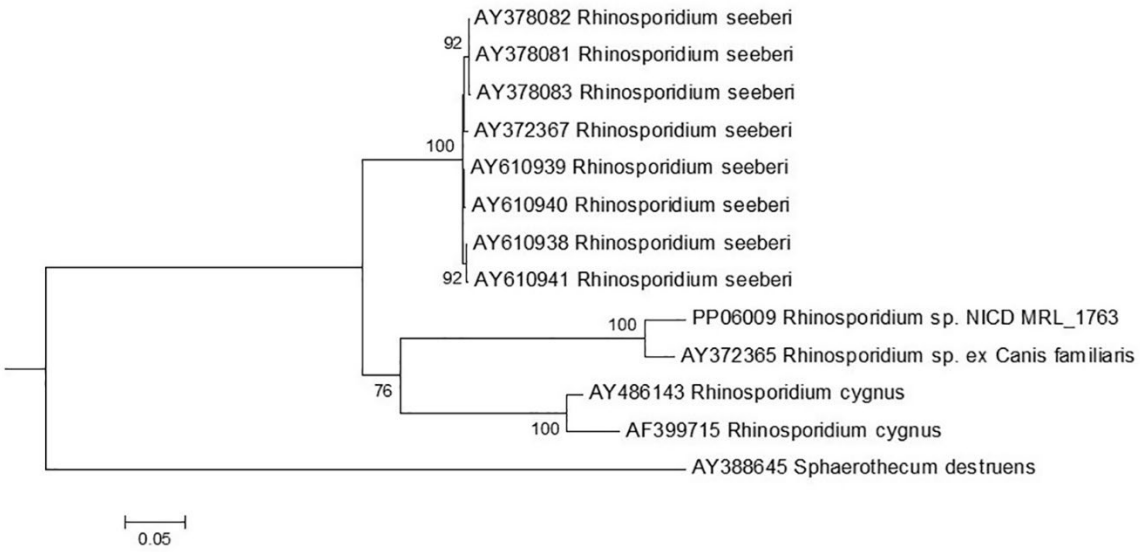
Region	Country	References
Africa	Burundi, Cameroon, Central African Republic, Chad, Congo – Brazzaville, Democratic Rep of Congo (Zaire), Ethiopia, Ghana, Ivory coast, Kenya, Madagascar, Malawi, Nigeria, Rwanda, South Africa, Sudan, Tanzania, Uganda, Zambia	*4–9*
Central and North America	Canada, Costa Rica, Cuba, Mexico, Panama, USA	*10,11*
South America	Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, French Guyana, Paraguay, Venezuela	*11*
Asia	India, Indonesia, Nepal, Pakistan, Philippines, Sri Lanka, Thailand, Vietnam	*12–17*
Europe	Serbia, Spain	*18,19*
Middle East	Egypt, Iran, Turkey	*20–22*



Appendix Figure 1. Computed tomography scan of the nasal cavity, axial section, showing a small homogenous mass with no epithelial extension occupying the anterior part of the right nasal cavity (yellow arrow).



Appendix Figure 2. Scanning electron micrograph of excised nasal polyp (sectioned face) showing the highly vascularized tissue (arrowheads) interspersed with spherical sporangia at various stages of development.



Appendix Figure 3. Phylogenetic relationships of human (*Rhinosporidium seeberi*), dog (*Rhinosporidium sp. canis*), and swan (*Rhinosporidium cygnus*) strains based on *ITS* sequences. The phylogenetic tree is constructed using a neighbor-joining algorithm with 1000 bootstrap replications, following the Jukes-Cantor model. The *ITS* sequence from the case patient is denoted as NICD MRL_1763 and clustered with the sequence obtained from a dog (*3*).