REVIEW ARTICLE: CLINICAL MYCOLOGY

Candida krusei: biology, epidemiology, pathogenicity and clinical manifestations of an emerging pathogen

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Summary. Early reports of Candida krusei in man describe the organism as a transient, infrequent isolate of minor clinical significance inhabiting the mucosal surfaces. More recently it has emerged as a notable pathogen with a spectrum of clinical manifestations such as fungaemia, endophthalmitis, arthritis and endocarditis, most of which usually occur in compromised patient groups in a nosocomial setting. The advent of human immunodeficiency virus infection and the widespread use of the newer triazole fluconazole to suppress fungal infections in these patients have contributed to a significant increase in C. krusei infection, particularly because of the high incidence of resistance of the yeast to this drug. Experimental studies have generally shown C. krusei to be less virulent than C. albicans in terms of its adherence to both epithelial and prosthetic surfaces, proteolytic potential and production of phospholipases. Furthermore, it would seem that C. krusei is significantly different from other medically important Candida spp. in its structural and metabolic features, and exhibits different behaviour patterns towards host defences, adding credence to the belief that it should be re-assigned taxonomically. An increased awareness of the pathogenic potential of this yeast coupled with the newer molecular biological approaches to its study may facilitate the continued exploration of the epidemiology and pathogenesis of C. krusei infections.

Introduction

There are nearly 150 asporogenous yeast species presently classified in the genus Candida. Of these, C. albicans, C. tropicalis and C. glabrata comprise > 80% of clinical Candida isolates while others such as C. krusei, C. parapsilosis, C. guilliermondii and C. kefyr are isolated sporadically and are thought to be less virulent. Nonetheless, their importance as medically important fungi has been recognised from the early twentieth century and recent data indicate that > 30% of nosocomial candida infections are due to species other than C. albicans.

The yeasts belonging to the genus Candida were first discovered by Langenbeck in 1839 from buccal aphthae in a patient with typhus, but the suggestion that C. krusei may cause disease in man was proposed by Castellani in 1919 more than 75 years later. Since then, this organism has been generally recognised as a commensal in warm-blooded animals with very low pathogenicity and virulence. However, there has been a remarkable increase in the reports of C. krusei as a human pathogen during the last two to three decades. For instance, from 1960 onwards, > 65 articles have been published implicating C. krusei as an aetiological agent in human disease. Although this may be due partly to increased awareness of the organism and improvements in laboratory identification methods, there is little doubt that a true increase in the numbers of C. krusei infections has occurred during this period. This review emphasises the features that distinguish C. krusei from other members of the genus, its pathogenicity, epidemiology and clinical manifestations.

Biology

In contrast to a majority of other Candida spp. which are ovoid in shape, the cells of C. krusei are generally elongated and have the appearance of “long grain rice” (fig. 1), a feature which they share with C. kefyr (formerly C. pseudotropicalis) amongst clinically important Candida spp. C. krusei (Castellani Berkhout) measures 2.2–5.6 × 4.3–15.2 μm, with wide variations in the length and the breadth of the isolates (fig. 1). Variation in colony morphology of C. krusei has also been observed.
The ultrastructure of *C. krusei* has been described in one article. This study indicated a multilayered cell wall comprising six layers and a few intracytoplasmic organelles such as small vesicles, lipid droplets, ribosomes and groups of dense intra-cytoplasmic granules, probably glycogen. The multilayered cell wall consisted of an outer irregular coat of flocculent material, an electron-dense zone, a granular layer, a less granular layer, a thin layer of dense granules and another sparsely granular layer outside the trilaminar cell membrane. The outer layer of flocculent material appears in abundance in some isolates as extracellular extensions linking the individual cells, especially during growth of colonies on solid media (fig. 1c).

The α-D-mannan of the cell wall of *Candida* spp. is an important constituent of its structure as it acts as a major antigen. The mannan usually has a (1-6)-linked main backbone with side chains containing (1-2) or (1-3) linkages, or both. Although Nishikawa et al. are of the opinion that *C. krusei* cell-wall mannans are...
comparable with those of other Candida spp., Kogan et al. have demonstrated recently that C. krusei mannan is different from that of other Candida spp. in being lightly branched and containing (1-2) and (1-6) side chains in the ratio of 3:1. They have proposed a modified structure for α-D-mannan of C. krusei where the main chain is lightly branched and consists of 2- and 6-linked units in the ratio 3:1. The low cross-reactivity of C. krusei with antisera raised against other pathogenic Candida spp. and its weak immunogenicity is more compatible with a short side chain structure than with the highly branched 6-linked structure with longer side chains. Such differences in the cell wall may account for the variable behaviour of C. krusei in biological fluids such as saliva and bronchial lavage fluid (see section on Pathogenicity) when compared with other Candida spp. Indeed, some argue that C. krusei should be re-classified into a different genus on the basis of its ultrastructure, cell wall composition and co-enzyme Q numbers. Recent molecular biological studies also tend to add credence to this contention, as the average chromosomal number of C. krusei appears to be eight, compared with 16 for C. albicans. Interestingly, Barns et al., who evaluated the evolutionary relationship among Candida spp., found C. krusei to be the most distantly related to C. albicans of the medically important Candida spp. Nonetheless, as only the medically important species were investigated in this taxonomic study, virtually excluding more than 100 other Candida spp. which comprise the genus, further research into the true taxonomic position of C. krusei appears to be warranted.

C. krusei is usually found in two basic morphological forms, as yeast and pseudohyphae. Both are frequently present simultaneously in growing cultures and may not be separated easily. Despite the fact that Candida is a genus of asexual yeasts, C. krusei is very closely related to a sexual species, and a sexual form—termed Issatchenkia orientalis—has been proposed for the organism.

**Growth and metabolism**

C. krusei grows at a maximum temperature of 43–45°C. Although most of the medically important Candida spp. require biotin for growth and some have additional vitamin requirements, only C. krusei can grow in vitamin-free media. C. krusei assimilates and ferments only glucose out of a large panel of carbohydrates. The only other yeast sometimes isolated from medical specimens which reacts similarly is C. pintolopesii. However, of the medically important Candida spp., C. krusei is perhaps the only species which grows on Sabouraud’s dextrose agar as spreading colonies with a matt or a rough whitish yellow surface, in contrast to the convex colonies of other Candida spp. This characteristic, together with its “long grain rice” appearance on microscopy, helps the definitive identification of the species.

A complex variety of fatty acids has been demonstrated as metabolites when C. krusei is grown in culture media containing lactose, it is also able to produce acetoin. It also produces a number of short-chain carboxylic acids when cultured in saliva supplemented with glucose; these include acetate, pyruvate, succinate, propionate, formate and lactate. The biological role of these, if any, is as yet unknown.

**Epidemiology**

Compared to the medically important Candida spp., C. krusei has been isolated from a large variety of
natural habitats such as the atmosphere, fruits, sewage, silage, soil, foods (including dairy and meat products, pickles, sugar and syrup-based products), wines and beer. Hence it is widely distributed in nature and considered to be a facultative saprophyte. It is also found in chickens and seagulls.

Generally, *C. krusei* is considered to be a transient commensal in man and has been isolated only infrequently from the mucosal surfaces of various patient groups and as a mucosal inhabitant in healthy individuals. In his comprehensive review of the literature on oral carriage of *Candida* spp., Odds concluded that *C. krusei* is the fifth most dominant species, with *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. parapsilosis* preceding it. In one large study, the most common yeast combinations isolated from oral samples comprised *C. albicans* with one or more of the following: *C. krusei*, *C. tropicalis* or *C. glabrata*. Tables I–III summarise the frequency of isolation of *C. krusei* from the oral cavity, gastrointestinal tract and the vaginal-anorectal area, respectively.

### Pathogenicity

#### Virulence factors

*Candida* spp. have several virulence attributes. Some of these include adherence to host surfaces, production

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### Table II. The frequencies of isolation of *C. krusei* and *C. albicans* from the gastrointestinal tract of various patient populations

<table>
<thead>
<tr>
<th>Date</th>
<th>Reference</th>
<th>Patient group</th>
<th>Sampling technique</th>
<th><em>C. krusei</em> (%)</th>
<th><em>C. albicans</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1968</td>
<td>Schonebeck</td>
<td>With or without gastric diseases</td>
<td>Aspirated by catheter</td>
<td>0.9</td>
<td>52.6</td>
</tr>
<tr>
<td>1968</td>
<td>Bernhardt</td>
<td>Not stated</td>
<td>Not stated</td>
<td>3.7</td>
<td>53.1</td>
</tr>
<tr>
<td>1969</td>
<td>Cohen et al</td>
<td>Normal adults</td>
<td>Aspirates from small intestine</td>
<td>2.9</td>
<td>71.4</td>
</tr>
<tr>
<td>1974</td>
<td>Brooks et al</td>
<td>Pre-vagotomy and post-vagotomy</td>
<td>Aspiration</td>
<td>10.3</td>
<td>79.5</td>
</tr>
<tr>
<td>1980</td>
<td>Gordon et al</td>
<td>Chronic lymphocytic leukaemic patient with intra-abdominal abscess</td>
<td>Pus samples</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>1985</td>
<td>Di Febo</td>
<td>Gastric ulcer</td>
<td>Biopsies of lesion border</td>
<td>26</td>
<td>47.5</td>
</tr>
</tbody>
</table>

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### Table III. The frequencies of isolation of *C. krusei* and *C. albicans* from various patient populations with and without symptoms of vaginitis

<table>
<thead>
<tr>
<th>Date</th>
<th>Reference</th>
<th>Patient group</th>
<th>Sampling technique</th>
<th><em>C. krusei</em> (%)</th>
<th><em>C. albicans</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1962</td>
<td>Stenderup and Pederson</td>
<td>Hospital patients</td>
<td>Not stated</td>
<td>5.6</td>
<td>55.6</td>
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<tr>
<td>1963</td>
<td>Sonck and Somersalo</td>
<td>Diabetic girls</td>
<td>Swabs</td>
<td>9.7</td>
<td>55.4</td>
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<tr>
<td>1963</td>
<td>Kearns and Gray</td>
<td>Gynaecology</td>
<td>Not stated</td>
<td>3.2</td>
<td>77.7</td>
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<td>1964</td>
<td>Hurley and Morris</td>
<td>Obstetrics</td>
<td>Not stated</td>
<td>54</td>
<td>54.9</td>
</tr>
<tr>
<td>1966</td>
<td>Timonen et al</td>
<td>Gynaecology</td>
<td>Smear</td>
<td>4.9</td>
<td>37.6</td>
</tr>
<tr>
<td>1967</td>
<td>Mahgoub</td>
<td>Pregnant women</td>
<td>Swabs</td>
<td>4.7</td>
<td>60.9</td>
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<td>1979</td>
<td>Knippenberger et al</td>
<td>Gynaecology</td>
<td>Not stated</td>
<td>48</td>
<td>76.9</td>
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<tr>
<td>1981</td>
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<td>Smear</td>
<td>11.7</td>
<td>83.3</td>
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<td>1983</td>
<td>Schonheyder et al</td>
<td>Pregnant women</td>
<td>Swabs</td>
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<td>64.0</td>
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<td>1984</td>
<td>Mendling and Schnell</td>
<td>Pregnant women</td>
<td>Not stated</td>
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<td>73.6</td>
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<td>1986</td>
<td>Mondello et al</td>
<td>Asymptomatic</td>
<td>Swabs</td>
<td>26</td>
<td>34.8</td>
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<tr>
<td>1986</td>
<td>Guaschino et al</td>
<td>Pregnant women</td>
<td>Swabs</td>
<td>5.0</td>
<td>66.3</td>
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</table>

### Asymptomatic women

<table>
<thead>
<tr>
<th>Date</th>
<th>Reference</th>
<th>Patient group</th>
<th>Sampling technique</th>
<th><em>C. krusei</em> (%)</th>
<th><em>C. albicans</em> (%)</th>
</tr>
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<tbody>
<tr>
<td>1972</td>
<td>Thierry et al</td>
<td>Vaginal candidosis</td>
<td>Swabs</td>
<td>12.5</td>
<td>77.2</td>
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<td>1972</td>
<td>Proost et al</td>
<td>Vaginal candidosis</td>
<td>Swabs</td>
<td>0.7</td>
<td>93.4</td>
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<td>1972</td>
<td>Peeters et al</td>
<td>Vaginal candidosis</td>
<td>Swabs</td>
<td>1.9</td>
<td>81.5</td>
</tr>
<tr>
<td>1973</td>
<td>Hurley et al</td>
<td>Pregnant women</td>
<td>Charcoal swabs</td>
<td>0.4</td>
<td>90.4</td>
</tr>
<tr>
<td>1975</td>
<td>Sparks et al</td>
<td>Obstetrics</td>
<td>Swabs</td>
<td>4.0</td>
<td>86.0</td>
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<tr>
<td>1985</td>
<td>Horowitz et al</td>
<td>Gynaecology</td>
<td>Vaginal specimens</td>
<td>0.6</td>
<td>67.9</td>
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<tr>
<td>1986</td>
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<td>Not stated</td>
<td>2.2</td>
<td>92.6</td>
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<td>Mondello et al</td>
<td>Gynaecology</td>
<td>Swabs</td>
<td>16.1</td>
<td>29.0</td>
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<td>1987</td>
<td>Sanz and Del Palacio</td>
<td>Gynaecology</td>
<td>Not stated</td>
<td>1.7</td>
<td>91.7</td>
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<tr>
<td>1989</td>
<td>Poria et al</td>
<td>Gynaecology</td>
<td>Not stated</td>
<td>11.4</td>
<td>56.8</td>
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<td>1989</td>
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<td>Not stated</td>
<td>54.0</td>
<td>9.2</td>
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of phospholipases and proteinases and formation of hyphae, which help evasion of the host immune defences. Unlike the more pathogenic *C. albicans*, relatively few studies have been conducted to determine the virulence potential of *C. krusei* in man and laboratory animals. An early investigation by Hurley and Stanley compared the cytopathic effect of *C. krusei* and other *Candida* spp. in cell cultures. *C. krusei*, *C. kefyr* and *C. parapsilosis* destroyed cultured mouse renal epithelial cells in 48–72 h, whereas the relatively more pathogenic *C. albicans*, *C. tropicalis* and *C. stellatoidea* (now re-assigned as *C. albicans*) induced degenerative changes rapidly, and totally destroyed the cultures in a much shorter period of 24 h.

Howlett determined the pathogenicity of *C. krusei* with an in-vitro organ culture system comprising the dorsal tongue mucosa of neonatal Sprague Dawley rats. In the tissues infected with *C. krusei*, the fungus grew in both the yeast and mycelial phases, but generally showed less invasiveness than *C. albicans* or *C. tropicalis*. *C. albicans* produced extensive epithelial invasion, penetrating all the layers of the epithelium but *C. krusei* did not penetrate the stratum corneum, although a heavy growth of the yeast was observed on the superficial epithelium. However, a scanty growth of *C. krusei* into the connective tissues was observed at the explant edges. Similar results were also obtained for two other *Candida* spp.—*C. parapsilosis* and *C. guilliermondii*.

Although adhesion to host surfaces is an essential prerequisite for colonisation and subsequent invasion, very few workers have investigated the adhesion of *C. krusei* to host epithelial cells. In one early study by King et al., where the in-vitro adhesion of *C. krusei* and other *Candida* spp. to buccal epithelial cells (BEC) was compared, it was observed that the adhesion of *C. krusei* was far less than that of *C. albicans*. Indeed, *C. krusei* failed to adhere to BEC whereas the other species tested—*C. tropicalis*, *C. parapsilosis*, *C. guilliermondii* and *C. kefyr*—adhered but demonstrated lower values than *C. albicans*. In another investigation, Ray et al. investigated the adhesion of five *Candida* spp.; *C. albicans* and *C. stellatoidea* exhibited marked adhesion to BEC whereas *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii* and *C. krusei* showed little or no adhesion. However, all these studies were conducted with only one or two isolates of *C. krusei* and hence it is not possible to conclude whether intra-species differences exist amongst different *Candida* spp.

Two recent investigations have employed a larger number of *C. krusei* isolates to evaluate the adhesion of the fungus to several surfaces. One of these, by Tobgi, revealed that seven *C. krusei* isolates had the lowest affinity to BEC when compared with a number of other *Candida* spp. that adhered to oral epithelial cells in the following hierarchical order: *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. guilliermondii* and *C. krusei*. In a second study with five isolates each of *C. krusei* and *C. albicans*, all *C. krusei* isolates were c. 15 times less adhesive than the *C. albicans* isolates. In a related clinical study where the adhesion of *C. krusei* and *C. albicans* isolates to BEC from healthy individuals and bone marrow transplant patients were compared, the adhesion of *C. albicans* to the latter cells was three-fold lower than to the control cells, although adhesion of *C. krusei* remained the same. This may possibly reflect a selective colonisation process which may operate in these patients, possibly due to drug therapy (see section on *C. krusei* and antifungal agents), and may explain to some extent the frequent isolation of *C. krusei* from the mouth in compromised patient groups.

A few workers have examined the adherence of *C. krusei* to non-biological surfaces. Tobgi, for instance, found that the adhesion of *C. krusei* to denture acrylic strips was the lowest in comparison with several other *Candida* spp.—including *C. albicans*. Other studies examining the relative adherence of different *Candida* spp. to acrylic have confirmed these findings. However, a recent study, with 20 isolates of *C. krusei*, demonstrated that the adhesion of the latter to acrylic is significantly greater than that of *C. albicans*. Although the variations in these results from different centres could be attributable to differences in the isolates, techniques and culture media, it is fair to conclude that *C. krusei* is more adherent to inert surfaces than to buccal epithelial cells.

Cell-surface hydrophobicity is considered to play a critical role in the initial events leading to colonisation of host surfaces by *Candida* spp. and this property, together with adherence, may have clinical implications in fungal infections related to plastic devices such as implants and catheters. Klotz et al. have ranked the hydrophobicity of different *Candida* spp. by measurement of adherence to an aqueous-hydrocarbon two-phase system. The two *C. krusei* strains they studied were more hydrophobic than *C. albicans*, *C. tropicalis*, *C. kefyr*, *C. parapsilosis*, *C. glabrata* or *C. lipolytica* isolates. Others have also found *C. krusei* to be more highly hydrophobic than the key, medically important *Candida* spp. Recently, we examined the cell surface hydrophobicity of 20 oral isolates of *C. krusei* with the aforementioned biphasic assay system and found the hydrophobicity of the latter to be five-fold greater than that of a panel of *C. albicans* (Y. H. Samaranayake, unpublished data). Minagi et al. also observed the cell surface hydrophobicity of *C. krusei* to be similar to that of *C. glabrata* and *C. tropicalis* but greater than that of either *C. albicans* or *C. parapsilosis*. A significant positive correlation has been reported between the adherence of *Candida* spp. to acrylic surfaces and their cell surface hydrophobicity. However, when saliva-coated acrylic plates were used, the cell surface hydrophobicity as well as the adherence of *C. krusei* to acrylic was significantly decreased. Therefore, it would seem that, of the medically important *Candida* spp., *C. krusei* is endowed with a greater ability to colonise...
inert surfaces such as implants and catheters by virtue of its cell surface hydrophobicity.

Once attached to an inert substrate, and if left undisturbed and provided with adequate nutrition, organisms multiply and colonise surfaces with resultant formation of thin pellicles or biofilms. This phenomenon of biofilm formation by microbes on implant materials (such as urinary catheters, prosthetic heart valves, cardiac pacemakers, silicone voice prostheses, endotracheal tubes and cerebrospinal fluid shunts) has received considerable attention in the last decade as there appears to be a direct relationship between the ability of an organism to form a biofilm and its pathogenicity. In a recent study, Hawser and Douglas demonstrated, with an in-vitro model, that the biofilm formation on the surface of different catheter materials varied among Candida spp., and also correlated to some extent with pathogenicity. Thus, less pathogenic species such as C. parapsilosis, C. pseudotropicalis (now C. kefyr) and C. glabrata produced significantly less biofilm than the more pathogenic C. albicans. Remarkably though, of the Candida spp. examined, C. krusei produced the most extensive biofilm on the surfaces of polyvinyl chloride catheter disks irrespective of the growth medium, which either suppressed or promoted extracellular polysaccharide formation. One reason for this may be the dual attributes of very high cell surface hydrophobicity and adherence of C. krusei to inert plastic surfaces compared with other species (see above) which may have facilitated biofilm development.

The ability to produce hydrolytic enzymes such as phospholipases and proteinases is considered a putative virulence factor of Candida spp. However, C. krusei, in contrast to species such as C. albicans, does not possess the ability to produce either of these enzymes.

From the foregoing review of the virulence attributes of C. krusei, it is evident that the organism is relatively less virulent than the commonly pathogenic Candida spp. such as C. albicans. The following section will further elucidate the relative vulnerability of C. krusei to host defence mechanisms.

**Host defence factors**

There are few studies on the effect of host defence factors on C. krusei. These mainly relate to the effect of constituents of biological fluids such as lysozyme and lactoferrin. Both are non-immune defence factors.
CANDIDA KRUSEI: AN EMERGING PATHOGEN

Fig. 3. The fungicidal activity of lysozyme (2 µg/ml) for different candidal isolates. The higher the Fly value the more sensitive the isolate is to lysozyme. Note the very high susceptibility of C. krusei to lysozyme when compared with the other species. (From reference 96, with permission.)

present in human external secretions (e.g., saliva, milk, tears), mucosal surfaces and secondary granules of polymorphonuclear leucocytes. Lactoferrin is highly bactericidal in nature and this activity is a direct result of iron sequestration and deprivation of this element that is essential for bacterial growth. Lactoferrin may also interact directly with bacteria, altering their permeability. Valenti et al. were the first to examine the effect of iron-free lactoferrin on a single isolate of C. krusei and they reported no inhibition of growth or adsorption of lactoferrin on to the fungal cell surface. However, a recent study indicated varying degrees of susceptibility to lactoferrin amongst C. albicans and C. krusei isolates. The authors demonstrated that C. krusei was almost 50% more sensitive to iron-free lactoferrin than C. albicans and, furthermore, they noted cell surface changes such as bleb-like structures and efflux of cellular protein into the test medium due to lactoferrin activity, implying that cell death may be a direct consequence of permeability changes (fig. 2).

The inhibitory effects of lysozyme on C. krusei and several other Candida spp. were first demonstrated by Kamaya. Similar studies have been conducted by Tobgi et al., comparing the antifungal activity of lysozyme against a battery of Candida spp. They showed that the Candida spp. were susceptible to lysozyme in the following order: C. krusei > C. parapsilosis > C. tropicalis > C. guilliermondii > C. albicans > C. glabrata, the latter being the most resistant to lysozyme (fig. 3). Furthermore, C. krusei pre-incubated in sucrose-supplemented media becomes highly sensitive to the killing effect of lysozyme in comparison to C. albicans. As it is known that C. albicans produces an extracellular floccular layer during growth in sucrose-supplemented media, the increased sensitivity of C. krusei to lysozyme under these conditions appears to be due to the absence of such extracellular material. These data tend to confirm the observations of Kogan et al. that the cell wall structure of C. krusei differs significantly from that of C. albicans (see above) while indicating that biological fluids such as saliva may exert a selective colonisation pressure on different Candida spp., thereby suppressing the growth of more susceptible species such as C. krusei.

Samaranayake et al. examined the fungicidal effect of murine bronchial lavage fluid to determine whether respiratory secretions could protect the surfaces of the lower respiratory tract. Of the five different Candida spp. examined, C. albicans was the most sensitive, whereas C. krusei and C. glabrata were highly resistant to the activity of bronchial lavage fluid.

After exposure to a primary barrage of anti-candidal...
defence factors in the biological fluids which bathe the superficial mucosae, the subsequent disposal of the yeast will be the remit of the immuno-effector cells such as polymorphonuclear leucocytes (PMNLs). Although several investigators have reported the importance of phagocytosis in host defence against invasive, deep seated candidosis in general (for a review, see Odds') the protective role of phagocytes in C. krusei infections has been evaluated only sparsely.

Vecchiarelli et al.\textsuperscript{109} demonstrated the in-vitro killing of C. krusei and several other Candida spp. by murine phagocytic cells by means of a radiolabel release micro-assay and measurement of colony forming units. They observed that C. krusei, C. guilliermondii and C. parapsilosis were killed by polymorphonuclear and bone marrow cells more rapidly (1 h) than C. albicans, C. tropicalis and C. viswanathi (4 h). Moreover, the effector to target cell ratio was significantly higher for the latter species. Another interesting observation was that C. krusei, C. guilliermondii and C. parapsilosis were more vulnerable to peritoneal resident macrophages and spleen cells, in particular, whereas C. albicans and C. tropicalis were not affected even in mice immunodepressed with cyclophosphamide. A few other workers have shown a similar hierarchy of resistance to intracellular killing of different Candida spp. by macrophages.\textsuperscript{110} In a cytotoxic colorimetric assay, Borg et al.\textsuperscript{111} demonstrated that C. krusei, C. guilliermondii, C. parapsilosis and C. glabrata were associated with a lower degree of cytotoxicity than C. albicans and C. tropicalis. In another report, in an in-vitro phagocytosis assay with rat peritoneal macrophages, phagocytic indices obtained for C. krusei and C. viswanathi were significantly lower than those for C. albicans and C. tropicalis.\textsuperscript{112}

In animal studies, Bistoni et al.\textsuperscript{113} tested the relative pathogenicity of C. albicans, C. krusei, C. parapsilosis, C. tropicalis and C. viswanathi in normal and cyclophosphamide-immunodepressed mice. In normal mice, only C. albicans, C. tropicalis and C. viswanathi were pathogenic on intravenous challenge, with increased virulence after cyclophosphamide treatment; the mice were consistently resistant to challenge with C. krusei, C. guilliermondii and C. parapsilosis, despite increasing doses of cyclophosphamide. Although the foregoing data are limited, a consensus view that C. krusei is highly vulnerable to immuno-effector cells, when compared with C. albicans, appears to be emerging.

Despite the limited information available on the pathogenic attributes of C. krusei and its host interactions, it can be concluded that in both these aspects, C. krusei appears to be a feeble pathogen when compared with other Candida spp., especially C. albicans. Hence, the predominant motive force in its conversion from commensalism to parasitism is likely to be related to the host within which it may lie dormant and cause disease in the event of complete or partial failure of host antimicrobial defences. The next section is an account of clinical manifestations that C. krusei may cause in hosts with such compromised defences.

**Clinical manifestations**

**Systemic infections**

In parallel with the increase in superficial candida infections, there has been a surge in the incidence of systemic candida infections in recent years. This has been related to several predisposing factors, including the use of immunosuppressive drugs, prolonged broad-spectrum antibiotic therapy, indwelling intravascular catheters, extensive periods of treatment in intensive care units and the pandemic of human immunodeficiency virus (HIV) infection.\textsuperscript{104-106} Although C. albicans and C. tropicalis infections have predominated in these patient populations, the emergence of the less virulent C. krusei as a systemic pathogen has been described in a number of patients with compromised host resistance.\textsuperscript{105-109}

In one of the earliest studies in which C. krusei was implicated as a systemic pathogen, Young et al.\textsuperscript{110} described 70 patients with fungal infections from the National Cancer Institute (Bethesda, MD, USA) during the decade beginning 1962. Of these, 28 patients had C. albicans- and one had C. krusei-associated disseminated fungal disease; the rest of the infections were other mycoses. C. krusei was observed histologically in one or more viscer al organs that were not considered to be the original portal of entry of the fungus. This is perhaps the earliest documentation of C. krusei fungaemia and systemic infection.

A prospective investigation of surgical and autopsy specimens submitted to the Mycology Laboratory of the Veterans Administration Centre in Wisconsin, USA, from 1963 to 1973 was undertaken by Rose and Varkey\textsuperscript{111} to study patients with possible deep fungal infections. Of 123 such patients, 55 (44.7\%) infections were caused by Candida spp. and 28 (22.8\%) by Aspergillus spp. There were 44 patients during the first 5 years and 79 during the second period—an increased number which was related primarily to the marked increase in deep-seated candida infections. The major aetiological agent of the 55 patients with candida infection was C. albicans. Of the 46 patients with positive blood cultures, C. albicans was isolated from 33 patients, C. parapsilosis from four, C. krusei from two, C. tropicalis from two and C. guilliermondii from one patient. The main underlying diseases among the 55 patients were general surgery, aspiration pneumonia and cerebral sclerosis, although they did not describe the condition of the patient with C. krusei infection.\textsuperscript{111}

Disseminated candidosis was seen in 39 patients who attended the Presbyterian-University Hospital, USA between 1963 and 1975.\textsuperscript{112} These investigators observed a six-fold increase in the incidence of disseminated candidosis during the last 4 years compared
with the first 4 years of the study period. This high incidence of disseminated candidosis occurred mainly in patients with acute leukaemia. Of the 11 cases where speciation of Candida isolates was considered reliable, C. krusei accounted for one case, C. stellatoidea for eight, and C. albicans and C. tropicalis were identified in one patient each.

Sanford et al.\textsuperscript{113} surveyed the presence of different species of yeasts in the urine, stool and respiratory specimens of 89 immunocompromised patients admitted to the Johns Hopkins Oncology Centre, Baltimore, MD, USA during the period 1977–1978. Of these, 37 were recipients of bone marrow transplants, 47 were patients with acute leukaemia and five had other haematological malignancies. C. krusei was isolated from one urine, 13 stool and 17 respiratory cultures. It was also observed that 67% of the patients were colonised by C. albicans and only 28% by C. tropicalis, C. krusei and C. glabrata. Of the six patients which were colonised with C. krusei, three produced positive surveillance cultures for 2 weeks or longer.

Episodes of fungaemia due to various Candida spp. have been documented by Meunier-Carpenter et al.\textsuperscript{114} at the Memorial Sloan Kettering Cancer Centre, USA, from 1974 to 1977. In total, there were three patients with C. krusei fungaemia in comparison to 53 with C. albicans, 29 C. tropicalis, 26 C. glabrata, 16 C. parapsilosis and one with C. kefyr (formerly C. pseudotropicalis) fungaemia. The underlying diseases for the C. krusei fungaemias were leukaemia (one patient) and solid tumour (two patients). They surmised that the most important predisposing factors in these patients were intravenous catheters, chemotherapy, neutropaenia and antibiotic administration; diabetes mellitus, adrenocorticosteroid treatment and radiation therapy accounted to a lesser degree. In comparison to patients with infections due to other Candida spp. who had multiple positive sites for the respective organisms, all three C. krusei fungaemia patients had only one positive site for the yeast other than the blood cultures and in all cases this was their respiratory tract, as the yeasts were isolated from sputum. Although all three patients with C. krusei fungaemia died, it was difficult to attribute death to the fungaemia. These early studies tended to suggest that C. krusei is not merely an innocuous commensal but has the potential to cause serious infection in patients with underlying immunosuppressive disease.

Since the realisation of the importance of C. krusei fungaemia in compromised patients, other studies have followed. Horn et al.\textsuperscript{108} reported 200 episodes of fungaemia in 188 patients in the Memorial Sloan Kettering Cancer Institute, USA, between 1978 and 1982, of which seven were due to C. krusei in comparison with 89 patients with C. albicans fungaemia. Whereas most episodes of C. krusei fungaemia occurred in patients with leukaemia, lymphoma or aplastic anaemia, most episodes of C. parapsilosis and C. glabrata fungaemia occurred in patients with solid tumour or non-neoplastic diseases; C. albicans fungaemias were evenly distributed between patients with haematological and non-haematological malignancies. It was also noted that other factors such as neutropaenia, chemotherapy, broad-spectrum parenteral antibiotics and oral nystatin therapy tended to precede C. krusei or C. tropicalis fungaemia.

Arguably, the landmark study that confirmed the importance of C. krusei fungaemia in compromised patients was that of Merz et al.\textsuperscript{109} They evaluated a total of 868 patients admitted to the Oncology Centre of the Johns Hopkins Medical Institution, Baltimore MD, USA (1977–1985) and receiving time sequential chemotherapy for haematological malignancies or bone marrow transplantation, to determine the role of C. krusei in systemic candidosis. The patients received chemotherapy that induced a period of profound granulocytopenia (\(<\) 100/mm\(^3\)) of at least 2 weeks, a condition conducive to candida infection. They were variably given platelet, blood and leucocyte transfusions and antibiotics, and urine, stool, rectal swabs and throat swabs were examined for fungal growth. The two criteria used to delineate C. krusei systemic infection in this population were isolation of the yeast from two or more blood cultures collected within a 72-h period during granulocytopenia, and isolation of the organism and evidence of candidosis from histopathological findings from tissues taken at biopsy or autopsy.

C. krusei was isolated from a total of 108 (12.4\%) patients during this 9-year study. However, only 46 of them had persistent colonisation and the number of patients colonised each year with C. krusei ranged from eight to 21. The gastrointestinal tract was most frequently colonised (73\%) followed by the upper respiratory tract (39\%) and the urinary tract (6\%). Seven of the patients died within 1 month of C. krusei sepsis; systemic candidosis was seen in four patients on whom autopsies were performed.

Finally, there is a single case report of disseminated C. krusei fungaemia in a 23-year-old male patient who had acute lymphosarcoma.\textsuperscript{112} The patient was diagnosed as having disseminated candidosis and treated with amphotericin B. A few days later C. krusei was grown from blood and faecal cultures. After recovering completely the patient died 2 months later; post-mortem examination did not reveal any evidence of disseminated candidosis.

A series of reports on C. krusei fungaemia in patients who are on the newer triazole antifungal agent, fluconazole, has appeared. This has wide therapeutic and clinical implications, as discussed in the penultimate section of this review.

\textbf{Ocular infections}

The most important ocular manifestation of C. krusei infection to date has been endophthalmitis. Previous citations on candidal endophthalmitis have largely been related to infections with C. albicans\textsuperscript{116,117}
although other species such as *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. guilliermondii* have been reported sporadically to be associated with haematogenous endophthalmitis.\textsuperscript{118}

There are three case reports of *C. krusei* endophthalmitis. The first describes *C. krusei* fungaemia together with endophthalmitis in a 70-year-old man who was admitted for elective abdominopereineal resection for a recurrent rectal adenocarcinoma.\textsuperscript{118} After surgery, total parenteral nutrition through a peripheral intravenous catheter was administered. The patient developed high fever 15 days after surgery and blood cultures yielded *C. krusei*. Endophthalmitis in the left eye due to *C. krusei* was diagnosed and the patient recovered after receiving intravenous amphotericin B.

In the second case, a 69-year-old man with acute myeloid leukaemia was found to have endophthalmitis due to *C. krusei*.\textsuperscript{118} The patient initially experienced recurrent fever despite negative culture, although he was persistently neutropenic. At this stage, piperacillin, gentamicin and induction chemotherapy was administered. Blood cultures on the 20th day of hospitalisation yielded *C. krusei*, although there was no evidence of retinitis. Cultures of the central venous line tip yielded a moderate growth of *C. krusei*. On day 32, fundoscopic examination revealed two white infiltrates near the macula of the right retina which was consistent with candidal retinitis. However, the patient recovered and by day 48 was no longer neutropenic. When the patient died 3 months later, there was no evidence of candidal infection.

A third case report of endophthalmitis due to *C. krusei* was in a man with acute myeloid leukaemia who was on induction chemotherapy.\textsuperscript{118} The patient became neutropenic, developed fever on day 20 and 2 days later several cutaneous lesions were observed. Two blood cultures obtained from a peripheral vein on day 20 were positive for *C. krusei*. In spite of treatment with liposomal amphotericin B, the fever persisted and on day 32 the patient developed candidal endophthalmitis. He subsequently recovered from endophthalmitis, but several months later experienced a relapse of leukaemia and died. Autopsy results revealed no sign of recurrent fungal infection.

A corneal ulcer due to *C. krusei* has also been described in another report.\textsuperscript{119} A 45-year-old female farmer complained of pain, redness, swelling and lacrimation in the left eye. Prior to the isolation of the aetiological agent the patient was given penicillin, oxytetracycline eye ointment and atropine drops and chloramphenicol. The condition deteriorated with swelling and diminution of vision and 10 days later fungal cultures were positive for *C. krusei*. The patient recovered her vision after topical and intravenous amphotericin B therapy. Interestingly, in animal experiments, the *C. krusei* isolate produced endophthalmitis in three compromised albino rabbits. Infection developed in all eyes that were tested within 24-72 h after inoculation.

**Miscellaneous infections**

Arthritis due to *Candida* spp. is uncommon and the involvement of *C. krusei* as a causative agent is extremely rare. There has been a report of arthritis caused by *C. krusei* in a heroin addict who had leukaemia and was neutropenic.\textsuperscript{119} The 41-year-old patient was undergoing cytotoxic drug therapy for relapse of acute myelogenous leukaemia. Following chemotherapy, he became neutropenic and was in an isolation chamber without prophylactic antibiotic or antifungal therapy. On the 10th day of hospitalisation, *C. krusei* was grown from five sputum cultures and a urine culture. However, blood cultures were negative for fungi. Fever persisted and on the 21st day swelling and tenderness of the right knee occurred and synovial fluid obtained by arthrocentesis revealed *C. krusei*. On the 27th day in hospital, after *C. krusei* was grown from the second aspirate, intravenous amphotericin B was started. On the 29th hospital day the patient was discharged to receive amphotericin B intravenously three times weekly as an outpatient. After cessation of the antifungal therapy, swelling and tenderness of the right knee recurred. Amphotericin therapy was re-instituted and after 3 months there was no clinical evidence of arthritis. Arthritis in this case was possibly a consequence of haematogenous spread, as the patient was colonised in the respiratory tract and the urinary tract with *C. krusei*. It is noteworthy that arthritis caused by *C. albicans* is more common as haematogenous joint invasion complicating disseminated candidosis, although it is more often polyarticular than mono-articular.\textsuperscript{121}

Candidal endocarditis is a frequent occurrence among intravenous drug abusers. In one survey Odds\textsuperscript{8} estimated that one-fifth of candidal endocarditis occurs in the latter group. *C. albicans* and *C. parapsilosis* accounted for the vast majority of reported cases,\textsuperscript{b} but 3-7\% of 163 cases in the latter survey were due to *C. krusei*. The detailed clinical features of these case reports are sparse. Rubinstein et al.\textsuperscript{112} described a case of *C. krusei* endocarditis in a 36-year-old male heroin addict who had fever and heart failure. Although the patient was treated with intravenous amphotericin B he subsequently died. Autopsy examination revealed myocardial abscesses, aortic vegetation and septic emboli due to *C. krusei*.

Renal candidosis is usually seen in patients with pre-existing renal pathology and diabetes is the most common single underlying condition. Candida infection is usually obstructive, with fungal material often referred to as "fungus balls" collecting in the renal pelvis. As in the case of other candida infections, *C. albicans* is the most common species implicated in renal candidosis and *C. krusei* infection is still a rarity. In a review of 74 cases of renal candida infection, 86\% were caused by *C. albicans* and 2.7\% by *C. krusei*.\textsuperscript{11} A case report by Thomalla et al.\textsuperscript{123} represents a rare involvement of *C. krusei* leading to the ureteral obstruction. An immunosuppressed 29-year-old
woman presented with renal dysfunction. On cystoscopic examination, an erythematous papillary lesion involving the ureteral orifice was observed; histological examination of a biopsy sample revealed chronic inflammation and oedema and \textit{C. krusei} was isolated on culture. The patient was given intravenous miconazole and bladder irrigation with amphotericin B. The infection proved to be very difficult to eradicate but resolved eventually after 11 weeks of combination therapy with ketoconazole, amphotericin B and fluconazole.

**Antifungal agents**

The most widely used antifungal agents are the polyenes and the azoles, which include the imidazoles and the newer triazoles. As it is beyond the scope of this review to dwell in detail on the antifungal susceptibility patterns of \textit{C. krusei} and their clinical implications, we provide for reference purposes, in table IV the relative MICs of various antifungal agents against \textit{C. krusei} and \textit{C. albicans}. However, because of the generally expressed concerns of the emergence of fluconazole-resistant \textit{C. krusei} isolates, some aspects related to this problem are discussed below.

Although fluconazole MICs for individual \textit{C. krusei} isolates have been reported as elevated by some authors, only one group has directly compared the MICs for \textit{C. krusei} with those of other \textit{Candida} spp., and they too report higher MICs for \textit{C. krusei} than for other species. This issue has been further compounded by the discordant correlation of in-vitro testing with outcome in vivo (for a recent review see Rex et al.). For the azole derivatives, especially, the outcome of the in-vitro susceptibility tests depends on the methods and media used and also on other variables such as the endpoint definition, inoculum size, inoculum preparation, incubation conditions and the nutritional requirement of the fungus. Another key problem in interpreting antifungal susceptibility test results is the partial inhibition of growth with azoles. According to Odds et al., the activity of fluconazole against \textit{Candida} spp. in vitro appears to be the hardest to determine meaningfully, being heavily dependent on the culture medium used. Hence, future workers need to review the available data by means of a standardised assay method such as the National Committee for Clinical Laboratory Standards (NCCLS) reference method for MIC determination.

Notwithstanding these reservations, there is an emerging consensus that \textit{C. krusei} isolates demonstrate a high level of resistance to fluconazole—widely used in the empirical treatment of patients with immunodeficiencies, especially HIV-infected individuals. The clinical implications of this problem are discussed in some detail below.
C. krusei and fluconazole therapy

Fluconazole is highly active against several pathogens that cause systemic mycoses. It is a triazole with a low mol. wt and unique pharmacokinetics—good water solubility, weak protein binding, long half-life and high level of cerebrospinal fluid penetration. It is well absorbed after oral administration and has been effective in treating both superficial and systemic candida infections. It has emerged as the drug of choice for prophylaxis of oropharyngeal and systemic candidosis in HIV-infected patients. Despite the initial claims of the efficacy of fluconazole in candida infections in general, studies in animals and man have now demonstrated both prophylactic and therapeutic failure of fluconazole against C. krusei. Furthermore, it is possible that the increase in colonisation and infection of human patients by C. krusei which has been documented in the last few years is partly attributable to the widespread use of this drug as a prophylactic antifungal agent.

Immediately after the approval of its use in early 1990, fluconazole was used as a prophylactic antifungal agent in recipients of heart and bone marrow transplants. In one study conducted by Goodman et al., patients receiving bone marrow transplants were randomly assigned to receive fluconazole (400 mg daily) or placebo. By the end of the treatment period 28 of 177 patients in the placebo group developed systemic fungal infections, two of which were due to C. krusei. In comparison, five of 179 patients who received fluconazole developed systemic fungal infections of which three were due to C. krusei. This study demonstrated that although fluconazole prevents infection with most pathogenic Candida spp. it does not eradicate C. krusei.

In another retrospective study of 463 bone marrow transplant and leukaemia patients, there was a seven-fold greater incidence of blood stream or visceral infection with C. krusei in 84 patients who received fluconazole prophylaxis than in 355 patients who received other modes of prophylaxis, including amphotericin B, miconazole and ketoconazole, or no prophylaxis. The foregoing observations suggest that the prophylactic use of fluconazole in immunocompromised patients, while decreasing the frequency of fungal infection caused by C. albicans and C. tropicalis may also promote the emergence of resistant pathogens such as C. krusei.

Several other reports have documented the development of resistant strains of Candida spp. after the use of fluconazole as a prophylactic agent or as primary therapy for superficial candidosis. A recent study by Casasnovas et al. also strongly supports these reports and suggests that fluconazole is not the ideal antifungal agent for preventing C. krusei infections. The author observed a high incidence (11%) of C. krusei septicaemia in patients with neutropenia who received fluconazole. Goodman et al. also concluded that fluconazole can be administered effectively to reduce the incidence of systemic mycoses in severely immunosuppressed patients, although they noted a tendency towards increased isolation of C. krusei during therapy and more episodes of candidaemia due to C. krusei in patients who received this drug.

Other case reports of failure of fluconazole therapy include the following. Roder et al. described consistent growth of C. krusei from at least six blood cultures of a patient during treatment with fluconazole, suggesting that this agent was ineffective against C. krusei. In another report reaffirming the failure of C. krusei infection to respond to therapy with fluconazole, Akova et al. quoted three immunosuppressed patients who developed oropharyngeal C. krusei infection with ulceration after fluconazole treatment. Furthermore, the development of C. krusei sepsis in an HIV-infected patient during fluconazole treatment was described by Stellbrink et al.

Taken together, these data strongly indicate that the administration of fluconazole, especially in low doses, as a prophylactic antifungal agent in immunocompromised patients may result in the emergence of resistant C. krusei strains. Hence, controlled clinical trials of prophylactic and therapeutic use of triazoles for disseminated candidosis appear to be warranted before their widespread recommendation as a primary therapeutic agent. Furthermore, it should now be mandatory to identify Candida isolates to species level whenever fluconazole is to be used for treatment of systemic mycoses.

Conclusions

The biology, epidemiology, pathogenicity and clinical manifestations of C. krusei have been reviewed. The available data indicate that C. krusei is significantly different from other medically important Candida spp. in its structural and metabolic features and that it exhibits different behaviour patterns towards host defences, thus adding credence to the belief that it should be taxonomically re-assigned. Experimental studies have generally shown that C. krusei is less virulent than C. albicans in terms of its adherence to epithelial and prosthetic surfaces, proteolytic potential and production of phospholipases.

Early reports described this organism as a sporadic isolate of minor clinical significance, but more recently it has emerged as an important nosocomial pathogen. The most common clinical manifestation of C. krusei is disseminated fungaemia in compromised patients, especially leukaemia patients. The advent of HIV infection and the widespread use of the newer triazole fluconazole to suppress fungal infections in these individuals have contributed to a significant increase in C. krusei infection, particularly because of the high incidence of resistance of the yeast to this drug. Other focal infections due to C. krusei include endophthalmitis, arthritis and endocarditis, which are usually
related to invasive procedures superimposed on a compromised host defence system. Thus the epithet "an emerging pathogen" could justifiably be given to this yeast, not least because of a putative increasing incidence of C. krusei infection, due partly to the HIV pandemic. An increased awareness of the pathogenic potential of this yeast coupled with the newer molecular biological approaches to its study should enhance our understanding of the epidemiology and pathogenicity of this important nosocomial pathogen.

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