Original Research Article

Mycological profile and risk factors for fungal keratitis: A three years study at a tertiary care teaching hospital

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Abstract

Background: The incidence of fungal keratitis has been significantly increased in many developing countries. As fungi causing keratitis, varies as per locale and climatic condition, the local epidemiological data often guide ophthalmologists in management of patients with mycotic keratitis. The present study was conducted with an aim to know the mycological profile and risk factors for fungal keratitis at a tertiary care teaching hospital. **Material and Methods:** Clinically suspected cases of keratitis attending ophthalmology out-patient department were included in the study. The corneal scrapings were used for the preparation of 10% potassium hydroxide mount, Gram staining and culture. The fungal isolates were identified as per standard protocol. **Results:** A total of 93 (34.8%) cases showed growth of fungus. *Fusarium* spp. (33.3%) followed by *Candida* spp. (22.6%) and *Aspergillus* spp. (22.6%) were common fungal isolates. Maximum patients were seen in age group 51-60 years (31.2%). Predominance of male gender (61.3%) was noted. Majority of patients with culture proven fungal keratitis were farmers. Ocular trauma due to vegetative matter was the most common predisposing factor. **Conclusion:** To minimize ocular morbidity, timely antimicrobial treatment must be initiated on the basis of clinical and microbiological evaluation.

Keywords: Keratitis, *Fusarium* spp., *Candida* spp., Corneal trauma.

Introduction

Eyes are considered as the most beautiful gift by the nature. Good vision is key to one's overall sense of well-being. Globally, various researchers have documented the negative impact of vision loss on quality of life. Cataract, glaucoma, infections, macular degeneration, diabetic retinopathy and uncorrected error are the most common causes of vision loss and impairment worldwide[1].Infective corneal diseases, especially keratitis are common cause of blindness worldwide next to cataract[2]. Keratitis is the superficial or deep, inflammation or infection of the cornea. The incidence of fungal keratitis has been significantly increased in many developing countries like India, China, Nepal and Ghana[2-5]. Predisposing factors that correlated with this increasing incidence include corneal trauma due to vegetative material or ophthalmic surgeries, insect fall, use of contaminated ophthalmic solutions, presence of contact lens, widespread spread abuse of broad spectrum antibiotics and steroids, traditional eye remedies, diabetes, immunocompromised condition and ocular disorders[6].As fungi causing keratitis, vary as per locale and climatic condition, the local epidemiological data often guide ophthalmologists in management of patients with mycotic keratitis[6]. The present study was conducted with an aim to know the mycological profile and risk factors for fungal keratitis at a tertiary care teaching hospital.

Material and methods

The present prospective cross-sectional, descriptive, hospital based study was conducted in the Department of Microbiology, Government Medical College, Aurangabad, Maharashtra. Clinically suspected cases of keratitis attending ophthalmology out-patient department (OPD) were included in the study.

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Information regarding sociodemographic features, duration of symptoms, risk factors, history of corneal trauma, traumatic agents and other ocular conditions was collected and analyzed.

After detailed ocular examination, corneal scrapings were obtained from these patients by an Ophthalmologist under aseptic conditions. The sample was collected from the active margins of the ulcer using a sterile Baird-Parker blade[4].

The corneal scrapings were used for the preparation of 10% potassium hydroxide (KOH) mount and Gram staining. Additionally, specimen was directly inoculated onto blood agar, chocolate agar and two sets of Sabourauddextrose agar (SDA) with chloramphenicol. C-shaped streak was made on culture plates to ensurethat growth was from the specimen and not of laboratorycontaminants. One set of SDA was incubated at 25°C while other at 37°C. Blood agar and chocolate agar plates were examined daily for seven days and discarded if no growth was observed. SDA plates were examined daily for twenty one days. Microbial growth if any was identified by standard bacteriological and mycological protocol[7].

A fungal culture was considered positive if the isolation of the fungus was corroborated by positive direct microscopy (KOH mount and Gram staining), growth of the same fungal species in 2 or more culture media, and/or its isolation on more than one occasion[4,8]. The filamentous fungi were identified on the basis of their macroscopic and microscopic features. Yeast isolates were identified upto species level by germ tube technique, colony color on Hichrom*Candida* agar and sugar assimilation test[9].

Results

During the study period, out of 4891 patients who attended ophthalmology OPD, a total of 267 (5.5%) were clinically diagnosed for keratitis. On examination of KOH mount, fungal elements were seen in total 89 (33.3%) cases whereas Gram staining demonstrated fungal elements in 67 cases. A total of 93 (34.8%) cases showed growth of fungus.

Considering culture as gold standard, the sensitivity KOH mount and Gram stainingfor detection of fungal elements in corneal scrapping was 95.7% (**95% CI, 89.3 to 98.8 %**) and 72.1% (**95% CI, 61.8% to 80.8 %**). Both KOH mount and Gram staining showed 100% specificity for detection of fungal elements from corneal scrapping.

Among 93 fungal isolates, a total of 68 were pure isolates whereas 25 isolates were mixed with bacteria. In the present study, there was no isolation of more than one fungus from single case. The fungal pathogens isolated in the present study are shown in table 1.

Table 1: Fungi isolates from keratitis cases.

Fungal isolate	Pure isolate (%)	Mixed with bacteria (%)	Total
Alternaria spp.	03 (75)	01 (25)	04
*Aspergillus*spp.	17 (80.9)	04 (19.1)	21
A. flavus	04 (100)	-	04
A. fumigatus	03 (100)	-	03
A. niger	07 (70)	03 (30)	10
A. nidulans	03 (75)	01 (25)	04
*Bipolaris*spp.	04 (80)	01 (20)	05
Candida spp.	14 (66.7)	07 (33.3)	21
C. tropicalis	08 (66.7)	04 (33.3)	12
C. albicans	04 (66.7)	02 (33.3)	06
C. krusei	02 (66.7)	01 (33.3)	03
*Fusarium*spp.	21 (67.7)	10 (32.3)	31
Mucor	05 (100)	-	05
Pseudallescheriaboydii	03 (75)	01 (25)	04
Penicillium spp.	01 (50)	01 (50)	02
Total	68 (73.1)	25 (26.9)	93

Fusarium spp. (33.3%) followed by *Candida* spp. (22.6%) and *Aspergillus* spp. (22.6%)were common fungal isolates. Predominance of non *albicans Candida* (NAC) spp. was noted. Among *Candida* spp., *C. tropicalis* was the most common isolate. A total of 8 (8.6%) fungi remained unidentified.

The age wise distribution of patients with culture proven fungal keratitis is shown in figure 1. Maximum patients were seen in age group 51-60 years (31.2%) followed by 41-50 years (22.6%) and 31-40 years (17.2%).

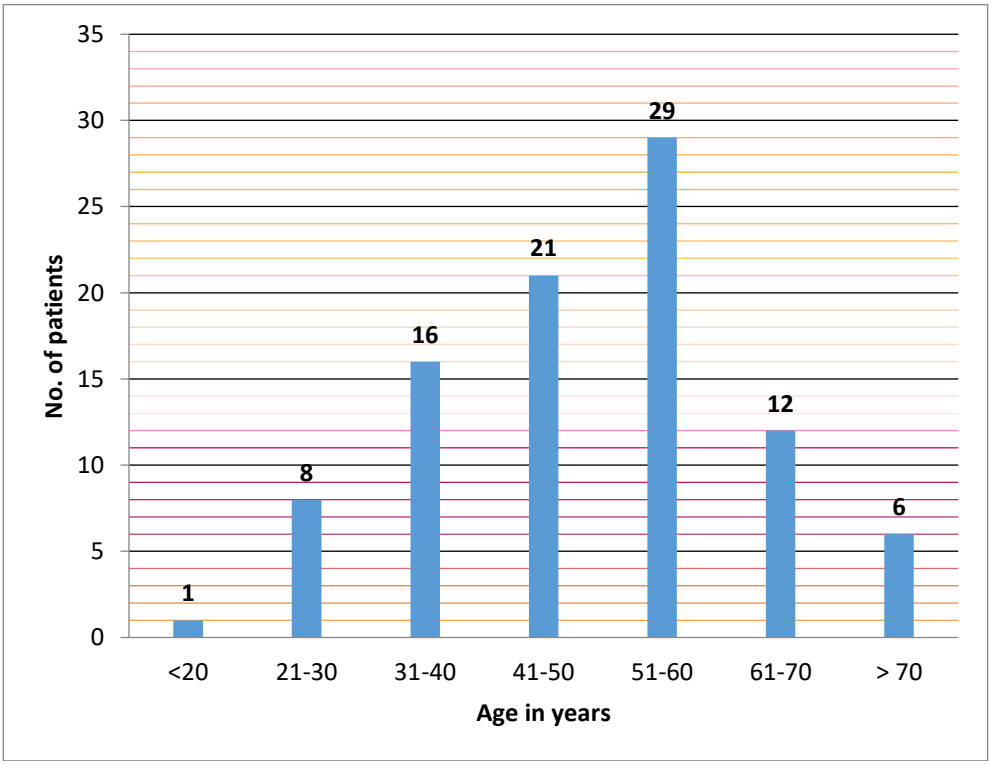

Figure 1: Age wise distribution of patients with culture proven fungal keratitis.

The sex wise distribution of patients with culture proven fungal keratitis is shown in figure 2. Predominance of male gender (61.3%) was noted in the present study.

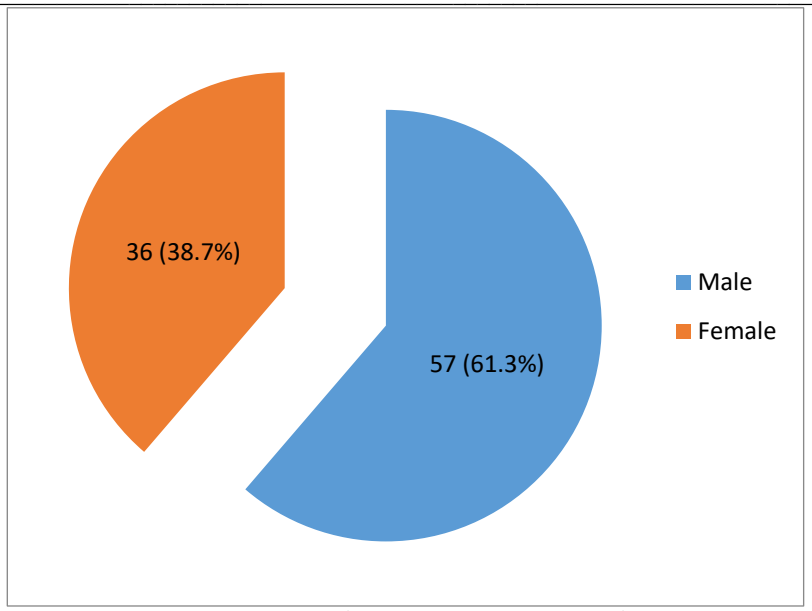

Figure 2: Sex wise distribution of patients with culture proven fungal keratitis.

Occupation distribution of patients with culture proven fungal keratitis is shown table 2. In the present study, majority of patients with culture proven fungal keratitis were farmers (49.5%). A total of 19 patients were manual laborer. Fungal keratitis was significantly high in farmers (Fischer's exact test, P value <0.05) compared to patients with other occupations.

Table 2: Occupation distribution of patients with culture proven fungal keratitis.

Occupation	No. of cases (%)
Farmer	46 (49.5)
Manual laborer	19 (20.4)
Carpenter	14 (15.1)
Homemaker	06 (6.5)
Factory worker	04 (4.3)
Welder	02 (2.2)
Student	01 (1.1)
Teacher	01 (1.1)
Total	**93**

A total of 68 (73.1%) patients with culture proven fungal keratitis belonged to rural area whereas 25 (26.9%) were from urban area. Predisposing factors for fungal keratitis in this study shown in figure 3.

Corneal trauma was significantly associated with fungal keratitis in present study (Chi Square test, *P* value <0.05). In the present study,2 patients with culture proven fungal keratitis had no associated factors. Ocular surgery and contact lens usage, though proven predisposing factors for fungal keratitis, were not seen in this study.

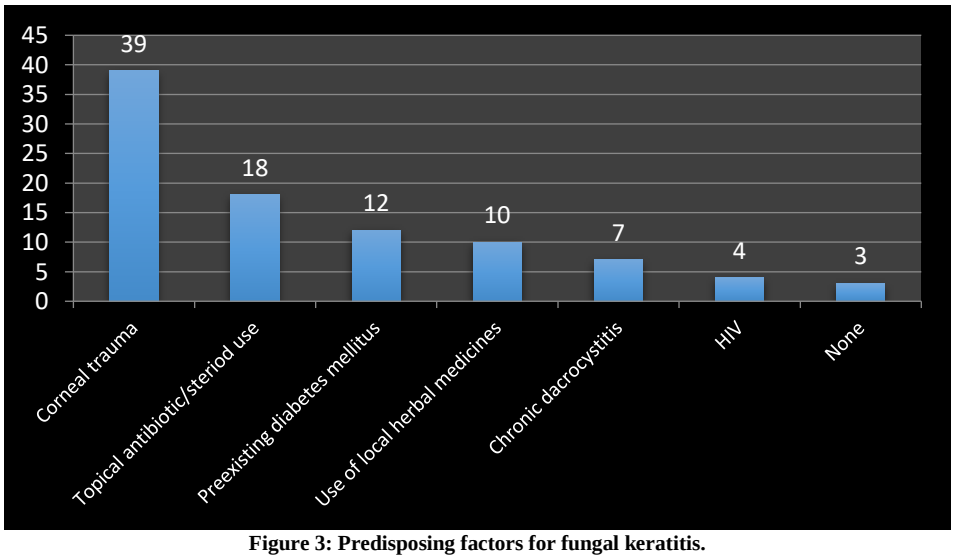

Figure 3: Predisposing factors for fungal keratitis.

Out of 39 cases of trauma, a total of 24 (61.5%) cases had trauma from vegetative matter whereas 15 (38.5%) had trauma due to foreign body injury. Trauma due to vegetative matter was significantly high in patients with culture proven fungal keratitis (Fischer's exact test, *P* value <0.05).

Injury due to sugarcane leaf was significant cause of injury due to organic matter (Fischer's exact test, *P* value <0.05) whereas there was no significant difference between various causes of injury due to foreign body (Fischer's exact test, *P* value >0.05). Table 3 shows the nature of trauma in patients with culture proven fungal keratitis.

Table 3: Nature of trauma in patients with culture proven fungal keratitis.

Nature of trauma	No. of cases (%)
Vegetative matter injury	**24 (61.5)**
Sugarcane leaf	14 (58.3)
Jowar leaf	08 (33.3)
Thorn prick	02 (8.4)
Foreign body injury	**15 (38.5)**
Wooden injury	03 (20)
Stone	03 (20)
Dust	04 (26.7)
Iron piece	01 (6.7)
Buffalo tail	02 (13.3)
Finger nail injury	02 (13.3)
Total	**39**

Discussion

Infectious keratitis may be caused by bacteria, viruses, fungi and parasites[10]. Fungal keratitis is an infection of the cornea by fungi. Keratomycosis, mycotic keratitis and fungal corneal ulcer are various synonyms used to describe this condition[11].

In India, the national program to control blindness has emphasized on treatment of trachoma however in recent years many researchers have underscored the importance of fungal keratitis as the major cause of vision disability[6]. In the present study, diagnosis of fungal keratitis was established in 93 (34.8%) out of the total of 267 suspected cases by positive culture. By considering fungal culture as a reliable method for diagnosis of fungal keratitis, Chowdhary*et al*. (2005)reported the incidence of fungal keratitis as 39%[6]. Deorukhka*et al*. (2012) reported fungal keratitis in 57.9% of clinically suspected cases of keratitis[4].

Direct microscopy of corneal scraping is rapid, simple and cost effective method for diagnosis of infective keratitis[12]. KOH mount, lacto phenol cotton blue preparation, Gram staining, fluorescent staining and special staining (Giemsa, periodic acid Schiff, Gomorimethenamine silver stain) are examples of direct microscopic methods employed for diagnosis of keratitis[13].

The sensitivity of KOHpreparation for diagnosis fungal keratitis has been reported to be 61–94%[14]. In the present study, Gram staining and KOH mount preparation were compared with culture for diagnosis of fungal keratitis. The sensitivity of KOH preparation was 95.7%. Addition of Calcofluor-white with KOH has been reported to increase sensitivity up to 98·3%[14]. Studies on utility of Gram staining for diagnosis of fungal keratitis have reported sensitivity of this technique to range between 36-50%[14]. In the present study, the sensitivity of Gram staining was 72.1%. Although Gram staining is less sensitive for diagnosis of fungal keratitis its utility should be underestimated especially in resource constrained setups[14].

In the present study, out of 93 fungal isolates from keratitis, 72 (77.4%) isolates were filamentous fungi whereas 21(22.6%) were yeast. Fungal keratitis can be caused due to any agent prevalent in air, soil, vegetative matter or water[15]. Globally, filamentous fungi are the major cause of fungal keratitis. *Candida* spp. was the yeast isolated.*Candida* is ubiquitous yeast in the current study. Infection with *Candida* is not linked to environmental factors as infection with filamentous fungi[16].

Predominance of NAC spp. was noted. As compared to other infections there is a dearth of information regarding speciation of *Candida* isolates from corneal ulcer.*C. tropicalis* was the major isolate. Many recent studies have reported increased isolation of *C. tropicalis* from candidemia, oropharyngeal candidiasis and candiduria[17]. The increased isolation of *C. tropicalis*is of great concern because of its ability to develop rapid resistance to fluconazole[17]. *C. krusei* were isolated from 3 cases. This NAC spp. is intrinsically resistant to fluconazole[17,18].

Fungi causing keratitis vary greatly as per different geographic area. However, most Indian studies have reported *Fusarium* spp. and *Aspergillus* spp. as most common filamentous fungi isolatedfrom keratitis cases[4,6]. Similarobservation was noted in the current study. In other developing countries like Nepal, Nigeria and China, *Fusarium* spp. and *Aspergillus* spp. has been the most common fungi isolated from keratitis[5].

Among the *Aspergillus* spp., *A. niger* was the most common isolate. Our observation is in accordance to that of Indian authors likeChowdhary*et al*. (2005)[4] and Deorukhka*et al*. (2012)[6] whereas Ghosh*et al.* (2016)[19] reported *A. flavus* as the common species.

In the present study, a total of 2 species phaeoid fungus was isolated. These included 4 isolates of *Alternaria* spp. and 5 of *Bipolaris* spp. *Alternaria* spp., *Bipolaris* spp., and *Curvularia* spp., are the commonest phaeoid fungi reported from fungal keratitis. Phaeoid fungi are saprophytic in nature and found in woods and decomposing plants[16]. Corneal manifestations with phaeoid fungi usually develop in healthy individuals[16].

In our study, the predominant age group suspected was 51-60 years (31.2%) followed by 41 to 50 years (22.6%) and 31-40 years (17.2%). Maximum number of cases was clustered in 31-60 years age group suggesting this age group is at maximum risk of developing fungal keratitis. This finding was in consistent to other researchers.[20, 21]The age range involved reflects the economically active period of life and hence the increased vulnerability to injury during outdoor activities.

In concordance to many other studies, male predominance was noted[4, 6, 20, 21]. This is may be due to the fact that men are more actively involved in outdoor activities like farming, construction etc., which subsequently increases their vulnerability to keratitis.

Unlike in western countries, wherein fungal keratitis is mostly seen in contact lens users, in the present and many other Indian studies this corneal disease was mainly seen in farmers and laborers. This occupational group is prone for trauma of eye as protective eyewear is rarely used by them.

Corneal trauma inflicted by vegetative matter was the predominant predisposing factor fungal keratitis in the present study. Agricultural material like sugarcane leaf, jowar leaf and thorn was the common cause of corneal trauma. Similar observation was noted by Bharati*et al*. (2003)[8] and Deorukhka*et al*. (2012)[12].

Conclusion

Infective keratitis is an ocular emergency that requires prompt and appropriate management to ensure the best visual outcome for the patient. To minimize ocular morbidity, timely antimicrobial treatment must be initiated on the basis of clinical and microbiological

evaluation. Although fungal culture is considered to be the gold standard for diagnosis of mycotic keratitis, direct microscopic evaluation of smears provides immediate information about the causative organisms. As corneal trauma due to vegetative matter was the predominant predisposing matter, the present study highlights the need to educate our farmers regarding use of protective eyewear while working in farm.

References

1. Bourne R, Stevens G, White R, Smith J, Flaxman S, Price H*et al*. Vision Loss Expert Group. Causes of vision loss worldwide, 1990-2010: a systematic analysis. *Lancet Glob Health* 2013; 1:e339-349.
2. Leck A, Thomas P, Hagan M, Kaliamurthy J, Ackuaku E, John M *et al*. Etiology of suppurative corneal ulcers in Ghana and south India, and epidemiology of fungal keratitis. *Br J Ophthalmol* 2002; 86:1211-1215.
3. Xie L, Zhai H, Zhao J, Sun S, Shi S, Dong X. Antifungal susceptibility for common pathogens of fungal keratitis in Shandong province, China. *Am JOphthalmol* 2008; 146: 260-226.
4. Deorukhkar S, katiyar R, Saini S. Epidemiological features and laboratory results of bacterial and fungal keratitis: A five-year study at a rural tertiary-care hospital in western hospital in western Maharashtra, India. Singap Med J. 2012; 53:264-267.
5. Upadhyay M, Karmacharya P, Koirala S,Tuladhar N, Bryan L, Smolin G*et al*. Epidemiological characteristics, predisposing factors and etiologic diagnosis of corneal ulceration in Nepal. Am J Ophthalmol. 1991;111:92-97.
6. Chowdhary A, Singh K. Spectrum of fungal keratitis in north India. Cornea 2005;24:8-15.
7. Jones D, Liesegang T, Robinson N. Laboratory diagnosis of ocular infections. Washington DC: American Society for Microbiology, 1981.
8. Bharathi M, Ramakrishnan R, Vasu S, Meenakshi R, Palaniappan R. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three year study. *Indian J Ophthalmol.*2003; 51:315-321.
9. Deorukhkar SC, Saini S (2016) Medical device-associated Candida infections in a rural tertiary care teaching hospital of India. *InterdiscipPerspect Infect Dis* 2016: 1854673: 5.
10. Jadhav SV, Gandham NR, Misra RN, Ujagare MT, Sharma M, Sardar. Prevalence of fungal keratitis from tertiary care hospital from western part of India. *Int J Microbiol Res*2012; 4:211-214.
11. Shokohi T, Dailami K, Haghighi T. Fungal keratitis in patients with corneal ulcer in Sari, northern Iran. *Arch Iranian Med* 2006; 9:222- 227.
12. Mahmoudi S, Masoomi A, Ahmadikia K, Rezaie S. Fungal keratitis: An overview of clinical and laboratory aspects. Mycoses. 2018;61:916-930.
13. Badiee P, Nejabat M, Alborzi A, Keshavarz F, Shakiba E. Comparative study of Gram stain, potassium hydroxide smear, culture and nested PCR in the diagnosis of fungal keratitis. Ophthalmic Res 2010; 44: 251-256.
14. Brown L, Leck A, Gichangi M, Burton M, Denning D. The global incidence and diagnosis of fungal keratitis.Lancet Infect Dis 2021; 21: e49-e57.
15. Thomas P. Fungal infections of the cornea. Eye 2003; 17:852-862.
16. Koneman EW, Allen SD, Janada WM, Schrckneberger PC, Winn WC. Colour atlas & Textbook of diagnostic Microbiology. 6th edition. Philadelphiae; Lippincott-raven publisher; 2006.
17. Deorukhkar SC, Saini S, Mathew S, Non-albicans Candida Infection: An emerging threat. *InterdiscipPerspect Infect Dis* 2014, Article ID 615958, 7 pages.
18. Krcmery V, Barnes AJ. Non-albicans Candida spp. causing fungaemia: Pathogenicity and antifungal resistance. J Hosp Infect 2002; 50:243-260.
19. Ghosh A, Gupta A, Rudramurthy S, Paul S, Hallur V, Chakrabarti A. Fungal keratitis in North India: spectrum of agents, risk factors and treatment. Mycopathologia 2016; 181:843-850.
20. Nath R, Baruah S, Saikia L, Devi B, Borthakur A K, Mahanta J. Mycotic corneal ulcers in upper Assam. Indian J Ophthalmol.2011; 59:367-371.
21. Gopinathan U, Garg P, Fernandes M, Sharma S, Athmanathan S, Rao G N. The epidemiological features and laboratory results of fungal keratitis. A 10-year review at a referral eye care center in south India. Cornea.2002; 21(6): 555-559.

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