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LEADING ARTICLE 1

Nocardia Update

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Summary

The application of molecular strategies in the identification of *Nocardia* has resulted in the recognition of some new species which are implicated in human disease. In 2003, the Queensland Mycobacterium Reference Laboratory, performed 16S rDNA sequencing analysis on all referred *Nocardia* isolates, in addition to determining phenotypic characteristics and drug susceptibility patterns for each strain. As DNA sequenced based identification is often restricted to reference laboratories we have used our sequence data to validate a phenotypic based identification strategy. This report aims to review recent taxonomic research and determine the incidence of the newer described species in Queensland and to present a simple strategy to identify *Nocardia* spp. in routine bacteriology laboratories.

I wish to respectfully acknowledge the passing of Zeta Blacklock on 12th July 2004. Zeta made significant advancements in the taxonomy of *Nocardia* both in Australia and internationally and wrote the first update on *Nocardia* for the *Mycoses Newsletter* back in 1997.

Introduction

Nocardia spp. are gram-positive, aerobic actinomycetes with a worldwide distribution in soil, and organic matter. The spectrum of disease caused by *Nocardia* is diverse and members of the genus are implicated in pulmonary, cutaneous and disseminated disease in both immunocompetent and immunocompromised patients. Disseminated disease which often involves the central nervous system can be seen in immunocompromised patients. Pulmonary infection is the most common manifestation of nocardia infection. It is usually found in patients with decreased immune function and is probably acquired by the inhalation of contaminated dust particles. Cutaneous disease is the most common presentation in immunocompetent individuals however such infections also occur in immunocompromised patients.

Nocardia was first isolated in 1888 by French veterinarian Edmond Nocard as a cause of bovine farcy in the Canary Islands. Nocard characterised this often fatal illness in cattle as a granulomatous process with draining sinuses, abscess formation with frequent pulmonary involvement (1). The aetiologic agent, which was characterised a year later by Trevisan, was named *Nocardia farcinica*, and in 1954 was made the type species of the genus. The first human nocardia infection was reported in a patient with pneumonia and brain abscess, and in 1896 the isolate was formally classified as *Nocardia asteroides*. Taxonomic studies of aerobic actinomycetes undertaken in the 1960s by Gordon

and Milne found marked differences between isolates of Nocard's original strain, *N. farcinica* (Trevisan), that were held in two culture collections. The isolate designated as ATCC 3318 had mycolic acids consistent with *Nocardia* spp. while NTCC 4524 was more characteristic of mycobacteria. Since the status of the type strain was uncertain, *N. asteroides* was chosen in 1980 to replace *N. farcinica* as the type strain for the genus. The name *N. farcinica* was reintroduced in 1990 and was used to characterise a distinct subgroup recognised as *N. asteroides* (2).

Laboratory Identification**Microscopy**

The standard bacteriological staining techniques such as Gram stain, Ziehl Neelson (ZN) and modified ZN (mod ZN) stains form the main first line approach to recognition and differentiation of *Nocardia* spp. from other aerobic actinomycetes in both clinical specimens and cultures. Table 1 (p 4) shows the key features differentiating *Nocardia* from other aerobic actinomycetes. *Nocardia* appear as Gram positive branching filaments which fragment to form rods and coccoid forms of varying sizes. The characteristic branching filaments of nocardia are usually more evident in direct clinical samples. The filaments may appear beaded on Gram stain. *Nocardia* are resistant to decolourisation with 1% sulphuric acid and thus are acid-fast by the modified ZN method. Although *Nocardia* may show partial acid-fastness (i.e.

showing both acid-fast and non-acid-fast filaments) individual filaments that stain acid-fast show uniform pink staining and do not have a beaded appearance. *Nocardia* are non-acid-fast by the standard ZN method. Interpretation of acid-fast-stains can be difficult. A well stained ZN stain will show good contrast between the carbol fuschin and counterstain. Smears from cultures often show greater fragmentation of filaments than direct smears ☞

from clinical specimens. *Nocardia* need to be distinguished from *Streptomyces* spp. which may show acid-fast coccoid forms and non-acid-fast hyphae but are considered non-acid-fast. *Nocardia* characteristically have fine filaments (<1µ diameter) with right angled branches. In contrast, the filaments of *Streptomyces* spp. are broader and do not show right-angled branches.

Table 1. Distinguishing features between *Nocardia* and affiliated genera

Genera	Colony Morphology	Gram	Mod ZN	ZN	Bacilli Morphology
<i>Nocardia</i>	cream ,yellow, pink indented , bloom, orange smooth	+	+	-	right angled branching filaments, coccoid forms or spores
<i>Actinomadura</i> <i>Nocardiosis</i> <i>Streptomyces</i>	various colours large colonies leathery, folded powdery surface, bloom	+	-	-	extensive filaments
<i>Gordonia</i> <i>Rhodococcus</i> <i>Tsakamurella</i>	cream to red rough or smooth not indented	+	+ or (+)	(+) or -	pleomorphic bacilli no branching or spores
<i>Mycobacterium</i>	cream, buff to yellow or orange rough or smooth	+	+	+ or (+)	pleomorphic bacilli no branching or spores

Nocardia spp. grow on a wide variety of laboratory media including sheep blood agar, Sabouraud's agar and Lowenstein Jensen media. Blood agar incorporating nalidixic acid and colistin is a useful media for isolating *Nocardia* from respiratory specimens that may contain normal flora. The gross morphology of *Nocardia* is extremely variable and differs depending on the growth medium or incubation temperature used. Colonies on routine media are rough, hard and indented into the media and often form a chalky white appearance or bloom, reflecting the growth of aerial hyphae. *Nocardia farcinica* is softer and does not indent.

The colour of colonies can vary from cream, yellow,

salmon pink to orange.

Conventional tests used in the identification of the *Nocardia* genus comprise the decomposition of various substrates, lysozyme resistance, acid production from selective carbohydrates and antibiotic susceptibility patterns. Table 2 (p 5) summarises the key phenotypic differentiating characteristics of *Nocardia* spp. The preparation of media used in this scheme is detailed in the publication of Gordon *et al.* 1974 (3).

Nocardia Update continued next page.

Table 2. Phenotypic differentiating characteristics of *Nocardia* spp

	Substrate Decomposition					Other Key Characteristic
	Adenine	Casein	Hypoxanthine	Tyrosine	Xanthine	
<i>N. asteroides</i> complex	-	-	-	-	-	Susceptibility patterns see Table 3.
<i>N. brasiliensis</i>	-	+	+	+	-	urease activity
<i>N. pseudobrasiliensis</i>	+	+	+	+	-	
<i>N. paucivorans</i>	-	-	-	-	-	Lysozyme susceptible
<i>N. brevicatena</i> complex	-	-	-	-	-	Gentamicin resistant
<i>N. otitidiscaviarum</i>	-	-	+	-	+	Cefamandole resistant
<i>N. veterana</i>	-	-	-	-	-	Inducible β lactamase
<i>N. africana</i>	-	+	-	-	-	Growth at 45°C
<i>N. transvalensis</i> complex (includes <i>N. asteroides</i> IV)	-	-	+	-	-	Aminoglycoside resistance

***Nocardia asteroides* complex**

The *Nocardia asteroides* complex comprises a heterogeneous group of bacteria which demonstrate resistance to lysozyme and fail to hydrolyse casein, xanthine, tyrosine, or hypoxanthine (4). In 1988, Wallace *et al.* recognised that there were six major patterns of antibiotic susceptibility among the complex and these are reproduced in Table 3 (p 6) (5). With the application of molecular analyses, these drug pattern types have been confirmed as distinct species by PCR restriction enzyme analysis and 16S

rDNA sequencing. However, drug patterns I and II may not be species-specific markers and further distinguishing phenotypic characteristics need to be determined (6). Molecular identification of *Nocardia* using 16S rDNA sequencing has allowed for the recognition of new species and allows us to accurately and rapidly identify clinically relevant *Nocardia* spp.

Nocardia Update continued next page.

Table 3. Drug susceptibility patterns recognised within the *N. asteroides* complex

Drug Pattern Type	Species name	Major Group Characteristics
I	<i>N. abscessus</i>	Susceptible to ampicillin, carbenicillin and broad spectrum cephalosporins; imipenem MICs for half of the isolates were high (8-32µg/ml)
II	Not characterised	Same as for Type I except that kanamycin MICs were low (<1µg/ml); susceptible to ciprofloxacin.
III	<i>N. nova</i>	Susceptible to ampicillin but resistant to carbenicillin; susceptible to erythromycin and cefamandole
IV	<i>N. transvalensis</i>	Resistant to all aminoglycosides, including amikacin; susceptible to ciprofloxacin
V	<i>N. farcinica</i>	Resistant to penicillin and broad-spectrum cephalosporins; resistant to all aminoglycosides except amikacin; susceptible to ciprofloxacin and imipenem.
VI	<i>N. cyriacigeorgica</i>	Resistant to penicillins but susceptible to broad-spectrum cephalosporins
Miscellaneous		Unable to group with the above

Nocardia abscessus is a newly named species which was isolated from a joint abscess in a patient with an endoprosthesis of the knee (7). Strains demonstrate a *N. asteroides* type 1 susceptibility pattern. Like *N. nova* and *N. veterana*, *N. abscessus* produces an inducible membrane-bound β lactamase with penicillinase activity which is induced by clavulanic acid. This is evident as these organisms display susceptibility to ampicillin and resistance to augmentin (8, 9). Only two *N. abscessus* isolates have been recognised in Queensland during 2003, therefore further investigation will be necessary to determine if all *N. asteroides* type 1 strains are *N. abscessus*.

Nocardia nova was first described by Tsukamura in 1982 as distinct from *N. farcinica* and other isolates of *N. asteroides*. It was not until 1991 that it was separated as a distinct species on the basis of DNA homology studies, a *N. asteroides* type III drug susceptibility pattern, susceptibility to erythromycin with a zone of inhibition greater than 40mm, and an inability to utilize citrate or acetamide. 75-80% of strains give a positive arylsulphatase reaction after 7 days (5, 9). *Nocardia nova* accounts for approximately 30% of respiratory nocardia isolations over the last five years. However, recent in-house data suggests that approximately one third of these isolates may be unrecognised strains of *N. veterana*.

Nocardia asteroides type IV susceptibility pattern is characterised by resistance to all aminoglycosides including amikacin and an ability to hydrolyse hypoxanthine. *Nocardia asteroides* IV represents an unknown taxon closely related to *N. transvalensis* but this taxon has not yet been validly described as a new species (10). *N. transvalensis* was initially described as a cause of mycetoma in an African patient in 1927 but has since been implicated in invasive and disseminated disease in immunocompromised patients. *Nocardia transvalensis* complex includes *N. asteroides* type IV and *N. transvalensis sensu stricto* and two new taxons 1 and 2 which have been differentiated on the basis of PCR-RFLP analysis. To date *N. asteroides* IV and the new taxon 2 have not been isolated in Australia (10).

N. farcinica was the first member of the *N. asteroides* complex to be separated as a distinct species. *Nocardia farcinica* is characterised by growth at 45°C at 3 days, utilization of acetamide, and an *N. asteroides* type V drug susceptibility pattern characterised by resistance to broad spectrum cephalosporins, all aminoglycosides except amikacin, and susceptibility to ciprofloxacin and imipenem (2, 5). Resistance to cefamandole is a key differentiating test for *N. farcinica*. *Nocardia farcinica* accounts for 20% of pulmonary nocardiosis isolations but it is also an important pathogen in disseminated disease and in cerebral nocardiosis. In the last five years

N. farcinica has been found as the aetiologic agent in 16 disseminated nocardia infections including three patients with brain abscesses.

N. cyriacigeorgica was isolated from the bronchial secretions of a patient with chronic bronchitis and was recognised as a distinct species within the *N. asteroides* complex on the basis of chemotaxonomy and 16S rDNA sequencing (11). The name was derived from *cyriaci* (church) and *georgicus* (of St George) refers to the origins of the name of the German town Gelsenkirchen where the type strain was isolated. *Nocardia cyriacigeorgica* demonstrates type V1 *N. asteroides* complex susceptibility and is the most common respiratory isolate encountered in Queensland. *Nocardia asteroides sensu stricto* demonstrates a *Nocardia asteroides* complex type VI susceptibility pattern.

Other clinically relevant *Nocardia* spp.

Nocardia brasiliensis is usually associated with localised cutaneous infections and accounted for approximately 32% of the 515 *Nocardia* isolates isolated in Queensland from 1998-2002 (Table 4, p 9). Although the majority of strains were isolated from wounds on the extremities of patients' arms and legs, 5 % of *N. brasiliensis* isolates were from systemic infections and a further 5% associated with pulmonary nocardiosis. *Nocardia brasiliensis* isolates are easily characterised phenotypically by their ability to hydrolyse different substrates, aminoglycoside susceptibility and resistance to imipenem. In 1995, a new taxon of *N. brasiliensis*-like organisms were found associated with cutaneous and disseminated disease and the following year were designated as the new species *N. pseudobrasiliensis* (12, 13). *Nocardia pseudobrasiliensis* is an uncommonly recognised pathogen with approximately one case per year being detected in Queensland. Of the 6 strains recognised in the previous five years, 3 were associated with disseminated disease.

N. otitidiscaviarum (formerly *N. caviae*) was initially isolated from the infected ear of a Sumatran guinea pig in 1924 (14). Only 12 isolates of this species have been recognised in Queensland in the last 5 years. The majority of cases were associated with wounds although four pulmonary isolates were recognised.

Nocardia brevicatena complex has not been fully characterised to date. *Nocardia brevicatena* has been described as a group of *Nocardia* that are resistant to gentamicin but demonstrate an *N. asteroides* type II susceptible pattern (i.e. susceptible to all other aminoglycosides) or are susceptible to lysozyme. PCR with restriction enzyme analysis of 32 strains in this group revealed three different groups (Brown *et al.* 1997. Abstr. 97th Gen Meet. Am Soc Microbiol). The lysozyme susceptible group now forms the new

species *N. paucivorans* and are discussed below. All of the *N. brevicatena* isolates reported in Table 4 were lysozyme susceptible and therefore are probable *N. paucivorans* isolates. A single isolate of *N. brevicatena* was isolated in 2003 from sputum of an immunocompromised patient. This organism was resistant to gentamicin and lysozyme and the identification was confirmed by DNA sequencing.

Nocardia paucivorans represents a newly described species of the genus *Nocardia* that was first reported from bronchial secretions of a patient with chronic lung disease (7). More recently systemic infection with *N. paucivorans* was reported in a patient with cerebellar nocardial abscess (15). All *N. paucivorans* strains isolated in Queensland have shown susceptibility to lysozyme which is the key discriminating test for this species. Ten lysozyme susceptible strains of *Nocardia* isolated in the Queensland Mycobacterium Reference Laboratory from 1985-1994 were previously characterised as *N. brevicatena* complex by Brown *et al.* 1997 (Abstr. 97th Gen Meet. Am Soc Microbiol). However, 16S sequencing showed these organisms to have 100% homology with *N. paucivorans* (DNA sequencing on these strains was kindly performed by R. Lumb, IMVS, Adelaide, SA). As four of these isolates were associated with cerebral abscesses in three patients it is important to recognise and differentiate this group from other *Nocardia*. Three isolates of *N. paucivorans* isolates have been recognised in our laboratory in 2003 and in one case was associated with disseminated infection.

Nocardia veterana was first reported in 2001, from a bronchoscopic lavage from a 78 year old returned serviceman and was named after the veteran's hospital in Heidleberg, Australia, where the organism was isolated (16). *Nocardia veterana* has subsequently been reported as a cause of mycetoma in an immunocompromised patient in Japan (17). More recently *N. veterana* was described as a "new emerging pathogen" being recognised as the cause of severe progressive pulmonary disease in immunocompromised patients and in the exacerbation of chronic pulmonary disease in an immunocompetent patient (8). *Nocardia veterana* is indistinguishable for *N. nova* using drug susceptibility pattern alone. Although these species are phylogenetically unrelated they both demonstrate a *N. asteroides* complex type III susceptibility pattern with a characteristically large zone of susceptibility to erythromycin. Also both species are variable in their arylsulphatase activity within 14 days. Current literature states that approximately 75% of *N. nova* strains demonstrate arylsulphatase activity (9) and this can be used as a differentiating test from *N. veterana* which gives a negative arylsulphatase test at 14-days (8, 16). In 2003, 6 strains of *N. veterana* and 15 strains of *N. nova* were identified by 16S rDNA sequencing of consecutive isolates. Of these, 12 (80%) *N. nova* strains and 3 (50%)

N. veterana strains showed arylsulphatase activity. *Nocardia veterana* is reported to be able to utilize rhamnose and inositol as sole carbon sources whereas *N. nova* strains cannot (16, 18). A recent report found that the type strain for *N. veterana* gave different carbohydrate results to those reported by Gurtler *et al.* but reported that the two species could be differentiated on the basis of esculin hydrolysis (19). Our laboratory work investigating bile esculin hydrolysis in 18 strains of *N. nova* and 7 strains of *N. veterana* has found all *N. veterana* strains are positive for bile esculin hydrolysis as were two thirds of the *N. nova* strains investigated. Further investigations are proceeding to determine a reliable test to distinguish between these two *Nocardia* spp. phenotypically. *Nocardia veterana* and *N. nova* can be differentiated by restriction enzyme analysis (REA) of the 5' end of the 16SrDNA gene. *Nocardia nova* has a restriction site for *BstE11* which is not present in strains of *N. veterana*.

Nocardia africana is another newly described species which was recognised causing pulmonary nocardiosis in patients in Sudan (20). *Nocardia africana* is unique among *Nocardia* in that it will only hydrolyse casein. Other distinguishing characteristics include the ability to grow at 45°C, susceptibility to erythromycin and gentamicin resistance. This species has not been recognised in Australia to date.

Identification scheme

Figure 1 (p 10) represents an identification strategy for recognising and differentiating *Nocardia* spp. using susceptibility profiles and a small range of phenotypic tests. The scheme initially relies on

determining the susceptibility pattern to tobramycin and amikacin to yield one of three patterns; susceptible to both (SS), susceptible only to Amikacin (RS) and resistant to both (RR). Susceptibility tests are performed as previously described (21). In both the SS and SR categories susceptibility to erythromycin with a zone of inhibition greater than 40mm differentiates *N. nova* and *N. veterana* from other *Nocardia* spp. In the SS category, resistance to imipenem is useful in recognising *N. brasiliensis* and *N. pseudobrasiliensis*. The latter organisms are then distinguished on the basis of substrate hydrolysis and susceptibility to clarithromycin and ciprofloxacin. The *N. brevicatena* complex shows a SS pattern but is resistant to gentamicin. *Nocardia paucivorans* is the only *Nocardia* sp. which is susceptible to lysozyme. The SR pattern with acetamide utilization is useful for identifying *N. farcinica*. Resistance to amikacin is uncommon and is seen only in the *N. transvalensis* complex.

The identification scheme presented should resolve nocardia identifications in the majority of cases. Susceptibility patterns can be used in the identification of *Nocardia* as they are environmentally acquired and the organisms have not been subjected to any selective antibiotic pressure in the majority of cases. Occasionally *Nocardia* isolates may demonstrate unusual susceptibility patterns and will require 16SrDNA sequencing for confirmatory identification. Organisms which are highly susceptible to all antibiotics should also be confirmed by DNA sequencing.

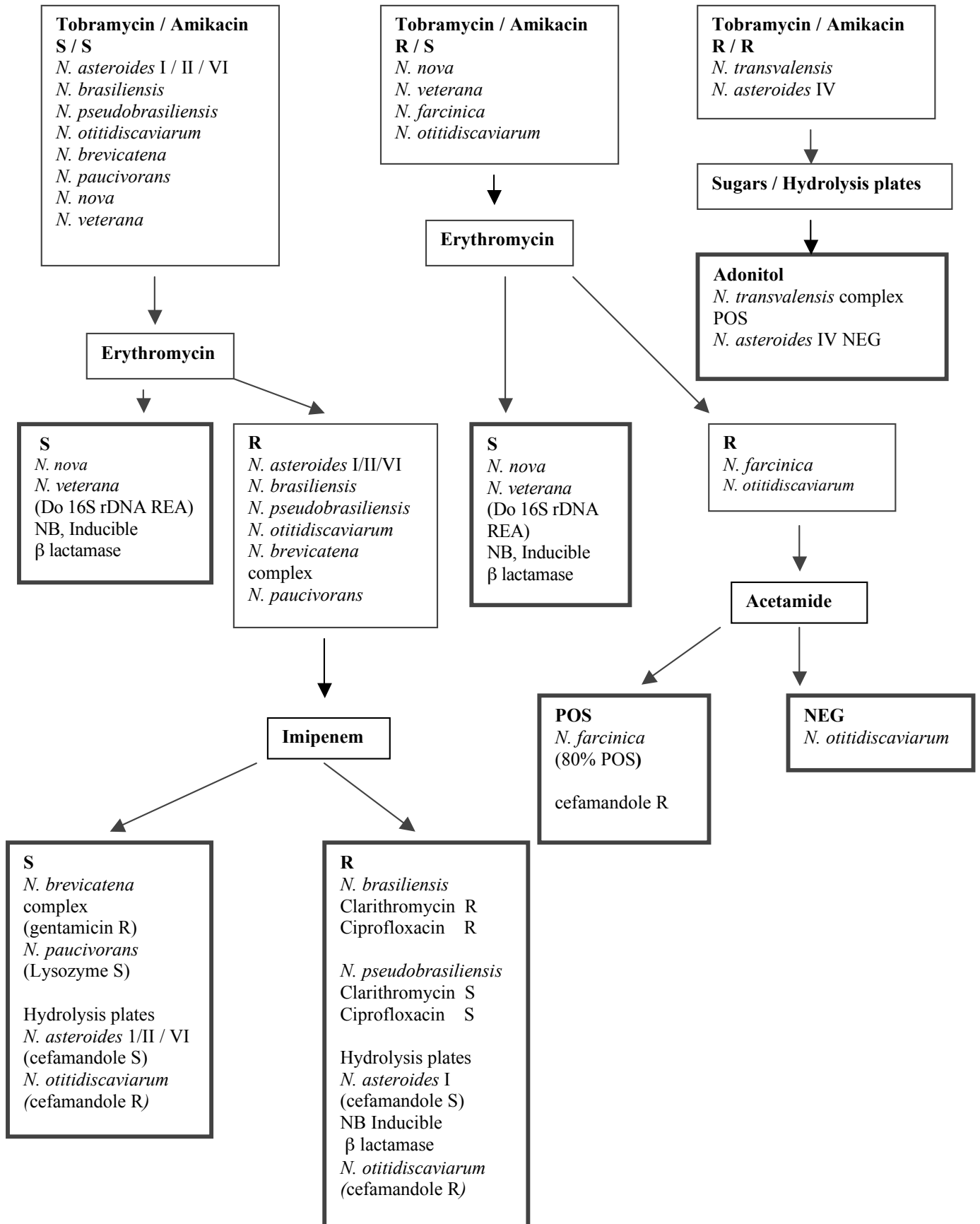
Nocardia Update continued next page.

Table 4: Queensland isolates of *Nocardia* spp. from 1998-2002

	Respiratory	Foot/Leg Wound	Hand/Arm Wound	Sterile Fluid	Blood	Other Wound	Brain abscess
<i>N. asteroides</i> Type I/II/VI n = 136	113	4	5	5	1	8	-
<i>N. brasiliensis</i> n = 176	8	58	71	8	1	29	1
<i>N. brevicatena</i> complex n = 10	4	1	2	1	1	-	1
<i>N. farcinica</i> N = 80	54	3	4	8	5	3	3
<i>N. nova</i> n = 97	74	7	8	6	1	-	1
<i>N. otitidiscaviarum</i> n = 12	4	2	4	-	1	1	-
<i>N. pseudobrasiliensis</i> n = 6	1	-	1	2	1	1	-
<i>N. transvalensis</i> n = 8	7	-	-	1	-	-	-
<i>Nocardia</i> spp. (unspeciated) n = 13	9	1	-	2	-	1	-

Nocardia Update continued next page.

Figure 1. Identification of *Nocardia* using Susceptibility Profiles



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LEADING ARTICLE 2

Malassezia Update

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Summary

The genus *Malassezia* has undergone a comprehensive revision in the past eight years. Prior to 1996, there were three *Malassezia* spp: *M. furfur*, *M. sympodialis* and *M. pachydermatis*. In 1996, four new species were described: *M. globosa*, *M. obtusa*, *M. restricta* and *M. slooffiae*. In recent years, two more new species have been described, *M. dermatis* in 2002 and *M. japonica* in 2003. In the majority of instances, the routine laboratory could, at present, legitimately report the lipodependent *Malassezia* spp. as *Malassezia furfur* complex, but under some circumstances, a full identification would be warranted. Details of the media used for the isolation of the lipodependent *Malassezia* spp. are given together with identification of the species. The diseases in which *Malassezia* are causal, or possibly contributory, are reviewed and where possible, an indication is given as to which species are involved.

Introduction

Malassezia yeasts are lipophilic, basidiomycetous yeasts which show percurrent budding from a broad base. A single species, *M. sympodialis*, may also show sympodial budding. Members of the genus are able to utilise lipids as carbon sources (1), and with the exception of *M. pachydermatis*, have an absolute requirement for the supplementation with medium to long chain fatty acids (C₁₂ – C₂₄) in culture media. *Malassezia pachydermatis* grows on normal media without the addition of lipid but its lipophilic nature is evident by the more luxuriant growth in the presence of lipid. Those species showing an absolute requirement for lipid in the growth medium might be described better as lipodependent.

Malassezia species are found on those areas of the skin where the presence of numerous sebaceous glands provides a source of lipid material essential for their growth. Sebaceous glands are most numerous on the face, scalp, chest and back and it is these areas which are most commonly affected by skin disorders in which *Malassezia* species have been documented as either causal, or are suspected to play an important role (2).

Prior to 1990, there were just two species within the genus *Malassezia*; *M. furfur* from human sources and *M. pachydermatis* from animal sources. During 1990 a third species was described, *M. sympodialis* which differed from the other two species by virtue of the sympodial development of buds (3). Members of the genus *Malassezia* were formerly classified as *Pityrosporum* species. Those from human sources, *P. ovale* (oval cells) and *P. orbiculare* (round cells), were isolated from seborrheic dermatitis and pityriasis versicolor respectively (4). It is now generally accepted that *Malassezia* is the correct name for this genus.

***Malassezia furfur* species complex**

Examination of *M. furfur* isolates using morphological, physiological tests and serotyping suggested that there were recognizable variants within the species. For example, using serotyping of surface antigens, Cunningham and colleagues found there were three serovars of *M. furfur*: A, B & C (5).

Confirmation that *M. furfur* was a species complex came from the application of molecular techniques in a reappraisal of the known *Malassezia* species by Guillot and his colleagues. This involved partial sequencing of the large subunit rRNA and nDNA comparisons (6) which showed that there were seven distinct genetic entities within the *Malassezia* genus. This led to the description of the four new species in 1996: *M. globosa*, *M. obtusa*, *M. restricta*, and *M. slooffiae* (1).

Since the pioneering work of Guillot and his colleagues, there have been a number of other developments in the application of molecular techniques to the identification of *Malassezia* spp.

A system based on PCR and restriction endonuclease analysis was developed which was able to identify five of the seven described species. The large subunit of the ribosomal gene and the internal transcribed spacer (ITS) region were amplified by PCR and the products digested by restriction endonucleases. *Malassezia globosa* and *M. restricta* were difficult to distinguish on the basis of ribosomal DNA analysis and the authors recommended that concurrent use of physiological tests and microscopy of isolates be used to clarify any ambiguities (7).

A further development was the use of a nested PCR technique as an alternative to the culture of *Malassezia* species from patients with/without atopic dermatitis. The fungal DNA was extracted directly from transparent dressings applied to the skin of normal subjects and then amplified in a specific

nested PCR assay. The results showed that individual *Malassezia* spp. could be identified directly from cutaneous samples without the need for culture (8).

A recent study utilizing terminal fragment length polymorphism (tFLP) analysis involving fluorescent nested PCR of the intergenic transcribed spacer (ITS) ITS 1 and ITS 2 region ribosomal gene clusters showed that each of the seven species had unique ITS fragment lengths. The technique was shown to be specific for fungi and was sufficiently sensitive to identify *Malassezia* species directly from scalp specimens in two working days (9).

Rationale for the identification of *Malassezia* species

Generally, culture is not essential for the diagnosis of the most common clinical manifestation of members of this genus, pityriasis versicolor. Routine reporting can be simplified as yeasts resembling the *Malassezia furfur* complex. As members of the *M. furfur* complex are found on normal skin, culture alone does not confirm a causal role in a disease process. Essentially evidence of a pathogenic role is deduced from the clinical appearance of the lesions, the finding of *Malassezia* yeasts in the microscopy of material collected, and in some other disease entities, the response to specific antifungal treatment. Even this approach is not fool-proof as will be evident in the following discussion of the various diseases caused by *Malassezia* spp. (p 15 -18)

There are occasions when routine reporting of isolates as belonging to the *M. furfur* complex would be inadequate.

There are only a small number of reports in the recent literature which document the newly described species in relation to diseases caused by the lipodependent species originally in the *M. furfur* complex. It is therefore important to be aware that in the older literature, it is often unclear which of the newly described *Malassezia* species is being referred to. Investigative studies to determine which of the newly described species are involved in various disease processes attributed to *Malassezia* will obviously require culture and full identification of isolates. However, as discussed above, the need for culture may be obviated by the application of molecular techniques which can be used to identify species directly from clinical specimens.

Where the microscopic appearance of a specimen is unusual further investigation of which species was involved would be of interest.

Culture and identification of *Malassezia* isolates from systemic infections is important and should be pursued rigorously. Following on from this is the scenario is where a patient fails to respond to antifungals given for a *Malassezia* infection and

where full identification would help guide the choice of the appropriate antifungal.

Malassezia species show some variation in their susceptibility to antifungals. While all strains of seven of the species (excluding *M. dermatis*, and *M. japonica*) showed *in vitro* susceptibility to ketoconazole, itraconazole and voriconazole at low concentrations, there was a range of susceptibilities for terbinafine. *Malassezia furfur*, *M. globosa* and *M. obtusa* were more tolerant of terbinafine. *Malassezia furfur* was found to be highly susceptible to relatively resistant to this antifungal whereas *M. sympodialis* was highly susceptible (10).

Media used for isolation

Sabouraud glucose agar with an overlay of sterile olive oil has been used in the past for the growth of *Malassezia* species. However, not all species will grow on this medium and its use cannot be recommended. For example, *Malassezia globosa*, *M. restricta*, and *M. obtusa* require more complex media for their isolation; the incorporation of bile salts in culture media facilitates the metabolism of lipids by these species (1). Conversely, *M. furfur sensu stricto* is the least fastidious of the lipodependent species and can be recovered on Sabouraud agar with an overlay of sterile olive oil (1, 11). The following media support the growth of all lipodependent *Malassezia* species.

Modified Dixon's medium

30 g malt extract, 3.0 g peptone, 20 g desiccated ox-bile, 10 ml Tween 40, 2 ml glycerol, 15 g agar, 1 L distilled water (12).

Leeming-Notman medium

10.0 g bacteriological peptone, 5.0 g glucose, 0.1 g yeast extract, 4.0 g ox-bile, 1.0 ml glycerol, 0.5 g glycerol monostearate, 0.5 mg Tween 60, 10 ml cows milk (whole fat), 12.0 g agar per litre, distilled water 1L, pH 6.2 (13).

Incubate in a moist atmosphere at 32-35°C for up to 2 weeks.

Conventional identification of *Malassezia* species

Since the description of the new species, two identification schemes based on morphology and physiological testing have been described.

The first of these, by the authors responsible for the description of the new species, was largely based on the utilization of various Tweens as lipid sources (4). Further tests which have been added to the scheme are the splitting of esculin and the utilization of Cremophor EL (castor oil) (11). A modification of this scheme is given in the Atlas of Clinical Fungi (14). A disadvantage of identification based on the

use Tweens is that a number of the reactions are quite subtle and require some experience in their interpretation (11).

[Requirements: Complete medium, either Dixon agar or Leeming & Notman agar; Sabouraud glucose agar; Tween 20; Tween 80; Tween 40; catalase reagent; bile esculin agar; Cremophor EL]

An alternative identification scheme, avoids the use of Tweens, and is claimed to give quicker results which are easier to interpret (15).

[Requirements: Complete medium – Leeming & Notman agar; Sabouraud glucose agar; catalase; Cremophor EL; bile esculin agar; Dixon agar]

From a personal point of view, I believe that with this group of yeasts, the identification of species is more easily accomplished with molecular methods. As noted above, culture is unnecessary for some of these methods, and the species present can be identified directly from the clinical specimens. As microbiologists/mycologists, the use of traditional techniques may be found to be more interesting, but the turn-around time from specimen to report is considerably longer than that of the molecular techniques. There is nothing to be accomplished in terms of accuracy and everything to be gained in terms of timeliness. As increasing numbers of laboratories start to use molecular methods on a routine basis, these will be preferred to traditional methods when a definitive identification of a *Malassezia* isolate is required.

Descriptions of species

In recent years, two more species have been described: *Malassezia dermatis* (16) and *M. japonica* (17) (both from patients with atopic dermatitis). An unnamed *Malassezia*, closely related to *M. sympodialis*, has been recently reported from horses (18).

The following descriptions are based on references 1, 4, 16, 17.

Malassezia furfur

Synonymy

Pityrosporum ovale

Colonies mDixon (7 d at 32°C)

Matt or dull, smooth, umbonate or slightly folded, cream.

Micromorphology

Variable micromorphology with long cylindrical (1.5-3.0 x 2.5-8.0 µm) ovoid or even globose cells (2.5-5.0 µm in diameter)

Few strains may produce filaments spontaneously.

Other properties

Good growth at 37°C, max at 40-41°C.

Cells survive lyophilization.

Pathogenic role

Cause of a much smaller proportion of cases of pityriasis versicolor than originally believed.

Malassezia pachydermatis

Synonymy

Pityrosporum pachydermatis

Colonies mDixon (7 d at 32°C)

Matt, convex, sometimes umbonate, cream

Micromorphology

Characterised by small ovoid cells

(2.0-2.5 x 4.0-5.0 µm) with a very broad base.

Other properties

Growth occurs at 37°C, max 40-41°C.

Cells survive lyophilization.

Pathogenic role

Systemic infection in humans.

Ear infections in animals.

Malassezia sympodialis

Synonymy

Malassezia furfur serovar A

Colonies on mDixon (7d at 32°C)

Glistening, smooth, flat or with slight central elevation

Micromorphology

Small ovoid cells (1.5-2.5 x 2.5-6.0 µm), repetitive or sympodial budding is diagnostic when present.

Other properties

Growth occurs at 37°C, max 40-41°C.

Cells survive lyophilization.

Pathogenic role

Cause of appreciable proportion of cases of pityriasis versicolor.

Malassezia globosa

Synonymy

Malassezia furfur serovar B.

Colonies on mDixon (7d at 32°C)

Raised, folded, rough

Micromorphology

Stable spherical cells (2.5-8.0 µm diameter) with buds on a narrow base.

Buds may elongate to form germ tubes or even short filaments comparable to those seen in scale from pityriasis versicolor.

Other properties

None, or very weak growth at 37°C.

Cells do not survive lyophilization.

Difficult to maintain *in vitro*.

Pathogenic role

Cause of appreciable proportion of cases of pityriasis versicolor.

Malassezia obtusa

Synonymy

Pityrosporum ovale

Colonies on mDixon (7d at 32°C)

Smooth, flat

Micromorphology

Large cylindrical cells (1.5-2.0 x 4.0-6.0

µm) with broad base of attachment between mother and daughter cells.

Other properties

Usually grows at 37°C, max of 38°C.
Unpredictable results with lyophilization.
Difficult to maintain *in vitro*.

Pathogenic role

Uncertain.

Malassezia restricta

Synonymy

Malassezia furfur serovar C.

Colonies on mDixon (7d at 32°C)

Small, dull, smooth to rough at edges

Micromorphology

Small spherical or ovoid cells (1.5-2.0 x 2.5-4.0 µm) with a relatively narrow base.

Other properties

Growth at 37°C, max 38-39°C.
Unpredictable results with lyophilization.
Difficult to maintain *in vitro*.
The only lipodependent species that is catalase negative.

Pathogenic role

Uncertain.

Malassezia slooffiae

Synonymy

Pityrosporum ovale

Colonies on mDixon (7 d 32°C)

Rough, usually with fine grooves

Micromorphology

Short cylindrical cells (1.0-2.0 x 1.5-4.0 µm) appearing ovoid under the light microscope with buds formed on a broad base.

Other properties

Growth at 37°C, max 40°C.
Cells survive lyophilization.

Pathogenic role

Isolated from human and animal skin. Most frequently associated with pigs where it represents a greater proportion of the cutaneous yeast flora than in humans

Malassezia dermatis

Synonymy

Unknown

Colonies on Leeming-Notman medium

Yellowish-white, semi-shining to dull, convex, butyrous, entire or lobed margin.

Micromorphology

Spherical, oval, ellipsoidal (2.0-8.0 x 2.0-10.0 µm)

Other properties

Growth at 37°C, ?max 40°C.
Closest to *M. sympodialis* in phylogenetic tree.

Pathogenic role

Isolated from patients with atopic dermatitis and from healthy subjects.

Malassezia japonica

Synonymy

Unknown

Colonies on Leeming-Notman medium

Pale yellowish, semi-shining to dull, wrinkled and butyrous with entire or lobed margin.

Micromorphology

Spherical, oval or ellipsoidal with sympodial budding (2-5 x 2-7 µm)

Other properties

Growth at 37°C but not 40°C.

Pathogenic role

Isolated from patients with atopic dermatitis and from healthy subjects.

Human Diseases Associated with *Malassezia* species

Malassezia species are well documented as being causal in the first two of the following disease states. Their role in the remaining diseases is uncertain.

Pityriasis versicolor^A

Prior to the description of the new species in 1996, the causal organism was believed to be *Malassezia furfur*. Identification of isolates from pityriasis versicolor (PV) using the new nomenclature indicates the causal species are more likely to be *M. globosa* or *M. sympodialis* (19-21).

Lesions on untanned white skin are darker (hyperpigmented) than the surrounding skin. On tanned skin, areas affected by pityriasis versicolor fail to tan (hypopigmented). Pityriasis versicolor in dark-skinned patients can also present as hypopigmented lesions.

Young adults are most susceptible and the infection is more common in tropical and temperate countries. Male and female patients appear equally susceptible to the infection but there are notable differences between the different age groups. PV is rare in children and such cases are more common in tropical countries. Facial involvement is more common in children than in adults. In older adults the prevalence declines corresponding with a reduction in sebum production.

Some studies have noted the lack of conjugal cases, and that the disease usually occurs in more than one family member, which suggests there is a genetic component to susceptibility.

^A In some texts this is sometimes incorrectly referred to as tinea versicolor. This nomenclature suggests a dermatophyte aetiology which is misleading.

Lesions are most frequently found on the upper trunk but are also commonly found on the upper arms, the neck, and abdomen. Less often, lesions can be found in the axillae, groins, thighs and genitalia. The lesions may also extend down the arms to involve the backs of the hands. The backs of the knees may also be affected. Facial and scalp lesions are well documented in the tropics and in occasional cases, these may be the only areas affected by the disease. Palmar lesions have also been reported from the tropics (22).

The lesions usually show a pale yellow coloured fluorescence with the Woods light. It is of interest to note that *in vitro* studies have shown that *M. furfur* is the only species to produce the indole compounds thought to be responsible for the fluorescence (23). This indicates that at least some cases are caused by *M. furfur*. Alternatively, the other causal species may produce indole compounds *in vivo* but not *in vitro*.

The yeasts responsible for PV occur as part of the normal flora. The formation of mycelial forms of the yeasts is a prerequisite to the development of PV which is believed to be controlled by exogenous and endogenous factors. The endogenous factors influencing the development of PV include malnutrition, the use of oral contraceptives, use of systemic corticosteroids or immunosuppressants and hyperhidrosis. The last of these would account for why the disease is more common in the tropics than in temperate countries, and for the higher prevalence in the summer months. Furthermore, lesions are usually restricted to those sites covered by clothes supporting the idea that heat and moisture probably play an important role in pathogenesis.

Opportunistic Systemic Infections

These have been mainly documented in premature neonates in intensive care units receiving lipid rich infusions via intravenous catheters and the first cases were reported in the early 1980s (24).

Although the majority of these infections occurred in neonates receiving lipid rich intravenous infusions, a number have been documented in adults receiving similar nutrition (25, 26). The isolates in most cases were grown on isolation media with overlays of sterile olive oil which suggests that the causal yeast was *M. furfur sensu stricto* which, as noted above, is the least fastidious of the lipodependent species.

Malassezia pachydermatis also been documented as cause of systemic infections in low birth weight infants receiving lipid rich infusions (27, 28). In the second of these reports there was evidence which indicated that the organism was introduced into the intensive care nursery via health care worker's hands colonized by the yeast originating from their pet dogs at home. The organism is believed to have persisted in the nursery by patient to patient transmission.

Seborrheic Dermatitis (including dandruff)

This disease is characterised by the development of red, scaly, greasy lesions most commonly affecting the scalp, nasolabial folds, ears, eyebrows and chest (2).

There have been many studies documenting the role of *Malassezia* yeasts in SD. Many of these have reported the use of antifungals which reduce the numbers of yeasts on the skin leading to a clinical improvement in most cases. Conversely, re-colonisation by yeasts leads to a recurrence in SD (15).

Microscopic examination of skin scale from lesions shows the presence of *Malassezia* yeasts only. The short robust filaments present in PV lesions are not found. There is a need to exercise caution in evaluating the microscopy because healthy subjects also harbour yeasts and these may be present in large numbers in the absence of SD lesions (2, 15).

There is an increased incidence of SD in patients with immunosuppressive disorders. This suggests that the interaction between yeasts and the immune response is an important component in the disease. It has been suggested that the yeasts might induce immunological changes, or behave as irritants. Thus even a modest reduction in yeast numbers will result in clinical improvement (2).

There have been a small number of reports which investigated the aetiology of SD using the newer nomenclature for the *Malassezia* yeasts. *Malassezia globosa*, *M. globosa* with *M. restricta*, and *M. sympodialis* were all been documented as being present in SD lesions. The situation is by no means clear as each of the species also occurs on normal skin and the relative prevalence of the six lipodependent species also appears to vary with geographical region (21).

Seborrheic blepharitis, a chronic inflammation of the eyelid margins, usually occurs in patients with SD of the scalp, brows and ears. Causal organisms were previously reported as *Pityrosporum ovale* corresponding to either *M. furfur* or *M. obtusa* using the new nomenclature.

Malassezia folliculitis

This is a chronic disease characterised by pruritic follicular papules and pustules located primarily on the upper trunk, neck and upper arms. Investigation has shown that there is extensive growth of *Malassezia* in hair follicles. Although *Malassezia* is frequently isolated from folliculitis, this may not be significant as it can also be isolated from normal hair

follicles. For some authors, the exact role of *Malassezia* in folliculitis remains uncertain (29).

It was initially thought that the inflammation may be due both to products of the yeast and free fatty acids produced as result of lipase activity of the yeasts but a subsequent study showed that the levels of free fatty acids present were insufficient to produce inflammation (Puvel & Sakamoto, as cited by Ashbee and Evans, 29).

Some authors claim that the evidence for an aetiological role of *Malassezia* in folliculitis comes from the direct microscopy of the lesions, isolation of the yeast, and the response to antifungal treatment. The effect of the latter is often dramatic. Some cases respond to topical treatment but difficult cases respond to systemic treatment with itraconazole or fluconazole (15).

It is uncertain which species are involved in this condition.

Onychomycosis

Distal and/or lateral onychomycosis was described in ten (5 male, 5 female) Australian patients presenting with subungual hyperkeratosis, onycholysis but without associated paronychia. Infection in the fingernails was more common than in toenails. Direct examination of the nails showed moderate to large numbers of *Malassezia* yeasts but no filaments were found. The causal yeast was isolated on Mycobiotic agar and Sabouraud glucose agar with an overlay of sterile olive oil suggesting that the causal yeast may have been *M. furfur*, which is the least fastidious of the lipodependent species. Each of the four patients treated responded with a decrease in the numbers of yeasts present together with an improvement in the appearance of the nails. Two patients received oral ketoconazole augmented with either topical 3% thymol in chloroform or 2% alcoholic solution of miconazole. One case treated with topical natamycin showed clinical cure and the fourth case treated showed improvement with topical 2% alcoholic solution of miconazole. The authors noted that in selected cases, initial colonization lead onto invasion which ultimately results in the development of onychomycosis (30).

In a study from Chile, fourteen cases of onychomycosis (6 males, 8 females) were reported to have been caused by *M. furfur*. Oval yeasts were seen in the direct examination from 11/14 cases. Short filaments were seen in two cases. *Malassezia furfur* was isolated in pure culture from 11 cases, in combination with *Candida albicans* in two cases, and in combination with *Trichophyton rubrum* in one case. Four cases were treated, two with topical ketoconazole, two with oral itraconazole, resulted in a reduction of the numbers of yeasts present in the nails together with an improvement in the appearance of the affected nails (31).

The short filaments seen in the KOH examination of two of the Chilean cases may have been accumulated subungual debris from scratching PV lesions elsewhere on the body.

The difficulty in ascribing an aetiological role to *Malassezia* in cases of suspected onychomycosis lies in distinguishing between infection and colonization. Nails affected by other disease processes, for example psoriasis, have irregularities in the surfaces of the nails which are readily colonised by micro-organisms. One approach where moderate to large numbers of yeasts resembling *Malassezia* are seen in the KOH examination would be to report this. Where such cases fail to grow any other pathogens on conventional media, a comment on the final report to the effect that a small number of cases of onychomycosis attributable to *Malassezia* have been reported in the literature, and that the presence of these yeasts in the present specimen is of uncertain significance.

Otitis externa

Malassezia pachydermatis is well documented as a cause of otitis externa in dogs, particularly those with floppy ears which occlude the ear canal producing incubator-like conditions.

A human case of otitis externa caused by *M. sympodialis* has been reported in the literature. The heavy growth of the yeast was associated with severe symptoms which abated with antifungal therapy (Chai et al, as cited by Midgley, 11).

A small number of cases of probable *Malassezia* otitis externa in human patients have been seen in a pathology laboratory in Melbourne. Numerous *Malassezia* yeasts were present in the Gram stain and no other significant organisms were cultured on conventional laboratory media (unpublished observation, AJW).

Atopic Dermatitis

Fungi are not thought to be the cause of AD but in some patients, especially adults who do not respond to traditional treatment, *Malassezia* may play a role. The presence of *Malassezia* may exacerbate some cases of AD by either a possible allergic or non-immunogenic mechanism (15)

Psoriasis & Confluent and Reticulate Papillomatosis

Malassezia yeasts are not generally accepted as having an aetiological role.

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BRIEF REPORT

Trichosporon – recent developments

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An isolate of the yeast *Trichosporon asahii* was included in a recent issue of the RCPA QAP in Mycology. This yeast has been well documented as a cause of disseminated infections in human patients so that its inclusion in the program was justified (1, 2).

However, there is a difficulty with some yeast identification schemes, an example being Microscan, that the only species listed in the respective databases is the organism *T. beigelii*. Laboratories using such kits will identify isolates of *T. asahii* as *T. beigelii*.

For a number of years most taxonomists working with the genus *Trichosporon* have been aware that the epithet *T. beigelii* is of doubtful authenticity. *Trichosporon cutaneum* was believed to be synonymous with *T. beigelii* and that the former name was the more correct (3). Unfortunately the name *T. beigelii* has persisted in the literature and occurs in databases used by various manufacturers of kits used in the identification of yeasts.

Prior to 1992, the genus *Trichosporon* was thought to comprise a few, variable, ubiquitous species which resulted in very broad species concepts. For example, in the case of *T. cutaneum*, 62% of the physiological characteristics were described as being variable. As a consequence of this, nearly all clinical isolates were identified as either *T. cutaneum* or as the synonymous *T. beigelii* (2). The same species was claimed to be the causal agent of white piedra, skin lesions and systemic infections, as well as being commonly isolated from environmental sources (1).

Evelyn Gueho and her colleagues examined isolates of both species, *T. cutaneum* and *T. beigelii*, from a number of different collections and showed that they were different species based on their ubiquinone systems and mole % G + C of DNA (1). Further examination of the two species showed that *T. cutaneum sensu stricto* was in fact a rare species found to be an infrequent cause of skin lesions and of axillary white piedra (1, 2). *Trichosporon beigelii* is historically associated with capital white piedra and the correct name of the causal yeast is *T. ovoides* (2). Many of the references to either *T. cutaneum* or *T. beigelii* in older literature very probably relate to different species. For example, the majority of *Trichosporon* isolates reported from disseminated infections were almost certainly *T. asahii* (1, 2).

The 1992 publication showed that a large series of species should be recognised and that these showed marked ecological differences. Isolates from humans were clearly very different species to those coming from environmental sources such as soil and water (1).

The *Trichosporon* species causing infection in humans showed remarkable specialisation in terms of their ecological preferences. In humans there are essentially two habitats for *Trichosporon* spp.

Trichosporon asahii is the only species regularly isolated from systemic mycoses and shows a predilection for haematogenous dissemination. *Trichosporon mucoides* is also isolated from human deep infections and has an apparent preference for the CNS. The second human habitat of *Trichosporon* spp. is that of the skin and its appendages. The majority of isolates of *T. asteroides*, *T. cutaneum*, *T. ovoides* and *T. inkin* are from superficial cutaneous infections, predominantly white piedra. *Trichosporon inkin* is confined to the genital area and is the leading cause of pubic white piedra. *Trichosporon ovoides* is the main cause of white piedra affecting scalp hairs (capital white piedra) (1).

The majority of commercially available yeast identification schemes/kits do a reasonable job of identifying *Candida* spp. and *Cryptococcus* spp. known to be of medical importance. When purchasing a yeast identification system, as well as checking to see how well they identify the more common *Candida*/*Cryptococcus* spp, check to see if they list *T. beigelii* in the database instead of *T. asahii*. Those that do are using a database that is 10 years out of date. This needs to be weighed against other factors when purchasing any of the yeast identification kits.

Essentially there is no such yeast known as *T. beigelii* and all efforts should be made to delete this epithet from databases in yeast identification and to substitute species names that are congruent with the current nomenclature.

Since the re-evaluation of the genus in 1992 (1), there have been three keys described for the identification of *Trichosporon* spp. (1, 2, 3). The most practical of these from the point of view of the clinical laboratory, is that of Gueho and her colleagues (2). One criticism of this scheme would be that it omits reference to *T. loubieri*. There are two reports in the literature documenting this yeast as an opportunistic human pathogen (5, 6). This yeast has also been isolated from the sputa of cystic fibrosis patients where its clinical significance is uncertain (Southern Cross Pathology, unpublished observation).

Salient characteristics of the genus *Trichosporon* based on descriptions from references 1, 2, & 7 are:

- all species form true mycelium which fragments to forms arthroconidia. The extent of arthroconidia formation depends on the species.
- isolates of *T. cutaneum* may revert to yeast growth consisting of sub-globose budding yeast cells
- some species produce other morphological characters such as appressoria, macroconidia and meristematic conidiation (see Table)

- basidiomycetous yeasts, urease positive. The latter can help distinguish *Trichosporon* species from *Geotrichum* species.
- all species able to assimilate a large number of carbon compounds. A further distinguishing feature from *Geotrichum*, species which do not assimilate large numbers of carbon sources.
- fermentation is absent
- most taxa occupy well defined ecological niches
- sexual reproduction has not been documented

Table 1. *Trichosporon* spp. of importance in human infections (adapted from reference 2)

SPECIES	Niche	Infections caused
<i>T. asahii</i>	Human	Predominantly systemic infections; less frequent as a cause of human or animal white piedra. Possible cause of summer-type hypersensitivity pneumonitis in Japan.
<i>T. asteroides</i>	Human	Skin*
<i>T. cutaneum</i>	Human	Skin*– rare. Axillary white piedra – rare.
<i>T. inkin</i>	Human	Nearly all strains from genital area where it is the cause of white piedra. Less commonly from urines, anal swabs of homosexuals, rare systemic infections including endocarditis & peritonitis. Skin*.
<i>T. loubieri</i>	Soil	Human systemic infections rare. Uncertain role in sputa of cystic fibrosis patients. Mastitis in cows.
<i>T. mucoides</i>	Human	Human systemic infections – rare. May have a predilection for CNS. Possible cause of summer-type hypersensitivity pneumonitis in Japan. Possible cause of onychomycosis.
<i>T. ovoides</i>	Human	Human capital white piedra

* These species have been documented as causing skin lesions in immunocompetent patients but this is doubtful. The source of one isolate of *T. inkin* from a well known collection is listed as tinea cruris (2) but it may well have been isolated as a contaminant.
Cutaneous infections secondary to disseminated infections have been documented but need to be considered separately to cutaneous infections occurring in normal immunocompetent individuals.

Table 2. The identification of *Trichosporon* spp. of medical importance (adapted from reference 2)

SPECIES	Morphology	Growth at 37°C	Growth at 42°C	L-arabinose	Sorbitol	Melibiose	Inositol	Rhamnose	Cyclo 0.01%	0.1%
<i>T. asahii</i> *		+	-	+	-	-	-	+	+	-
<i>T. asteroides</i>		v	-	+	-	-	-	+	v	v
<i>T. cutaneum</i>		-	-	+	+	+	+	+	-	-
<i>T. inkin</i>	S-M/A	+	v	-	-	-	+	-	v	-
<i>T. loubieri</i>	GFC	+	+	+	?	+	+	+	+	+
<i>T. mucoides</i>		+	-	+	+	+	+	+	+	+
<i>T. ovoides</i>	A	v	-	v	-	-	-	+	+	-

Abbreviations:

S/M, sarcinae – meristematic conidiation; A, appressoria; GFC, giant fusiform cells 80 x 15 µm “macroconidia”; v, variable; cyclo, cycloheximide.

* *T. asahii* can be difficult to distinguish from *T. asteroides*. *Trichosporon asahii* are farinose (having minute granules like flour from the formation of aerial mycelium) and have regularly rectangular arthroconidia.

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ANNUAL SUMMARY OF OPPORTUNISTIC MYCOSES

TABLE 1. NEW ZEALAND DATA FOR JULY - DECEMBER 2003

Organism	No. of cases	Site	Clinical data
Filamentous fungi			
<i>Acremonium kiliense</i>	1	CAPD	Diabetes mellitus, ESRF.
<i>Aspergillus fumigatus</i>	4	Back (1)	DE+, myelodysplastic syndrome & immunocompromised, previously isolated August 2001 & treated with AmpB. Patient deceased.
		Bronchial washings (1)	DE+, oncology patient, brain tumour, on high dose steroids, also positive for Pneumocystis.
		Corneal scrape (1)	DE-, NHL.
		CT guided FNA – lung (1)	DE-, grew a light growth.
<i>Curvularia pallescens</i>	1	Maxillary crust & ethmoid polyp	DE+, NR.
<i>Exophiala jeanselmei</i>	1	Forearm aspirate & tissue	DE+, isolated 3x, immuno-suppressed.
<i>Fusarium oxysporum</i>	1	Corneal scrape (1)	DE-, NR.
<i>Geotrichum capitatum</i> formerly <i>Blastoschizomyces capitatus</i>	1	Sputum	DE+ (arthroconidia, yeast cells & <i>Aspergillus</i> hyphae seen), also isolated with <i>Aspergillus fumigatus</i> & <i>Candida glabrata</i> . Oncology patient with LUL pneumonia. Isolated 3x.
<i>Mucor hiemalis</i>	1	Ear	DE+, NR.
<i>Paecilomyces variotii</i>	1	CAPD	DE-, isolated 2x, ESRF.
<i>Penicillium citrinum</i>	1	CAPD	DE+, isolated 4x & from Tenckhoff catheter tip. Identity confirmed by 18S sequencing.
<i>Schizophyllum commune</i>	1	Sinus material	DE+, NR.
<i>Scopulariopsis koningii</i>	1	Bronchial aspirate	DE+, CF, post-lung transplant. Multiple isolations.

Continued next page

Organism	No. of cases	Site	Clinical data
Yeasts			
<i>Candida albicans</i>	27	Blood culture (20)	Febrile post-op following removal of staghorn calculus (1), febrile, ulcerative colitis (1), valve vegetation (1), SAH, post-op (1), CABG, post-op line infection (1), oesophageal obstruction due to old injury, thoracic injury during surgery, ICU stay (1), acute peritonitis & peritoneal adhesions, bladder surgery (1), trauma, head injury, subdural haemorrhage, ICU stay (1), abdominal absorption problems, TPN, multiple <i>Candida</i> infections previously, also isolated with <i>Candida glabrata</i> (1), post-abdominal surgery (1), premature baby, NICU patient (2), renal transplant (1), known carcinoma of prostate, urinary retention following CVA, catheterised (1), trauma, head injury, pneumonia, ARDS, ICU stay (1), ?sepsis (1), PUO (1), CF, also isolated with <i>Candida parapsilosis</i> (1), NR (2)
		Abdominal aspirate & CAPD (1)	DE-, gastrointestinal haemorrhage.
		CAPD (2)	ESRF (1), DE-, diabetes, ESRF, deceased (1)
		Chest pus (2)	DE+, post-lung transplant (1), ALL, catheter in-situ, also isolated with <i>Citrobacter freundii</i> (1)
		Corneal scrape (2)	DE+, corneal ulcer (1), corneal keratitis (1).
<i>Candida glabrata</i>	3	Blood (1)	Bowel obstruction.
		CAPD (1)	ESRF.
		Pleural fluid (1)	DE+, adenocarcinoma oesophagus, CHD, hypercholesterolemia.
<i>Candida guilliermondii</i>	1	CAPD	DE+, ESRF.
<i>Candida haemulonii</i>	1	CAPD	DE+, ESRF. Isolated 2x & also from Tenckhoff catheter tip.
<i>Candida lusitanae</i>	2	Blood	Renal patient (1), NR (1)
<i>Candida parapsilosis</i>	10	Blood (5)	Line sepsis (1), cystic fibrosis (1), cystic fibrosis, on steroids, also isolated from catheter tip (1), NR (2)
<i>Candida parapsilosis</i>		CAPD (5)	Diabetes mellitus with ESRF (1), ESRF (4)

Organism	No. of cases	Site	Clinical data
<i>Candida tropicalis</i>	7	Blood (5)	Previous breast Ca, post-treatment developed AML, neutropenic, febrile (1), pneumonia, deceased (1), IVDU (1), cystic fibrosis (1), febrile with confusion (1)
		Bone marrow (1)	NR.
		CAPD (1)	ESRF.
<i>Rhodotorula mucilaginosa</i> - formerly <i>R. rubra</i>	1	Blood	On TPN, CVL sepsis.
<i>Pneumocystis jirovecii</i> (<i>carinii</i>)	10	Sputum (1)	Breast Ca, on steroids (1)
		Early morning sputum (1)	Brain tumour, on high dose steroids.
		Induced sputum (3)	HIV+ (2), significant risk factors, not HIV tested (1)
		BW (5)	HIV+ (1), CNS lymphoma, on high dose steroids (1), lymphoma, on steroids (1), renal transplant patient on methotrexate (1), CLL, BMT patient (1)
Aerobic Actinomycetes			
<i>Nocardia brasiliensis</i>	1	Index finger	Post spider bite
<i>Nocardia cyriageorgica</i> - formerly <i>N. asteroides</i> type VI	2	Sputum (1)	Bronchiectasis, isolated 2x
		Abdo incision line (1)	DE+, and re-isolated, post-op
<i>Nocardia farcinica</i>	2	Sputum	Productive cough, abnormality on CXR (1), NR (1)
<i>Nocardia nova</i>	6	Sputum (5)	Pneumonia, DE+ (1), CXR changes ?TB (1), NR (3)
		Olecranon bursa abscess (1)	Bursitis
<i>Nocardia pseudovaccinii</i>	1	Hand pustule	NR, identified by 16S sequencing
<i>Gordona bronchialis</i>	1	Breast	Abscess

KEY:

ALL	Acute lymphoblastic leukaemia	CXR	Chest X-ray
AML	Acute myeloid leukaemia	DE	Direct examination
AmpB	Amphotericin B	ESRF	End stage renal failure
ARDS	Acute respiratory distress syndrome	FNA	Fine needle aspirate
BMT	Bone marrow transplant	HIV	Human immunodeficiency virus
BW	Bronchial washing	ICU	Intensive care unit
Ca	Carcinoma	IVDU	Intravenous drug user
CABG	Coronary artery bypass graft	LUL	Left upper lobe
CAPD	Continuous ambulatory peritoneal dialysis	NHL	Non-Hodgkin's Lymphoma
CF	Cystic fibrosis	NICU	Neonatal intensive care unit
CHD	Congenital heart disease	NR	Clinical data not received
CLL	Chronic lymphoblastic leukaemia	PUO	Pyrexia of unknown origin
CVA	Cardiovascular accident	SAH	Sub-arachnoid haemorrhage
CVL	Central venous line	TB	Tuberculosis
		TPN	Total parenteral nutrition

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Note: The above data were supplied by the following participating sentinel laboratories: Auckland and Children's Hospital; Canterbury Health Laboratories; Dunedin Hospital; Gisborne Hospital; Greenlane Hospital; MedLab Central (serving Palmerston North Hospital); MedLab, Bay of Plenty (serving Tauranga Hospital); Memorial Hospital Hastings; Middlemore Hospital; Northland Base Hospital; North Shore Hospital; Rotorua Hospital; Southland Hospital; Waikato Hospital; Wellington Hospital; Whakatane Hospital.

