Characterisation and Antifungal susceptibility pattern of Candida species isolated from various clinical samples at a tertiary care centre in South India

Sumita Rajeevan[1,*], Marina Thomas[2], Boppe Appalaraju[3]

[1]Assistant Professor, MES medical college, Perinthalmanna, Kerala
[2]Professor, [3]Professor and HOD, Dept. of Microbiology,
PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India

***Corresponding Author:**
E-mail: sumitarajeevan@gmail.com

Abstract

Background: Over the last 20 years, the rates of fungal infection have increased and Candida has emerged as a major cause of human disease. Furthermore there is an increase in nonalbicans candida species in many countries. The emerging pathogens are resistant to conventional antifungal therapy.

Objective: To find out which species of Candida is predominant in clinical infection and determinate their antifungal susceptibility patter.

Materials and method: All Candida isolates recovered from various clinical samples during the period from February 2011 to August 2012 were studied. The isolates were speciated using, both conventional and automated techniques. Antifungal susceptibility testing was done by disk diffusion methods according to Clinical and Laboratory Standards Institute guidelines. Various risk factors associated with the candida infection were also looked into.

Results: A total of 269 Candida isolates were isolated. (Prevalence of 5.3%). 42% of isolates were from patients above 60 years of age Candida tropicalis (46%) was the most common isolate. All the 269 Candida isolates (100%) were sensitive to amphotericin B. Resistance of more than 70% to the three azoles tested was noted amongst the C albicans and the C parapsilosis spp. The risk factors commonly associated with candida infection were Catheterization (87.5%), ventilator assistance (75%) and Diabetes mellitus (31%).

Conclusion: In our study Candida tropicalis was the predominant isolate. All candidal isolates were sensitive to Amphotericin B however marked resistance to azole derivative was found with Candida albicans than C.tropicalis. CHROMagar candida medium and tetrazolium reduction medium are found to be useful in identification of Candida species. All candidal isolates were sensitive to Amphotericin B.

Keywords: Candida species, Anti-fungal susceptibility, Amphotericin B, Azole resistance

Access this article online	
Quick Response Code:	**Website:** www.innovativepublication.com
	DOI: 10.5958/2394-5478.2016.00014.5

Introduction

The incidence and prevalence of fungal infections have increased since the 1980s especially in immunocom-promised patients and those hospitalized with serious underlying diseases.[1] The mortality rate associated with candidemia worldwide is also high ranging from 10% to 49%.[2] In India, the picture is not very clear due to lack of multicentre studies.

Candida species which are ubiquitous human commensals are responsible for various clinical manifestations from mucocutaneous overgrowth to bloodstream infections.[3] The genus consist of a heterogeneous group of organisms, and approximately 20 different Candida species are known to be aetiological agents of human infection. However, 90% of invasive infections are caused by Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis and Candida krusei.[4] Candidiasis is a growing medical problem which requires prompt diagnosis and early treatment. Clinical laboratories may thus need to expand their yeast identification capabilities to detect simultaneously *C.albicans* and the other major Candida species in clinical specimens.

The large population of immunocompromised patients using intravenous catheters, catheterization of urinary tract/vascular system, renal failure, hepatic failure, prolonged hospital stay, prolonged use of broad-spectrum antibiotics, cytotoxic chemotherapies and transplantation are factors that contribute to the increase of these infections.[5] Diabetes mellitus, the leading endocrine dysfunction provides a potent soil for Candidial infection. Specific association has been found between patients with leukemia and *C.tropicalis* infections and bone marrow transplant recipients and *C.krusei* infections.[5]

With the emergence of non-*albicans* species of *Candida* worldwide antifungal drug resistance has become a major concern in the management of candida infection.[6] There have been a few reports of *Candida* species being resistant to amphotericin B and echinocandins.

Materials and Methods

A total of 269 isolates of Candida species recovered from clinical samples during the period from February 2011 to August 2012 were studied after obtaining ethical clearance from the Human Institutional Ethics Committee. The clinical significance of the isolate was ascertained by ensuring that no other primary pathogen was isolated in that site, repeated isolations, association with pus cells and other co-factors such as, prolonged antibiotic therapy, total parenteral nutrition (TPN) and any immunocompromised conditions like diabetes, malignancy, neutropenia, steroid therapy and others.

Standard methods of direct microscopy and culture on Sabouraud's Dextrose Agar (SDA) with Gentamycin and Chloramphenicol were done for isolation.[1] Speciation of the *Candida* isolates was done by germ tube test, sugar fermentation and assimilation tests, color of growth on tetrazolium reduction medium and CHROM agar *Candida* (CHROMagar, Paris, France). Out of the 32 isolates from blood, 24 were subjected to VITEK 2 system for identification and 30 selected isolates were simultaneously subjected to Matrix assisted Laser Desorption Ionization Time of Flight analysis (MALDI-TOF). Antifungal susceptibility testing was performed by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[7]

Results

During the study period, among 5051 samples which yielded significant growth, 269 (5.3%) belonged to the Candida species. The study group included patients from new born infants to 93 years of age and 42% of isolates were from patients above 60 years of age. Out of these 269 isolates, 85.1% were from Inpatients and 14.8 % were from Outpatients. Among the isolates from inpatients 76 (27.63%), 69 (25.09%), 34 (12.36%), 32 (11.6%) were from Medical, surgical, trauma ward and ICU respectively.

Species identification revealed that, *Candida tropicalis* constituted 46% and *C.albicans* 35% of all the isolates and both together constituted 81% of isolates. Non albicans Candida species constituted 65% (178) of the isolates. *Candida tropicalis* was the predominant species in all samples except in the respiratory samples of tracheal aspirates and bronchoalveolar lavage fluids, where *C albicans* was the predominant isolate. Candida glabrata and krusei were not isolated from the urinary samples (**Table 1**). Germ tube formation was shown by all *C.albicans* isolates. On CHROM agar (Fig. 1) after 48hrs of incubation 98% of *C.albicans* showed the characteristic light green color and 95% of *C.tropicalis* showed the characteristic metallic blue color. On TRM (Fig. 2) all *C.tropicalis* (100%) produced maroon colored colonies and 95% of *C.albicans* produced the typical cream to pink colored glistening colonies.

Among the 128 (47.5%) patients from whom the urinary isolates of Candida species were identified, 87 (67.9%) were from catheterized samples and 41 (32%) were from mid-stream samples of urine, and in both samples the isolates were grown in significant numbers and were associated with pus cells. Catheterisation was the risk factor in all the catheterized samples of urine. The species distributions among urinary isolates are as follows: *C.tropicalis* 85 (66.9%), *C.albicans* 36 (28.3%), *C. parapsilosis*, *C.kefyr* and *C.guillermondi* 2 (1.57%) each and 1 was *C.lusitaniae*. Diabetes mellitus (29.2%) and malignancy (4.8%) were the risk factors among the urinary isolates in midstream samples. There was no significant difference in species distribution among the two samples.

Out of the 269 isolates of Candida, a total of 109 (40.52%) were grown from respiratory samples of tracheal aspirate and bronchoalveolar lavage fluid in significant numbers of $\geq 10^5$ cfu/ml. *Candida albicans* (45.87%) was the predominant species isolated in both samples. Other species included *C.tropicalis* (26%), *C.krusei* ((9%), *C.glabrata* (6%), *C.parapsilosis* (6%), *C.kefyr* (6%), *C.guillermondi* (1.8%) and *C.lusitanae* (1%). There was no significant difference in species distribution among the two samples.

Among the 269 isolates of Candida species 32 (11.89%) were from blood cultures and this formed the 5th most common microbe isolated from blood during the same period of time. All the isolates were from ICU areas. Out of 32 isolates from blood, 24 of them were subjected to Vitek 2 system analysis, 21 strains out of 24 (87.5%) identified by phenotypic ID were identified as same by Vitek 2 system where as 2 isolates *C.parapsilosis* and one isolate of *C.glabrata* identified as so by phenotypic ID were unidentified by Vitek 2 system. There was 100% correlation between identification by phenotypic methods and MALDITOF. Out of the 32 isolates, 11 (34.3%) were *C.tropicalis*, 8 (25%) were *C.albicans*, 6 (18.7%) were *C.glabrata*, 4 (12.5%) were *C.parapsilosis*, 2 (6.2%) were *C.guillermondi* and 1 (3.1%) was *C.krusei*. Catheterization (87.5%), ventilator assistance (75%) and Diabetes mellitus (31%) were the associated factors in the patients from whom these were isolated.

All the 269 isolates were sensitive to amphotericin-B. A total of 55%, 56% and 63% of all Candida isolates were sensitive to fluconazole, itraconazole and ketokonazole respectively. Resistance of more than 70% to the three azoles tested was noted amongst the *C albicans* and the *C parapsilosis* species. Table 2

Table 1: Candida species Isolated and its Distribution among various samples

Species	Urine	TA+ BAL	Blood	Total
C.tropicalis	85(66.92%)	28(25.68%)	11(34.3%)	124 (46%)
C.albicans	36(28.34%)	50(45.87%)	8(25%)	94(34.94%)
C.glabrata	-	7(6.42%)	6(18.7%)	13 (4.83%)
C.parapsilosis	2(1.57%)	7(6.42%)	4(12.5%)	13(4.83 %)
C.krusei	-	11(10.09%)	1(3.12%)	12(4.46%)
C.guillermondi	2(1.57%)	2(1.83%)	2(6.25%)	6(2.23%)
C.kefyr	2(1.57%)	3(2.75%)	-	5(1.85%)
C.lusitanae	1	1(0.91%)	-	2((0.74%)
Total	128 (47.5%)	109(40.52%)	32(11.89%)	269

TA: Tracheal aspirate**, BAL:** Bronchoalveolar lavage fluid

Table 2: Antifungal susceptibility pattern for Candida species isolated in the study:

Species	AP		CC		Fu		It		Kt		Ns	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
C.tropicalis	124 100%	-	117 94%	7	111 89%	13	102 82%	22	115 92%	9	124 100%	-
C. albicans	94 100%	-	70 74%	24	28 30%	66 70%	23 24%	71 76%	25 27%	69 73%	80 85%	14
C.glabrata	13 100%	-	13 100 %	-	3 23%	10 77%	13 100 %	-	13 100%	-	13 100%	-
C.parapsilosis	14 100%	-	10 71%	4	4 29%	10 71%	3 21%	11 79%	4 29%	10 71%	14 100%	-
C.krusei	12 100%	-	9 75%	3	-	12 100%	3 25%	9	2 16%	10	10 83%	2
C.guilliermondii	5 100%	-	.4 80%	1	3 60%	2	4 80%	1	5 100 %	-	5 100%	-
C.kefyr	5 100%		5 100 %	-	3 60%	2	4 80%	1	5 100%	-	5 100%	-
C.lusitaniae	2 100%		-	2	-	2	2 100 %	-	2 100%	-	2 100%	-
Total	**269 100%**	-	**228 83%**	**41**	**152 55%**	**117 45%**	**154 56%**	**115 44%**	**171 63%**	**98 37%**	**253 93%**	**16**

Ap – amphotericin B, **CC**-clotrimazole, **Fu**- fluconazole, **It**- Itraconazole, **Kt**- ketoconazole, **Ns**-nystatin

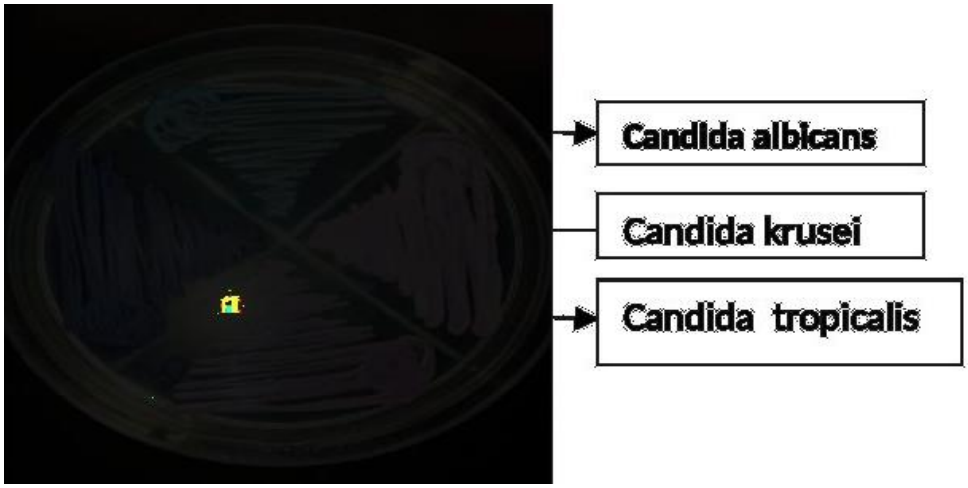

Fig. 1: Shows growth of Candida species on CHROMAgar. C.albicans produced light green C.tropicalis - metallic blue, C.krusei – dry pink color

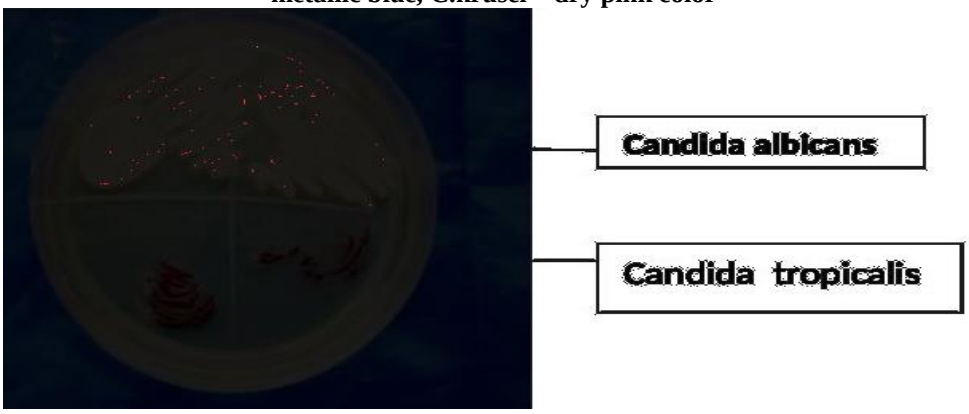

Fig. 2: shows growth of Candida species on Tetrazolium reduction medium (TRM).C. albicans produced pale pink, C.tropicalis – maroon color.

Discussion

Over the last two decades, fungal infections have increased at an alarming rate. Candida species are commensals and act as opportunistic pathogen only on interruption of normal host defenses. They are capable of causing a wide range of superficial and deep seated mycoses such as cutaneous, mucocutaneous and systemic candidiasis.

Out of 5051clinical samples which yielded significant growth during the study period, Candida species constituted 5.3% (269) of the isolates, all of which constituted the study material. In our study Candidiasis occurred in all age groups, but predominance (48%) was seen among those above 60 years age of age. The aging population is especially susceptible to fungal infection mainly due to underlying medical conditions and the associated increase in the rates of hospitalization. In a large European based study 28% of candidemic episodes were diagnosed in subjects over the age of 70 years[8]**.** These are comparable to other studies also[9].

Vitek 2 system and MALDITOF are expensive means for identifying Candida species. Use of CHROM agar Candida has been advocated in many studies to be used for primary isolation of Candida species and its rapid identification [10]**.** Using CHROMagar for primary isolation has the added advantage of being able to detect mixed Candida cultures in the same sample, which is a possibility in 3.4% of Candida isolations [11]. In our study only 95% of *C.tropicalis* showed the characteristic metallic blue color and 98% of *C.albicans* the characteristic green color on CHROMagar. In our study, all 100% of *C.tropicalis* was identified on tetrazolium reduction medium based[12] on its classic maroon color which is similar to other studies. All *C.albicans* were germ tube positive. *Candida dubliensis* are also germ tube positive, but they can be differentiated on CHROM agar by a darker green color, inability to grow at 45ºC and on Tobacco agar *Candida albicans* produce white to cream colored colonies without hyphal hinges were as *Candida dubliensis* produce yellow to brown colonies with peripheral hinges after incubation for 48 – 72 hours[13]. Since *Candida tropicalis* is the predominant isolate among the Candida species followed by *C. albicans*, therefore, TRM for identification of *C.tropicalis* and germ tube for *C.albicans* would be an ideal combination of tests in routine diagnostics as these together constitute 81% of all Candida isolates. The other Candida species may be identified by a combination of assimilation, fermentation tests, CHROMagar, TRM and MALDI-TOF methods.

Among the 32 blood isolates, non albicans Candida species constituted 75% of the isolates. *C.tropicalis* (34.37%) was the predominant species followed by *C.albicans* 25%. This is comparable to other Indian studies which showed C.tropicalis(35-45%) as the predominant isolate[14,15,16]. The risk factors associated were use of medical devices - urinary catheter (78%) and ventillation (71%), Indwelling vascular catheters (48%), diabetes mellitus (31.25%), surgical intervention (25%) and in 15.6% decompensated chronic liver disease (DCLD). *Candida* species adhere to materials used in intravascular devices and provide a potential nidus of infection [17]. The reasons for susceptibility to Candida infection in diabetics are many. They include associated illnesses, increased use of antibiotics, and hyperglycemia itself which can lead to disseminated disease[18]. A multi-centre observational study done by Blumberg et al. found surgery as an independent risk factor associated with candidiasis[19].

Candida species is one of the most common causes of nosocomial urinary tract infection[20]. Various factors like immunocompromised status, prolonged hospital stay, catheterisations have all contributed for increase in number of cases of candiduria. Catheterisation process increases chances of UTIs by allowing migration of the organisms into the bladder from external periurethral surface. This finding is in concordance with the studies done by Iman et al[21]. In our study among all the

urinary isolates of Candida, 67.9% were from catheterized samples. Diabetes mellitus was the predominant (29%) risk factor among the midstream samples. However, among the respiratory samples (tracheal aspirate and BAL fluids), *C.albicans* 50 (51.5%) was the predominant species as was found in other studies also[22].

In India, the scenario of antifungal drug resistance to *Candida* isolates is not very clear due to lack of multicentric studies over a period of many years. Resistance to amphotericin B among *Candida* isolates was not found in our study which is similar to other Indian studies, although very few instances of amphotericin B resistance in *Candida* isolates from cases of candidemia are reported from India [23]. Although fluconazole still remains a safe and effective choice for the treatment of candidemia, an increasing trend of fluconazole resistance in Candida isolates from blood has been reported. In our hospital the commonly prescribed antifungal drugs are fluconazole and itraconazole but susceptibility to these was only 55% and 56% respectively. Resistance to azole derivative was found to be more with *Candida albicans* (68% - 73%) than that for *C.tropicalis* (17% -25%) to all the three azoles. Chakrabarti A *et al*. observed that Candida species with fluconazole resistance have been seen more commonly in recent years. In another study from New Delhi by Kothari *et al*., fluconazole resistance was reported in 36% of candidemia isolates. Fluconazole resistance in *Candida* isolates from blood has been reported by Pfaller MA et al also.

Conflict of Interest: None

Source of Support: Nil

Reference:

1. Arendrup, M. C., Fuursted, K., Gahrn-Hansen, B., Jensen, I. M., Knudsen, J. D., Lundgren, B., Schønheyder, H. C. & Tvede, M.(2005). Seminational surveillance of fungemia in Denmark: notably high rates of fungemia and numbers of isolates with reduced Azole susceptibility. J Clin Microbiol 43,4434–4440.
2. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. Clin Microbiol Rev 2007;20:133-63.
3. Eggimann,P.,Garbino,J.& Pittet,D.(2003). Epidemiology of Candida species infections in critically ill non-immunosuppressed patients. Lancet Infect Dis 3,685–702.
4. Ortega, M., Marco, F., Soriano, A., Almela, M., Martı´nez, J. A., Lo´ pez, J., Pitart, C. & Mensa, J. (2011). Candida species bloodstream infection: epidemiology and outcome in a single institution from 1991 to 2008. J Hosp Infect 77,157–161.
5. Jaine R Maenza and William G. Merz Infectious disease-3rd edition-2004 Sheerwood L. Gorbach, John G. Bartlett, Neil R. Bkackiow (LWW).
6. Pfaller MA, Diekema DJ, International Fungal Surveillance Participant Group. Twelve years of fluconazole in clinical practice: Global trends in species distribution and fluconazole susceptibility of bloodstream isolates of *Candida.* Clin Microbiol Infect 2004; 10 Suppl 1:11-23.
7. Rex JH, Ghannoum MA, Alexander BD, Andes D, Brown SD, Diekema DJ, *et al*. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts: Approved Guideline M44-A2. In: Rex JH, Ghannoum MA, Alexander BD, Andes D, Brown SD, Diekema DJ, editors. M44-A2 [29]. Pennsylvania, USA: CLSI; 2009. Ref Type: Serial (Book, Monograph).
8. Tortorano AM, Peman J, Bernhardt et al. Epidemilogy of Candidemia in Europe; results of 28 month European Confedration of Medical Mycology (ECMM) hospital based surveilliance study Eur J Clin Microbial Infect Dis 2004;23:317-32.
9. Pinho Resende JC, De Resende MA, Saliba JL. Prevalence of Candida species in hospitalized patients and their risk factors. Mycoses. 2002;45:306.
10. Lynn L. Horvath, Duane R. Hospenthal, Clinton K. Murray et al. Direct isolation of Candida spp. from blood cultures on the Chromogenic medium CHROMagar candida Journal of Clinical Microbiology June 2003 p 2629-2632.
11. Yang YL, Chu WL, Lin CC, Tsai SH, Chang TP, Lo HJ. An emerging issue of mixed yeast cultures. J Microbiol Immunol Infect 2014;47(4):339-344).
12. MAIRE J. DENNY AND BETTY M. PARTRIDGE From the Mycology Research Laboratory, Department of Bacteriology, Charing Cross Hospital Medical School, Tetrazolium medium as an aid in the routine diagnosis of Candida. J. clin. Path. (1968),21,383-386.
13. Khan ZU.et al. Tobacco Agar a new medium for differentiating Candida dubliniensis from Candida albicans. J Clin Microbiol 2004,42:4796-4798.
14. Chakrabarti A, Chatterjee SS, Rao KL, Zameer MM, Shivaprakash MR, Singhi S, et al. Recent experience with fungaemia: Change in species distribution and azole resistance. Scand J Infect Dis.2009;41:275–84.
15. Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of candidemia in a tertiary care center of North India: 5 year study. Infection. 2007;35:256–9.
16. Singh RI, Xess I, Mathur P, Behera B, Gupta B, Misra MC. Epidemiology of candidemia in critically ill trauma patients: Experiences of a level I trauma center in North India. J Med Microbiol. 2011;60:342–8.
17. Esther Segal and Daniel Elad Topley and Wilsons Microbiology and Microbial Infections- 10th edition by-William G Merz and Rodrick J. Hay –2005 (Hodder Arnold Publishers-Ltd)-p5.
18. Al-Attas SA, Amro SO. Candidal colonization, strain diversity, and antifungal susceptibility among adult diabetic patients. Ann Saudi Med 2010;30:101-8.
19. Blumberg HM, Jarvis WR, Soucie JM, et.al. Risk factors for candidal bloodstream infections in surgical intensive care unit patients: the NEMIS prospective multicenter study. *Clin Infect Dis.* 2001;33:177-86.
20. Jacqueline M. Achkar and Bettina C. Fries *Candida* Infections of the Genitourinary Tract Clin Microbiol Rev. 2010 Apr;23(2):253–273. doi: 10.1128/CMR.00076-09.
21. Iman KB, Shorouk KEH, Muhmoud M.*Candida* infection associated with urinary catheter in critically ill patients. Identification, antifungal susceptibility and risk factors. Res. J. of Med & Med sciences.2010;5(1):79–86.
22. Vinitha M et al. Distribution of Candida species In Different Clinical Samples and Their Virulence: Biofilm Formation, Proteinase and Phospholipase Production: A Study on Hospitalized Patients in Southern India. J Glob Infect Dis. 2011 Jan-Mar;3(1):4–8.
23. Adhikary R, Joshi S. Species distribution and anti-fungal susceptibility of Candidaemia at a multi super-specialty center in Southern India. Indian J Med Microbiol 2011;29:309-11.

How to cite this article: Rajeevan S, Thomas M, Appalaraju B. Characterisation and Antifungal susceptibility pattern of Candida species isolated from various clinical samples at a tertiary care centre in South India. Indian J Microbiol Res 2016;3(1):53-57.