

## Review

*Candida tropicalis*: its prevalence, pathogenicity and increasing resistance to fluconazoleRajendra J. Kothavade,<sup>1</sup> M. M. Kura,<sup>2</sup> Arvind G. Valand<sup>3</sup> and M. H. Panthaki<sup>4</sup>

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*Candida tropicalis* has been identified as the most prevalent pathogenic yeast species of the *Candida*-non-*albicans* group. Historically, *Candida albicans* has been the major species responsible for causing candidiasis in immunocompromised and immunocompetent patients. However, infections (candidiasis) due to *C. tropicalis* have increased dramatically on a global scale thus proclaiming this organism to be an emerging pathogenic yeast. The reasons for this organism's dominance and its resistance to fluconazole have been difficult to elucidate. In addition, the mechanism of this organism's pathogenicity and the consequent immune response remain to be clarified. This paper describes certain predisposing factors potentially responsible for these characteristics and presents a 'root cause analysis' to explain the increasing prevalence of *C. tropicalis* in developed and undeveloped countries, as well as the organism's acquired drug resistance. Control measures against fluconazole resistance in clinical management have also been discussed.

## Introduction

Fungi are widespread in the environment. Some are associated with animals and humans as commensals, but turn pathogenic or opportunistic after alteration of the host immune system (Krasner, 2002). Therapeutic applications of immunosuppressive drugs, the use of broad-spectrum antibiotics in various clinical conditions and other predisposing factors are responsible for an increasing number of immunocompromised patients and consequent opportunistic infections globally. A weakened or impaired immune system provides favourable conditions for pathogenic and non-pathogenic micro-organisms. AIDS due to human immunodeficiency virus (HIV-I and HIV-II) is one of the major contributing factors for the increasing number of patients with fungal infections (Nissapatorn *et al.*, 2003; Singh *et al.*, 2003; Vazquez & Sobel, 1995). The extensive use of antifungals for prophylaxis in these patients became the leading cause of colonization of *Candida*-non-*albicans* (CNA) species and increasing resistance to antifungal drugs (Hsueh *et al.*, 2005; Perfect, 2004). In India, *Candida tropicalis* is the most common cause of nosocomial candidaemia. Epidemiological data from the Indian subcontinent showed that 67–90% of nosocomial

candidaemia cases were due to CNA species of which *C. tropicalis* was the most dominant (Kothari & Sagar, 2009; Verma *et al.*, 2003). This review demonstrates the increasing importance of CNA species, particularly *C. tropicalis*, in terms of (1) pathogenic potential, (2) ability to cause systemic life-threatening infections – candidiasis/candidaemia and (3) acquired fluconazole resistance and consequent mortality rate.

## Clinical and laboratory diagnosis of candidiasis

The clinical features of candidiasis are dependent on the sites of infection. Oropharyngeal candidiasis, angular cheilitis, balanoposthitis, oral thrush and vulvovaginal candidiasis are features of mucous membrane (mucosal candidiasis) infection; interdigital candidiasis, paronychia and nappy rash are features of cutaneous candidiasis; and pulmonary candidiasis, disseminated candidiasis, gastrointestinal candidiasis and candidaemia are features of systemic candidiasis, involving internal body fluids and organs (Jacobs & Nall, 1990).

Microscopic examination is the basic test for diagnosis of a fungal infection, by using a 10% potassium hydroxide wet mount prepared from skin scrapings or crushed autopsy

specimens and a simple wet mount preparation for body fluids such as blood, cerebrospinal fluid (CSF) or urine. Haematoxylin and eosin, periodic acid–Schiff and Gomori–Grocott methenamine silver stains are the preferred stains used for differential diagnosis of fungal infection in tissue sections taken from biopsy or autopsy specimens. Processed clinical samples are cultured on Sabouraud dextrose agar, which allows growth of any fungi and their isolation. Biochemical tests such as the rapid API 20 microtube system are readily available for species identification of the isolated colonies (Buesching *et al.*, 1979). There is an automated continuous-monitoring blood culture system available for critically ill patients. The rapid molecular and more specific techniques such as PCR offer genotyping and allow for browsing of any drug resistance acquired by clinical isolates of *Candida* species (Krawczyk *et al.*, 2009; Antonopoulou *et al.*, 2009).

### ***C. tropicalis* and *C. albicans* infections in clinical settings**

Patients with chronic mucocutaneous candidiasis are occasionally associated with other disseminated or systemic fungal infections such as *C. neoformans*, but never with any other species of *Candida* (Stein & Sugar, 1989). However, disseminated *C. tropicalis* infections have been reported in immunocompromised patients who have had chronic mucocutaneous candidiasis (Dixon *et al.*, 2004).

Acute leukaemia (immunocompromised) patients with gastrointestinal infections are more prone to invasive candidiasis (Bodey, 1984; Myerowitz *et al.*, 1977). Some rare cases of cancer and other underlying conditions associated with candidiasis are summarized in Table 1. In addition to the oral mucosa, the gastrointestinal tract seems to be a favourable site for penetration of *Candida* species through gastric mucosal layers and subsequent disseminated candidiasis in immunocompromised patients (Stone *et al.*, 1974). Autopsy studies in such patients have demonstrated culture-proven disseminated candidiasis due to *C. albicans* or *C. tropicalis* infection with the involvement of the gastrointestinal tract. The classical morphological features of *Candida* yeasts (budding yeast and pseudohyphal forms) in submucosal layers are usually preceded by a band of progressive necrotic tissue. These necrotic bands at the advancing mycelial margin have been observed with *C. tropicalis* but not with *C. albicans* (Walsh & Merz, 1986). Details of such studies are summarized in Table 2. *C. tropicalis* has progressively been observed to be the commonest cause of invasive candidiasis in neutropenic patients such as those with acute leukaemia or those who have undergone bone marrow transplantation (Sandford *et al.*, 1980; Wingard *et al.*, 1979). However, the mechanism of the mutual role of the microbes and associated host factors in creating opportunistic conditions for *C. tropicalis* to cause gastrointestinal colonization or infection and any consequent dissemination in such patients (Fromtling *et al.*, 1987) remains obscure.

Purulent pericarditis often remains undiagnosed because such infections due to CNA species are a rare phenomenon in cardiac patients, particularly those with pre- or post-thoracic surgical conditions. However, one hospitalized patient receiving chemotherapy for Hodgkin's disease, who had a history of febrile episodes, failed to respond to the spectrum of antibiotics. The purulent pericardial infection in this patient remained unresolved but *C. tropicalis* was present in pre-mortem blood, urine and CSF. Autopsy specimens of this patient demonstrated pericarditis accompanied by endocarditis and myocarditis. Purulent pericarditis caused by *C. albicans* has been observed in post-operative thoracic surgery patients. Nevertheless, the prognosis for such patients has been improved after surgical procedures and chemotherapy (Gronemeyer *et al.*, 1982). There are 24 published cases of purulent pericarditis caused by *Candida* species in patients that have undergone thoracic surgery and had disseminated candidiasis. From these, only 13 patients described after 1980 survived and the remaining 11 patients died prior to 1990. Patients who survived did so only due to pericardectomy (five of six survivors) and pericardiocentesis (one survivor) with a full course of amphotericin B therapy. Application of echocardiography has demonstrated its efficiency as a powerful tool in the early detection of pericarditis caused by *Candida* species. A combination of novel antifungal drugs such as echinocandins, capsosungin or voriconazole and pericardectomy may be an ideal approach for achieving speedy recovery in such patients (Schrank & Dooley, 1995).

Amongst organ transplant patients, almost 60 % of the liver transplant patients were found to be infected with *Candida* species (Paya, 1993; Singh *et al.*, 2003). Persistent candidiasis in such patients has been associated with haemodialysis, fungal colonization, exposure to broad-spectrum antibiotics, intensive care unit (ICU) hospitalization, acute hepatic failure and other surgical events. In addition, during several post-operative surgical procedures, bacterial and viral (cytomegalovirus and human herpesvirus 6) infections also occurred in such patients (Winston *et al.*, 1999; Husain *et al.*, 2003). *C. albicans* has been observed to be the most prevalent species associated with candidal pericarditis followed by *C. glabrata* and *C. tropicalis*. A 30–100 % mortality rate in such patients has been reported to be exclusively due to CNA species (Paya, 1993; Fung, 2002). However, widespread use of fluconazole and the emerging trend of CNA species pose a major risk of colonization or infections in liver transplant patients. This scenario warns that the use of fluconazole should be focused more on high-risk patients (Husain *et al.*, 2003).

A considerable risk of colonization by CNA species in the neonatal ICU may lead to the predominance of *C. tropicalis* as a subsequent cause of neonatal fungaemia (Roilides *et al.*, 2003). Acquisition of *C. tropicalis* very likely occurs in the neonatal ICU by cross-contamination. There are several reports of nosocomial cross-infections due to *C. albicans* or *Candida parapsilosis* in the neonatal ICU

**Table 1.** Comparative clinical features of *C. tropicalis* and *C. albicans* infections, underlying clinical conditions, treatment and outcome in cancer patients

FL, Fluconazole; AmB, amphotericin B; CSFG, caspofungin; NS, not specified.

Group of patients	Year	No. of cases	<i>C. tropicalis</i>						<i>C. albicans</i>					
			No. of cases	Affected area	Underlying disease/clinical features*	Therapy	Outcome		No. of cases	Affected area	Underlying disease/clinical features*	Therapy	Outcome	
							Resolved	Resistant					Resolved	Resistant
Candidial arthritis (Sim <i>et al.</i> , 2005)	1978–2005	12	3	Knee	ALL	AmB, FL, FL in combination with CSFG	1 case, AmB; 1 FL-resistant case resolved with CSFG	1 case (but resolved with a combination of CSFG and FL)	1	Knee	ALL	AmB and 5-flucytosine	1 case	
			2	Knee	AML	AmB and miconazole	1 case, miconazole; 1 case, FL	AmB	2	Knee	CML- BC	AmB, FL and liposomal AmB	1 case, liposomal AmB (resistant to FL)	1 case, early death (AmB)
			1	Knee	CML- BC	Ketoconazole	1 case	1					Knee	Smoldering leukaemia
			1	Knee	SLL	AmB, miconazole	1 case, miconazole (resistant to AmB)		1	Knee	CLL	FL	1 case	
Spondylodiscitis (Parthiban <i>et al.</i> , 2008; Khazim <i>et al.</i> , 2006)	2008	4	1	Vertebrae	(i) Spondylodiscitis of L23 and L12 vertebrae; (ii) sigmoid colon	FL (i.v., oral)	1 case	1 3	Knee Vertebrae					
Oral candidiasis in children and adolescents with cancer (González-Gravina <i>et al.</i> , 2007)	2007	26	6	Oral mucosa	(i) 5 cases with pseudomembranous and 1 case with erythematous candidiasis; (ii) 2 with ALL, 1 with meduloblastoma and 1 with grade 1 astrocytoma	NS	NS	NS	20	Oral mucosa	(i) 14 cases with pseudomembranous and 6 cases with erythematous candidiasis; (ii) 13 with ALL, 1 with meduloblastoma, 1 with grade 1 astrocytoma, 2 with retinoblastoma and 1 with rhabdomyosarcoma	NS	NS	NS

\*ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; CML-BC, chronic myeloid leukaemia in blastic crisis; SLL, small lymphocytic lymphoma.

**Table 2.** Disseminated candidiasis due to *C. tropicalis* and *C. albicans* infections in neutropenic and non-neutropenic patients

Results represent the numbers of patients.

Stage of gastrointestinal infection	<i>C. tropicalis</i>		<i>C. albicans</i>	
	Neutropenic	Non-neutropenic	Neutropenic	Non-neutropenic
Gastrointestinal invasion	8	0	9	6
Colonization in entire gastrointestinal tract	4	0	1	0
Submucosal penetration	6	0	2	0

setting. *C. tropicalis* associated with candidiasis is not often encountered in neonates. However, adults and children with haematological malignancies have a higher mortality rate due to such an infection. The epidemiology of *C. tropicalis* in neonates is uncertain, but the probability for nosocomial spread must be carefully measured. Nosocomial transmission of a predominant genotype of *C. tropicalis* may occur among neonates most likely via cross-contamination. It is noteworthy that neonatal *C. tropicalis* isolates tested in some studies were susceptible to amphotericin B and flucytosine. A significant proportion of *C. tropicalis* isolates from neonates demonstrated reduced susceptibility to azoles compared to *C. albicans* (Roilides *et al.*, 2003). In addition to weakened immunity, high virulence and low azole susceptibility characteristics of *C. tropicalis* may be the leading cause of disseminated candidiasis in patients with haematological malignancies. Under such debilitating conditions, as well as infections caused by *C. albicans* alone, *C. tropicalis* infection could be augmenting the high mortality rates.

### Pathogenicity of *C. tropicalis*

Increased virulence of *C. tropicalis* isolates was observed when given orally to compromised mice, which parallels clinical observations in immunocompromised patients. Some studies showed that *C. tropicalis* is even more invasive than *C. albicans* in the human intestine, particularly in oncology patients (Walsh & Merz, 1986; Wingard *et al.*, 1979).

Secreted aspartyl proteinase 5 and 9 (SAP5 and SAP9) activity occurs in all *Candida* species, in the following order: *C. albicans*>*C. tropicalis*>*Candida kefyr*>*Candida krusei*. A few experimental studies have suggested that, following ingestion of yeast cells by phagocytic cells, SAP antigens are expressed by *C. albicans* and *C. tropicalis*, but not by *C. parapsilosis* (Borg & Ruchel, 1990; Ruchel, 1986; Ruchel *et al.*, 1986). The aspartic proteinases secreted by *C. tropicalis* have also been demonstrated on the surface of the fungal cell walls before invading tissues during disseminated infections and invading macrophages after phagocytosis of yeast cells (Borg & Ruchel, 1990; Borg-von Zepelin *et al.*, 1998; Ruchel *et al.*, 1991).

Some CNA species that were previously thought to be SAP-negative were in fact proteolytic. The purified tropiase

from *C. tropicalis* (a novel acid proteinase) demonstrated haemorrhagic activity and the ability to increase capillary permeability (Capobianco *et al.*, 1992; Okumura *et al.*, 2007). In order to examine these detrimental properties of tropiase in humans, comprehensive evaluation in a clinical setting (gastroenterology) needs to be focused on *C. tropicalis*-infected patients who are also suffering from ulcerative colitis or varying levels of gastrointestinal illnesses or complications.

Recent studies on three specific key virulence factors (proteinase, phospholipase and biofilm) suggest that the detection of hydrolytic enzymes and the ability of *Candida* yeasts to form biofilm may be useful indicators of possible haematogenous infection. Such findings may support clinicians in the management of patients who have a high risk of haematogenous *Candida* infection (Gokce *et al.*, 2007).

### Host defence

The oral cavity possesses physical barriers such as epithelial cells, saliva and salivary immunoglobulin (IgA), lysozyme, histidine-rich polypeptide, lactoferrin and lactoperoxidase for antagonistic action against *Candida* overgrowth (Jorge *et al.*, 1993). Although there is a clear understanding of the dominant host defence mechanisms against infections caused by *C. albicans*, less is understood about those against infections caused by *C. tropicalis* which has proven almost always to be the concomitant agent in the development of mycotic infections (Wingard *et al.*, 1979).

Invasive candidiasis due to *C. tropicalis* has been found more frequently in neutropenic patients receiving treatment for acute leukaemia or bone marrow transplants (Sandford *et al.*, 1980; Wingard *et al.*, 1979). This indicates that polymorphonuclear leukocytes are the first line of defence against *C. tropicalis* (Odds, 1988). However, the mutual role of microbial and other predisposing host factors which allow *C. tropicalis* to colonize the gastrointestinal tract remains uncertain (Fromtling *et al.*, 1987). There have been an insufficient number of studies describing the fungal cell morphology and the histopathological manifestations associated with infections caused by *C. tropicalis*. In several cases, the histopathological manifestation has been described only in broad terms.

Epidermal keratinocytes play an important role in the cutaneous immune response through the production of cytokines and chemokines, including interferon (IFN)- $\gamma$ -inducible protein 10 (IP-10). Recent investigations demonstrated that *C. albicans* and *C. tropicalis* impaired IFN- $\gamma$ -induced IP-10 expression but *C. glabrata* did not. *C. albicans* and *C. tropicalis* produced marked levels of prostaglandins, while *C. glabrata* produced a very low level. The prostaglandin antagonist restores IFN- $\gamma$ -induced IP-10 expression in *C. albicans*-infected normal human epidermal keratinocytes (NHEKs). These experimental findings suggest that prostaglandin E2 may be a major predisposing factor for diminishing IFN- $\gamma$ -induced IP-10 expression in NHEKs (Shiraki *et al.*, 2008).

Based on clinical observations and experimental studies on mucosal (chronic mucocutaneous and gastrointestinal) candidiasis, T-cell (CD4<sup>+</sup> and CD8<sup>+</sup>) and cell-mediated immunity is the predominant host defence mechanism against *C. albicans* (Barnett *et al.*, 1990; Cantorna & Balish, 1990; Kirkpatrick, 1989; Kirkpatrick *et al.*, 1971). It is believed that vaginal candidiasis is affected by T-cell response. Experimental studies also suggest that T cells are important in the local T-cell response, such as in vaginal candidiasis caused by *C. albicans*. However, these findings do not correlate with decreased T-cell (CD4<sup>+</sup>) counts in HIV-infected women with vaginal candidiasis (White, 1996; Rhoads *et al.*, 1987; Imam *et al.*, 1990; Clark *et al.*, 1995). As far as the B-cell-mediated immune response to candidiasis is concerned, B-cell deficiency does not support susceptibility to *C. albicans* infection. Interestingly, CNA species, particularly *C. tropicalis*, and systemic candidiasis are becoming more prevalent in oral and gastrointestinal candidiasis (Wingard *et al.*, 1979, 1980; Sandford *et al.*, 1980; Fromtling *et al.*, 1987; Prasad *et al.*, 1999; Nucci & Colombo, 2007).

Clinical and experimental observations suggest that morbidity and mortality rates are higher due to *C. tropicalis* infection than to *C. albicans* infection (Wingard *et al.*, 1979; Fromtling *et al.*, 1987). These findings support the hypothesis that there must be some additional *C. tropicalis* secretory products that are probably more pronounced in a T-cell-deficient host. Such secretory products from *C. tropicalis* could be intensely cytotoxic or there could be synergistic interactions with the host cells that culminate in the deaths of immunocompromised patients. There are two basic queries that need to be addressed by clinical and experimental studies: why is *C. tropicalis* becoming an emerging pathogen and why is it causing higher mortality rates than *C. albicans*?

From the group of CNA species, *C. tropicalis* is becoming an emerging pathogen globally. The major contributory factors in the emergence of *C. tropicalis* include the following: (1) increasing use of an antifungal regimen, (2) the increasing number of immunocompromised patients, (3) long-term use of catheters, (4) use of broad-spectrum antibiotics and (5) complexity in treating underlying subclinical conditions

coupled with antifungal drug intolerance and resultant recurrent infections and nosocomial outbreaks. In addition, drug resistance in immunocompromised patients that survive longer has increased alongside the resultant mortality.

Poor cellular transportation of antifungal agents and inadequate immune response are the major contributory factors that allow yeasts to colonize extensively in immunocompromised patients such as AIDS sufferers. As a result, antifungal drug intolerance and toxicity has become the leading cause of increasing morbidity and mortality in patients infected with *C. tropicalis*. To investigate a more specific root cause, histochemical or biochemical (toxicity) studies on autopsy specimens recovered from immunocompromised or HIV/AIDS patients concomitantly infected with *C. tropicalis* may be more conclusive than experimental studies.

### ***C. tropicalis* and fluconazole resistance**

*C. albicans* is the cause of a wide spectrum of infections such as superficial, cutaneous, subcutaneous and systemic candidiasis in immunocompromised patients. Infections caused by non-*albicans* species of *Candida* such as *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *Candida lusitanae*, *Candida inconspicua*, *Candida lipolytica* and *Candida norvegensis* are numerically dominant over those caused by *C. albicans* (Weinberger *et al.*, 1997; Ng *et al.*, 1998). *C. tropicalis* alone or in association with *C. parapsilosis* is the second most prevalent *Candida* species after *C. albicans* (Ellis, 2002; Kao *et al.*, 1999; Kontoyiannis *et al.*, 2001). In a large surveillance study conducted by Pfaller *et al.* (2009), *C. tropicalis* showed a moderate level of fluconazole resistance. This indicates that there may be a risk of fluconazole resistance through upregulation of efflux transporters upon exposure to increasing concentrations of the drug (Barchiesi *et al.*, 2000). There are several factors probably responsible for the development of drug resistance in various clinical conditions. It is possible that higher MICs in strains from patients who have received antifungal regimens in the past in either a consistent or inconsistent manner or widespread use of fluconazole within community care facilities might have facilitated resistance to fluconazole (Joseph-Horne & Hollomon, 1997). The epidemiology appears complex and varies among the different patient care units.

Resistance to fluconazole in clinical isolates of *C. tropicalis* has increased (Tortorano *et al.*, 2003; Yang *et al.*, 2004; Myoken *et al.*, 2004). There are several reports on azole resistance, specifically in *C. albicans* and *C. tropicalis* (Brun *et al.*, 2004; Yang *et al.*, 2004; Vermitsky & Edlind, 2004). The precise mechanisms responsible for drug resistance in *Candida* species have been described by Sanglard & Odds (2002). Acquired resistance to azole in *C. tropicalis* could be due to overexpression of *CtERG11* gene associated missense mutation.

There is a significant correlation between phospholipase activity of clinical isolates of *C. albicans* recovered from

mucocutaneous lesions with infectivity in albino mice (Kothavade & Panthaki, 1998). However, *Candida* species exposed to antimycotic agents have shown a significant reduction of their phospholipase activity. Nystatin and amphotericin B, but not fluconazole, significantly reduce the phospholipase activity of both *C. albicans* and *C. tropicalis* species (Anil & Samaranayake, 2003). In HIV-infected patients, HIV-1 interacts with different clinically important *Candida* species and this could be affecting the clinical efficacy of fluconazole or other antifungal agents (Gruber *et al.*, 2003). The basic query here is: does fluconazole remain fungistatic because of its poor antiphospholipase activity? The exact mechanism that fluconazole has against phospholipase activity is not clearly understood, and hence additional clinico-mycological observations are needed for a more definitive conclusion.

### Measures for controlling fluconazole resistance in clinical management

The increasing use of fluconazole globally in various clinical conditions (candidiasis) is a major cause of CNA dominance over *C. albicans*. (1) Therapeutic application of fluconazole should be limited to selected high-risk patients to minimize the risk of emergence of azole-resistant strains of *Candida*. (2) Patients with recurrent candidiasis, but recently treated with fluconazole, should not be treated again with the same drug as counteractive treatment for a presumed or proven incidence of systemic candidiasis. (3) To control nosocomial outbreaks of drug-resistant strains of *C. tropicalis*, rapid detection followed by a drug susceptibility test needs to be performed on the clinical isolates. (4) Use of various antifungal agents and their interactions with other drugs for treating subclinical or underlying conditions needs to be carefully thought out prior to their application in the clinical management of high-risk patients. (5) The best way to improve antifungal drug therapy is to improve immunity of the host. In many cases, this is not possible, but there are clinical scenarios in which improving immune responses may improve clinical prognosis. For example, granulocyte colony-stimulating factor, a cytokine, in combination with fluconazole may be useful in the management of fungal diseases. (6) The removal of fungus-contaminated foreign objects or the surgical removal of abscesses can reduce the fungal burden and allow the host and antifungal drugs to clear the infection even with strains that are resistant to standard antifungal therapy. (11) In surgical patients, new antifungal agents such as azoles (voriconazole) and echinocandins are less-toxic therapeutic options for prevention and optimized therapy for *Candida* infections. (12) In invasive candidiasis (paediatric intensive care patients), quick removal of lines (e.g. parenteral nutrition, arterial lines, central venous catheter lines) and instigation of treatment with novel antifungal agents such as second-generation triazole and echinocandins may be preferred in clinical management of hospitalized patients. (13) Increased awareness of risk factors for CNA species can provide

guidance for appropriate choices of antifungal therapy (Davis *et al.*, 2007).

### Conclusions

Use of fluconazole should not be continued in patients with recurrent *Candida* infections. Rapid species identification from clinical specimens and standard drug susceptibility testing would be an effective approach for controlling nosocomial outbreaks caused by *C. tropicalis* or CNA species in different clinical settings. Consistent application of standard techniques, guidelines for the use of antifungal agents and control measures for predisposing factors or risk factors may potentially reduce the risk of drug-resistant life-threatening infections. However, application of standardized technology and its consistent use in diagnostics and research still remains a major global challenge.

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