Journal of Critical Care 51 (2019) 64–70

Contents lists available at ScienceDirect

Journal of Critical Care

journal homepage: www.journals.elsevier.com/journal-of-critical-care

Epidemiology and clinical outcomes of invasive mould infections in Indian intensive care units (FISF study)

Arunaloke Chakrabarti [a,*], Harsimran Kaur [a], Jayanthi Savio [b], Shivaprakash M. Rudramurthy [a], Atul Patel [c], Prakash Shastri [d], Umabala Pamidimukkala [e], Rajiv Karthik [f], Sanjay Bhattacharya [g], Anupma Jyoti Kindo [h], Pradip Bhattacharya [i], Subhash Todi [j], Ram Gopalakrishan [k], Pankaj Singh [a], Abhishek Pandey [a], Ritesh Agarwal [l]

[a] Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India
[b] Department of Microbiology, St John's Medical College, Bengaluru, India
[c] Infectious Diseases Clinic, Sterling Hospital, Ahmedabad, India
[d] Department of Critical Care Medicine, Sir Gangaram Hospital, Delhi, India
[e] Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, India
[f] Department of Infectious Diseases, Christian Medical College, Vellore, India
[g] Department of Microbiology, Tata Medical Center, Kolkata, India
[h] Department of Microbiology, Sri Ramachandra Medical University, Chennai, India
[i] Department of Critical Care Medicine, Chirayu Medical College, Bhopal, India
[j] Department of Critical Care, AMRI Hospital, Kolkata, India
[k] Department of Infectious Diseases, Apollo Hospital, Chennai, India
[l] Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India

ARTICLE INFO

Available online xxxx

Keywords:
Mould infections
Intensive care unit
Aspergillosis
Mucormycosis
Risk factors
Therapy

ABSTRACT

Background and aim: Due to limited data on invasive mould infections (IMIs) in the intensive care units (ICUs) of developing countries, we ascertain epidemiology and management of IMIs at 11 ICUs across India.
Methods: Consecutive patients with proven or probable/putative IMIs were enrolled during the study period. Subjects were categorized into classical (neutropenia, malignancy, transplant recipients on immunosuppression) and non-classical (chronic obstructive pulmonary disease, diabetes, liver disease and glucocorticoids) risk groups. We analyzed the demographic, laboratory variables and outcomes of these patients.
Results: 398 patients with IMIs (96 proven, 302 probable) were identified, amounting to a prevalence of 9.5 cases/1000 ICU admissions. The mean ± SD age of the participants was 45.6 ± 21.9 years. The mean ± SD APACHE II score was 14.3 ± 11.4. The IMIs were diagnosed at a median of 4 days after ICU admission. There were 145 and 253 subjects with classical and non-classical risk groups, respectively. Although *Aspergillus* spp. were the commonest (82.1%) isolates, *Mucorales* were detected in 14.4% subjects. A high APACHE II score and IMI due to mucormycosis were significant predictors of mortality.
Conclusions: The study highlights the distinct epidemiology of IMIs in India ICUs with high burden, new susceptible patient groups and considerable number of non-*Aspergillus* mould infections. [clinicaltrials.gov: NCT02683642].

1. Background

Invasive fungal infections (IFIs) are a major cause of morbidity and mortality in hospitalized critically ill patients [1-3]. Although the exact burden of IFIs in developing countries remains unknown, the rate of IFIs is presumed to be high in intensive care units (ICUs) [4,5]. The environment in developing countries is probably even more conducive for fungal infections. In fact, a recent study reported a high spore count (average of 82 CFU/m^3) in an ICU from India [6].

While *Candida* is the commonest agent causing IFI in patients admitted to the ICUs, invasive mould infections (IMIs) are increasingly being reported. IMIs have not only been reported in those with classical risk

Abbreviations: APACHE II, Acute physiology and chronic health evaluation II; ANOVA, Analysis of variance; BAL, Bronchoalveolar lavage; COPD, Chronic obstructive pulmonary disease; CT, Computed tomography; EORTC-MSG, European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycoses Study Group; FISF, Fungal infection study forum; GM, Galactomannan; HIV, Human immunodeficiency virus; ICD-10, International classification of diseases, Tenth revision; ICU, Intensive care unit; IFI, Invasive fungal infection; IMI, Invasive mould infection; IQR, Interquartile range; ODI, Optical density index; PGIMER, Postgraduate Institute of Medical Education and Research; SD, Standard deviation.

* Corresponding author at: Department of Medical Microbiology, Postgraduate Institute of Medical Education & Research, Chandigarh 160012, India.
E-mail address: arunaloke@hotmail.com (A. Chakrabarti).

https://doi.org/10.1016/j.jcrc.2019.02.005
0883-9441/

factors but also in newer susceptible groups including chronic obstructive pulmonary disease (COPD), diabetes mellitus, chronic liver and kidney diseases and influenza [4,5,7-9]. A recent multicenter retrospective study from five Asian countries reported glucocorticoid use, diabetes mellitus, acute myeloid leukemia and rheumatological conditions as common underlying diseases in patients developing IMIs [9]. The diagnosis of IMI in ICU remains a challenge due to the difficulty in obtaining tissue samples in critically ill patients. Thoracic imaging has limitations in non-neutropenic patients due to the absence of classical radiological presentation (halo and air crescent signs) [10,11]. Moreover, the mere presence of fungi in respiratory tract does not distinguish between colonization and invasion. Therefore, a large number (60%) of patients with invasive aspergillosis were diagnosed only on autopsy due to the lack of reliable diagnostic tests [12]. There is little data on IMIs from developing countries including India. We had previously published our experience with candidemia in Indian ICUs, which brought out several unique epidemiological determinants of the disease [8]. Herein, we report our multicenter experience with the epidemiology, risk factors and outcomes of IMIs in Indian ICUs.

2. Methods

This was a prospective observational study conducted between April 2016 and September 2017 in the ICUs of 11 tertiary care centers across India (Fig. 1). The study protocol was approved by the Institutional Ethics Review Board of the individual centers. An informed consent was taken from the patient or their relatives as applicable. The study was registered at clinicaltrials.gov (NCT02683642).

2.1. Study sites

The sites were selected based on a survey of tertiary care hospitals in India, and had to meet the following criteria: (a) willingness to participate; (b) use of International Classification of Diseases, 10th revision (ICD 10) coding; (c) access to high-resolution computed tomography (CT) scan; (d) mycology laboratory performing galactomannan (GM) detection; and, (e) facilities for histopathology.

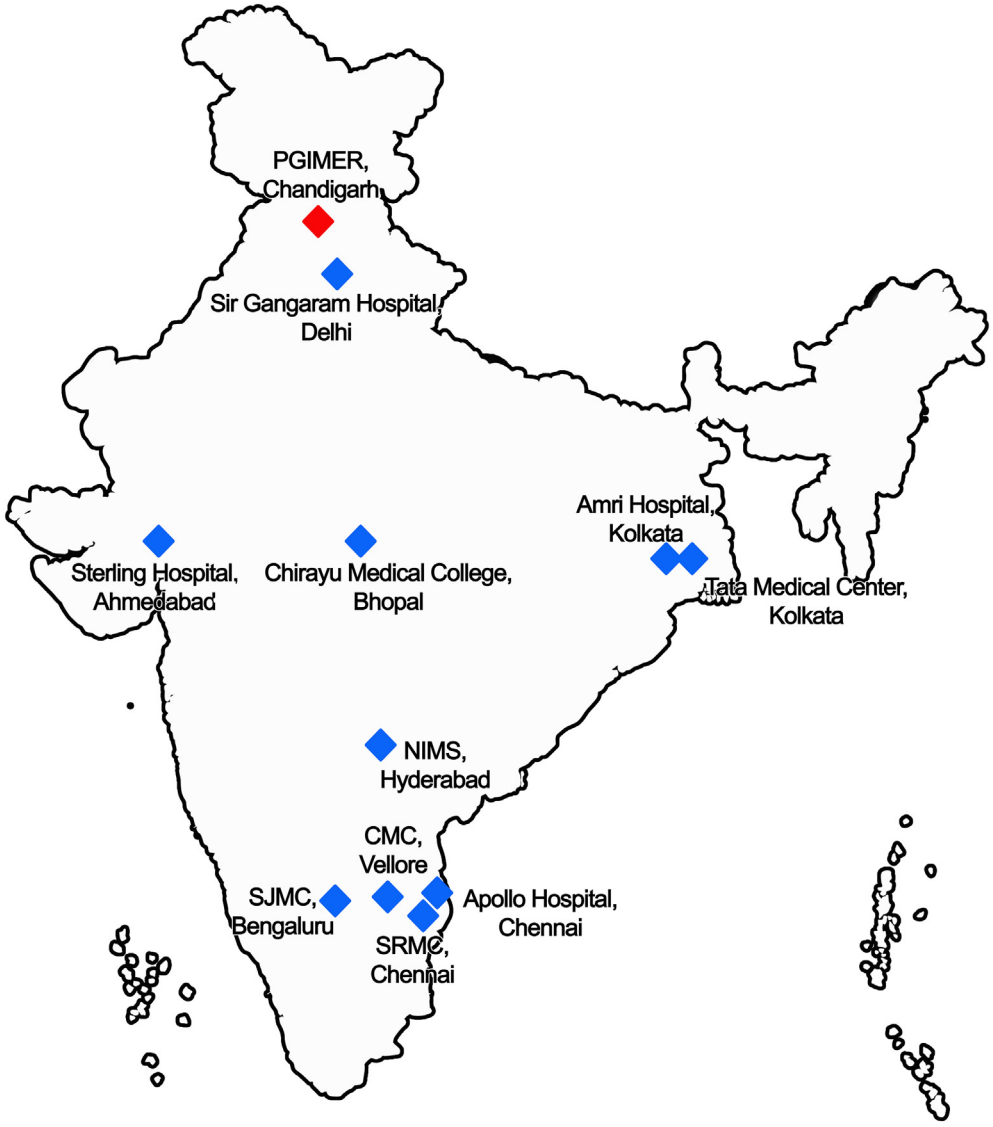

Fig. 1. Map of India depicting the 11 centers participated in the study. Red diamond represents the coordinating centre. *CMC* Christian Medical College; *NIMS* Nizam's Institute of Medical Sciences; *PGIMER* Postgraduate Institute of Medical Education and Research; *SJMC* St John's Medical College; *SRMC* Sri Ramachandra Medical College. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Study procedure

Demographic information such as age, gender, comorbid illness and clinical status at admission to the ICU were recorded. The severity of the underlying illness was scored using the Acute Physiology and Chronic Health Evaluation (APACHE) II score. All consecutive subjects in ICUs were screened for enrolment and subjects with a diagnosis of IMIs were enrolled. Subjects with isolated dimorphic fungal disease or yeast infections or allergic fungal disease were excluded from enrolment. IMI was defined based on the EORTC-MSG criteria in the classical immunosuppressed subjects [13], the criteria proposed by Bulpa et al. in subjects with COPD [14], and the criteria proposed by Vandewoude et al. in non-COPD subjects admitted to the ICU [15]. Briefly, a "proven" IMI was defined by the presence of septate or aseptate hyphae in the biopsied sample from deep tissue or isolation of mould from sterile sites. "Probable or putative" IMI was defined based on the host factors, radiological findings and demonstration of the fungi or its components (by either culture or cytology or galactomannan). For the current study, we defined the classical risk factors as any of the following: (i) neutropenic subjects with absolute neutrophil count <500 cells/μL (ii) subjects on cytotoxic chemotherapy for malignancies; (iii) immunosuppressive therapy for solid organ transplantation; and, (iv) hematopoietic stem cell transplantation. All the remaining conditions including diabetes mellitus, COPD, chronic liver and kidney diseases, influenza, sepsis, and glucocorticoid use >0.3 mg/kg/day of prednisolone (or equivalent) for at least three weeks were taken as non-classic risk factors. A diagnosis of IMI in the non-classical group was made using the criteria proposed by Vandewoude et al. or Bulpa et al. depending on absence or presence of COPD respectively [14,15].

2.3. Data collection

The demographic, clinical, treatment and outcome data for each patient were captured in a standard proforma designed for the study (supplementary file proforma I, II, III). The data collected from all the participating centers were analyzed at the coordinating centre (Postgraduate Institute of Medical Education and Research, Chandigarh, India).

2.4. Microbiological methods

The identity of the isolates from subjects with IMIs was confirmed at the coordinating center initially using conventional methods, and finally by sequencing of conserved regions for all isolates. If the diagnosis of mould infection was by histopathology alone, an attempt was made to identify the fungus by extraction of DNA from tissue and sequencing [16]. The detection of galactomannan in serum or bronchoalveolar lavage fluid (BAL) was performed as a part of the routine investigation at the participating centers. The test was considered positive when the serum and BAL optical density index (ODI) values were >0.5 and >0.8, respectively [17].

2.5. Study outcomes

The study outcomes included the prevalence of IMI in the ICU defined as the number of IMIs as a proportion of the total ICU admissions. We also compared the clinical and radiological parameters between those with classical and non-classical risk factors for IMI. We recorded the 42-day and 84-day hospital mortality; and, analyzed the factors predicting mortality in subjects with IMI.

2.6. Statistical analysis

Data are presented as frequencies, mean with standard deviation (or 95% confidence intervals [CI]), and median with interquartile range. The differences between categorical variables were determined by Pearson's chi square test (or Fischer's exact-test). The differences between continuous data were analyzed using student t-test, Mann-Whitney *U* test and analysis of variance (ANOVA), as applicable. Survival curves were constructed to study the effect of classic vs. non-classic risk factors on the time to hospital mortality using Kaplan-Meier analysis, and the group differences were analyzed using the log-rank test. Multivariate logistic regression was performed for identifying factors predicting mortality. Two tailed p values ≤.05 were taken as significant.

3. Results

During the study period, 41,879 subjects were admitted in ICU at the 11 participating centres. 398 cases (proven 96, probable 302) of IMI were diagnosed, amounting to a prevalence of 9.5 (range, 4.3–29.0) cases per 1000 ICU admissions. The mean ± SD age of the study participants (64% males) was 45.6 ± 21.9 years (Table 1). The mean ± SD APACHE II score was 14.3 ± 11.4. The classical and non-classical risk factor groups with IMI consisted of 145 (36.4%) and 253 (63.6%) subjects, respectively (Table 1). The diagnosis of IMIs was made at a median (IQR) duration of 4 [1-7] days after ICU admission. Underlying diseases were noted in 356 (89.4%) subjects; one risk factor in 108 (30.3%), two risk factors in 126 (35.4%), three risk factors in 77 (21.6%), and four or more risk factors in 45 (12.6%) subjects. The distribution of various illnesses in those with classic and non-classic risk factors is shown in Table 1. High-resolution CT scan of the thorax was performed in 375 (94.2%) subjects (Table 1). The most common radiological finding on CT chest was consolidation followed by nodule and pleural effusion. The occurrence of nodules was significantly higher in those with classic risk factors. The involvement of paranasal sinuses on MRI was seen in 56 subjects and was significantly higher in the non-classic risk group (17 vs. 9%, $p = .027$). Brain involvement was however similar in the two groups.

3.1. Site of mould infection, diagnosis and microbiology

The clear majority ($n = 321$, 80.7%) of the subjects had pulmonary disease. Other sites involved were rhino-orbito-cerebral (7.5%), restricted to sinuses only (5.8%), subcutaneous tissue (2%), central nervous system (1.3%), kidney (1%), gastrointestinal tract (0.8%), spleen (0.5%) and heart (0. 3%). The probable cases were diagnosed based on EORTC-MSG criteria in 145 (48.1%), Bulpa et al. criteria in 28 (9.3%) and Vandewoude et al. criteria in 129 (42.7%) subjects. Serum and bronchoalveolar lavage fluid galactomannan were performed in 242 (60.8%) and 56 subjects, respectively, at the time of diagnosis. In 31 subjects, both serum and bronchoalveolar lavage fluid galactomannan were performed at the time of diagnosis.

Moulds were isolated on culture in 190 (47.7%) subjects and visualized only on direct microscopy/ histopathology in 44 cases (Table 2). A single mould species was encountered in 173 (43.4%) subjects and more than one mould from 17 (4.3%) subjects. *Aspergillus* were the commonest (82.1%) species isolated followed by *Mucorales* (14.4%). Three of the eight cases, where molecular technique was utilized to identify the fungus from paraffin block, revealed *Aspergillus* spp. (2 *A. flavus,* 1 *A. fumigatus*); fungal DNA could not be extracted from the paraffin blocks of five subjects. *Mucorales* were the common isolates (66.7%) in the proven group. Among the 44 cases diagnosed only on direct microscopy/histopathology, 29 had aseptate hyphae (indicating *Mucorales*).

3.2. Treatment

Antifungal therapy (empiric and targeted) was provided to 321 (80.6%) subjects, 61 subjects died before the confirmation of diagnosis, and 16 subjects with probable mould infection recovered without any antifungal therapy. The details of antifungal therapy are provided in

Table 1
The comparison of clinical and radiological parameters between classical and non-classical risk factors for IMI.

Factor	Total	Classical Immunosuppressed ($n = 145$)	Non-classical group ($n = 253$)	p value
Age (years) (mean ± SD)	43.5 ± 21.5	39.8 ± 20.2	45.6 ± 21.9	0.01
Days from hospital admission to diagnosis (median)	6	8	5	0.002
APACHE II (mean ± SD)	14.1 ± 11.4	13.65 ± 11.5	14.28 ± 11.4	0.60
Days of treatment after diagnosis (Median)	4	4	4	0.78
Diabetes mellitus	115 (28.9)	30 (20.7)	85 (33.6)	0.006
Respiratory disease	73 (18.3)	9 (6.2)	64 (25.3)	0.001
Chronic obstructive pulmonary disease (COPD)	46 (11.6)	3 (2.1)	43 (17)	0.001
Asthma	12 (3)	2 (1.4)	10 (4)	0.13
H1N1 influenza	12 (3)	2 (1.4)	10 (4)	0.13
Interstitial lung disease	10 (2.5)	1 (0.7)	9 (3.6)	0.07
Renal Disease	90 (22.6)	30 (20.7)	60 (23.7)	0.49
Chronic kidney disease	48 (12.1)	20 (13.8)	28 (11.1)	0.42
Acute kidney disease	42 (10.6)	10 (6.9)	32 (12.6)	0.07
Liver disease	28 (7)	11 (7.6)	17 (6.7)	0.75
Chronic liver disease	17 (4.3)	7 (4.8)	10 (4)	0.68
Carditis	13 (3.3)	5 (3.4)	8 (3.2)	0.55
Cerebrovascular accident	11 (2.8)	3 (2.1)	8 (3.2)	0.52
Steroids	190 (47.7)	72 (49.7)	118 (46.6)	0.56
Ventilator use	189 (47.5)	65 (44.8)	124 (49)	0.42
Broad spectrum antibiotics	340 (85.4)	129 (89)	211 (83.4)	0.42
Dialysis	62 (15.6)	19 (13.1)	43 (17)	0.30
Pulmonary presentation	321 (80.7)	125 (86.2)	196 (77.5)	0.03
Pulmonary mucormycosis	17 (11.2)	9 (19.6)	8 (7.5)	0.03
Pulmonary aspergillosis	135 (88.8)	37 (80.4)	98 (92.5)	0.03
Rhino-orbital mucormycosis	29 (7.3)	4 (2.8)	25 (9.9)	0.009
Radiology (High resolution CT chest imaging)				
Nodule	118 (29.6)	57 (39.3)	61 (24.1)	0.001
Halo sign	30 (7.5)	14 (9.7)	16 (6.3)	0.23
Air crescent	6 (1.5)	4 (2.8)	2 (0.8)	0.13
Cavity	51 (12.8)	22 (15.2)	29 (11.5)	0.29
Consolidation	185 (46.5)	68 (46.9)	117 (46.2)	0.90
Pleural effusion	94 (23.6)	33 (22.8)	61 (24.1)	0.76
MRI Paranasal Sinuses: Sinusitis	56 (14.1)	13 (9)	43 (17)	0.03
MRI Brain: Brain involvement	43 (10.8)	11 (7.6)	32 (12.6)	0.12
Mortality	268 (67.3)	90 (62.1)	178 (70.4)	0.09

SD standard deviation, *APACHE* acute physiology and chronic health evaluation, *CT* computed tomography, *MRI* magnetic resonance imaging.

Supplemental Table 1. Fourteen subjects had received antifungal prophylaxis (5 developed proven and 9 probable mould infections). The infection developed after a mean of 14.1 days of prophylaxis. Empirical antifungal therapy was prescribed in 29 subjects; the antifungal agent was modified in 13 subjects after diagnosis, 12 subjects died before diagnosis and four subjects with probable mould infection recovered without change of therapy.

3.3. Outcome and mortality predictors

Overall, 42-day and 84-day mortality was 64.8% and 65.8%, respectively. The time to hospital mortality was significantly lower (log rank chi-square 4.2, $p = .04$) in those with non-classic risk factors (median [95% CI], 21 [17.6–24.4] days) compared to those with classic risk factors (median [95% CI], 26 [19.5–32.5] days) (Fig. 2). On a multivariate logistic regression analysis, high APACHE II score and IMI due to mucormycosis were predictors of mortality after adjusting for the type of risk factor (classic vs. non-classic), the need for dialysis and the time from ICU admission to the diagnosis of IMI (Table 3).

4. Discussion

The current study found a prevalence of 9.5 cases of IMI per 1000 ICU admissions in Indian ICUs. The majority (63.6%) of the IMIs were in the non-classical risk group including COPD, renal disease, diabetes mellitus, liver disease, and recent H1N1 influenza. Though *Aspergillus* species were the commonest (82.1%) mould isolated, *Mucorales* were isolated from a considerable number (14.4%) of subjects. The mortality remained very high (64.8% at 42-day; 65.8% at 84-day). A high APACHE II score and the occurrence of mucormycosis were independent predictors of mortality in subjects with IMI.

The incidence of IMI is high in patients with hematological malignancies, stem cell and solid organ transplant recipients [18,19]. However, in recent years, IMI especially invasive aspergillosis is increasingly being reported in patients with COPD, liver failure, cirrhosis, and recent H1N1 influenza [1,20]. The prevalence of invasive pulmonary aspergillosis in ICU patients has been reported between 0.3% and 19% [7,10]. Understandably, the case mix would have profound impact on the epidemiology of IMI. In a surveillance of 18 ICUs in Italy, only 12 (0.2%) cases with invasive aspergillosis were reported in 5561 patients [21], whereas from a single ICU at Belgium, 127 (7%) cases of invasive aspergillosis were reported [11]. Another important factor for varying prevalence could be the diagnostic criteria used for identifying IMIs. The definitions proposed by EORTC-MSG, are primarily for classical immunosuppressed patients, and cannot be generally extrapolated to the emerging non-classical groups [13]. In fact, a few authors have proposed a clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients [14,15,22]. We diagnosed 302 probable/putative cases of IMI using either of the three (EORTC-MSG/Bulpa et al./Vanderwoude et al.) diagnostic algorithm in the respective patient groups [13-15]. However, the clinical algorithms in non-classical risk group would require further refinement and validation [14,15]. Interestingly, 16 of our probable/putative cases recovered without antifungal therapy, attesting to the poor specificity (61%) of these criteria [22].

Among the moulds causing invasive infection in ICU, attention is focused mainly on *Aspergillus* spp. [1,7] However, fungi other than *Aspergillus* are gaining importance in certain geographical locations. For example, *Fusarium* in Brazil and United States [23], *Scedosporium* in

Table 2
Summary of the results of direct microscopy and fungal isolation of 234 cases (single isolate in 173 subjects, multiple isolates in 17 subjects; and only direct microscopy positive in 44 subjects).

Fungal species isolated (total no. of subjects = 190)		Number of subjects
Aspergillus spp.		142
	A. flavus	67
	A. fumigatus	56
	A. terreus	8
	A. niger	6
	Unidentified *Aspergillus* species	5
Mucorales		25
Rhizopus spp.		19
	R. arrhizus	11
	R. microsporus	1
	Unidentified *Rhizopus* species	7
Mucor spp.		4
Apophysomyces variabilis		2
Fusarium spp.		4
Curvularia lunata		1
Pythium insidiosum		1
Multiple isolates		17
	A. flavus, A. niger	2
	Multiple *Aspergillus* species (not identified)	3
	A. flavus, A. fumigatus	5
	A. flavus, A. terreus	1
	A. flavus, A. terreus, and aseptate hyphae on smear	1
	Aspergillus spp., *Rhizopus* spp.	1
	A. fumigatus, R. arrhizus	1
	A. flavus and aseptate hyphae on smear	1
	A. fumigatus, Mucor spp.	1
	Fusarium solani, A. flavus	1
Only direct microscopy positive (no. of subjects = 44)	Aseptate hyphae	25
	Septate hyphae	15
	Septate + aseptate	4

spp Species.

Spain and Australia [24], and *Mucorales* in India [25]. Even in the present study, although *Aspergillus* species were the commonest isolates, *Mucorales* were isolated in 14.4% cases, and *Fusarium* species in 2.3%

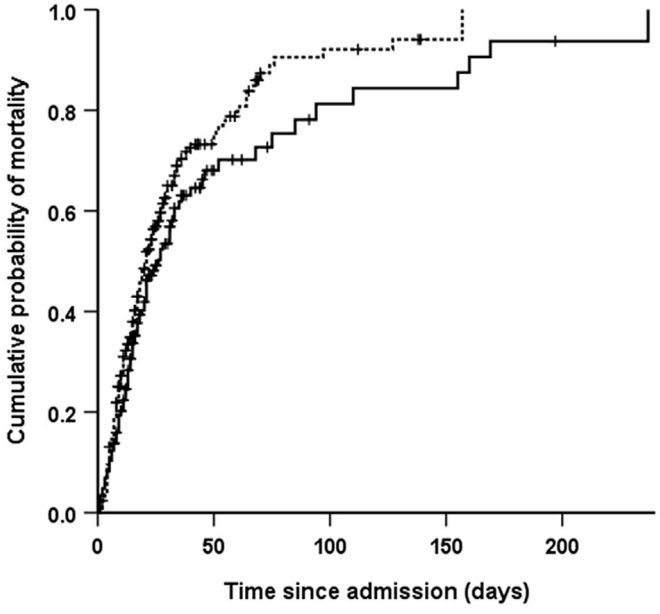

Fig. 2. Time to hospital mortality in subjects with non-classic risk factors (solid line) versus classic risk factors (dotted line) in subjects with IMI. The time to death was significantly lower in those with non-classic risk factors.

Table 3
Univariate and multivariate logistic regression for prediction of mortality.

Variable	Crude odds ratio (95% Confidence interval)	Adjusted odds ratio (95% Confidence interval)	p value
APACHE II score	1.04 (1.02–1.06)	1.05 (1.02–1.09)	0.001
Classical immunosuppression	0.69 (0.45–1.06)	1.20 (0.60–2.43)	0.61
Dialysis	1.63 (0.87–3.04)	1.99 (0.73–5.43)	0.18
Aspergillosis or mucormycosis	1.78 (0.86–3.68)	2.33 (1.05–5.14)	0.03
Days from ICU admission to diagnosis	0.99 (0.99–1.01)	0.99 (0.97–1.02)	0.77

APACHE acute physiology and chronic health evaluation.

cases. The number of mucormycosis cases may be even higher, as aseptate hyphae were detected in 29 of 44 cases that were diagnosed only by direct microscopy. Thus, identification of the fungus is important to choose proper antifungal therapy.

The present study also confirms the emergence of new susceptible groups like COPD, diabetes, liver failure and chronic liver disease in India for the development of IMIs in the ICU [1,7,10]. Glucocorticoid use, mechanical ventilation, and dialysis were important risk factors in 47.7%, 47.5%, and 15.6% patients, respectively. In a large cohort of 6424 patients with invasive aspergillosis in the ICU in the United States, steroid use was noted in 77% patients, renal failure in 41%, COPD in 37% and septicemia in 36% patients [26]. Similarly, in a prospective Italian study, steroid use was the commonest (43.9%) risk factor for invasive aspergillosis [27]. In the present study, 12 patients acquired the invasive mould infection after H1N1 influenza infection. This is a new emerging susceptible group for invasive aspergillosis [1,28-30]. The findings of newer risk factors in the present study and the available literature stress the need for continuous surveillance of IMIs in ICU to identify new risk groups.

Despite newer diagnostic modalities and treatment strategies [1,7,10,20], the overall mortality of IMIs in ICU remains high (40–90%) [1,30,31]. In the present study, the overall 42-day (64.8%) and 84-day (65.8%) mortality was high, despite the fact that 76.6% of the patients received targeted antifungal therapy. In critically ill patients, it is difficult to determine whether mortality was attributable to fungi or the comorbidities of the patients. We found high APACHE II score and mucormycosis as significant risk factors for mortality. Our population cohort was relatively young, evidenced by a mean age of 45.8 ± 20.7 years and lower APACHE II score of 14.1 ± 11.4. In contrast, other studies reported invasive aspergillosis in those with APACHE II score >20 [20,32]. However, the APACHE II score depends on the age, and the lower age of our study cohort might have lowered the score. Another matter of concern from this study is that the patients suffered from IMIs in early days of ICU stay (median, 4 days). The early occurrence of IMI, however, did not affect the outcome on a multivariate analysis.

Finally, our study is not without limitations. We collected data only for patients with IMIs and not for those without IMI. Thus, we were unable to calculate the attributable risk for mortality due to IMIs, which would have been a novel result from the current study. We also do not have the ICU severity scores for patient during the illness and are thus unable to assess the contribution of new organ dysfunction towards ICU mortality [33]. The strength of the study includes the fact that this is the first prospective study evaluating the epidemiology of IMIs in critically ill patients in India. Also, our findings may be generalizable to other developing countries as well, as the environment of ICUs is generally similar in those countries.

In conclusion, the current study highlights a high prevalence, new susceptible groups, and a considerable number of non-*Aspergillus* moulds causing IMIs. The study results suggest that comparatively young patients with few co-morbidities may suffer from IMIs within

the early days of ICU stay in India. Finally, IMI due to *Mucorales* is a significant predictor of mortality.

Acknowledgements

We wish to acknowledge Fungal Infection Study Forum (FISF) for providing us invaluable logistic support to accomplish the study. Other members of the FISF Invasive mould infections in Indian ICUs Network include: Arpita Bhakta, Mahua Bhattacharya (AMRI Hospital, Kolkata, India); Madhumita R, Ramasubramanian V (Apollo Hospital, Chennai, India); Deepak Mendiratta (Chirayu Medical College, Bhopal, India); Joy Michael (Christian Medical College, Vellore, India); Ramchandran Gopinath, Paramjyothi GK, M V S Subalaxmi, Shantiveer G Uppin (Nizam's Institute of Medical Sciences, Hyderabad, India), L N Yaddanapudi, Praveen Kumar, Jayashree M (Postgraduate Institute of Medical Education and Research, Chandigarh, India); Ashok Anand, Chand Wattal, Jaswinder Oberoi (Sir Gangaram Hospital, Delhi, India); S Arunkumar, M K Renuka, T Dhansekar (Sri Ramchandra Medical Universtiy, Chennai, India); Sriram Sampath, Priya Ramachandran, Poonam Panjwani (St John Medical College, Bengaluru, India); Mukesh Patel, Kamalesh Patel (Sterling Hospital, Ahmedabad, India); Kingshuk Dhar, Parijat Das, Gaurav Goel, Sudipta Mukherjee (Tata Medical Center, Kolkata, India) are also acknowledged for their continuous support and help to conduct the study.

Funding

The work was supported by the Educational Grant of MSD Pharmaceuticals Pvt. Ltd. through Fungal Infection Study Forum, a non-profit scientific trust in India. MSD did not play any role in the study design, data collection, data analysis or writing of the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Study conception and design: AC. Data collection: all authors contributed in data collection, Analysis and interpretation: AC, HK, RA, SMR analyzed the data, then sent to all authors who approved it. Writing the manuscript: AC. Critical revision: All authors read and approved the final manuscript.

Ethics approval and consent to participate

This observational study was conducted according to the terms and regulations of the local institutional review boards. Ethical approval was obtained separately from each Institute. Waiver for informed consent was received from each institutional review board, as the study was observational only and did not modify the physician's treatment decisions. Moreover, identity of each individual was anonymized with unique number at respective number. The Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India was the coordinating centre.

Consent for publication

While taking ethical clearance, consent of publication was received from each Institutional Review Board with following guideline. In the event of publication, the order of authorship will be according to the number of cases enrolled in the study. All the co-investigators of this study will be cited in the appendix of each publication. The sponsor of the study will be acknowledged in each publication. None of the sites is permitted to make any publication on its site's data, based solely on the parameters included in this study for the cases included in the present study. If there is any breach of this understanding, the data from that center will not be included in the final data analysis.

Competing interests

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcrc.2019.02.005.

References

[1] Bassetti M, Garnacho-Montero J, Calandra T, et al. Intensive care medicine research agenda on invasive fungal infection in critically ill patients. Intensive Care Med 2017;43:1225–38.
[2] Paramythiotou E, Frantzeskaki F, Flevari A, et al. Invasive fungal infections in the ICU: how to approach, how to treat. Molecules 2014;19:1085–119.
[3] Trof RJ, Beishuizen A, Debets-Ossenkopp YJ, et al. Management of invasive pulmonary aspergillosis in non-neutropenic critically ill patients. Intensive Care Med 2007;33:1694–703.
[4] Chakrabarti A, Singh R. The emerging epidemiology of mould infections in developing countries. Curr Opin Infect Dis 2011;24:521–6.
[5] Chakrabarti A, Chatterjee SS, Das A, et al. Invasive aspergillosis in developing countries. Med Mycol 2011;49(Suppl. 1):S35–47.
[6] Rudramurthy SM, Singh G, Hallur V, et al. High fungal spore burden with predominance of *Aspergillus* in hospital air of a tertiary care hospital in Chandigarh. Indian J Med Microbiol 2016;34:529–32.
[7] Bassetti M, Bouza E. Invasive mould infections in the ICU setting: complexities and solutions. J Antimicrob Chemother 2017;72:i39–47.
[8] Chakrabarti A, Sood P, Rudramurthy SM, et al. Incidence, characteristics and outcome of ICU-acquired candidemia in India. Intensive Care Med 2015;41:285–95.
[9] Rotjanapan P, Chen YC, Chakrabarti A, et al. Epidemiology and clinical characteristics of invasive mould infections: a multicenter, retrospective analysis in five Asian countries. Med Mycol 2018;56:186–96.
[10] Taccone FS, Van den Abeele AM, Bulpa P, et al. Epidemiology of invasive aspergillosis in critically ill patients: clinical presentation, underlying conditions, and outcomes. Crit Care 2015;19(7).
[11] Meersseman W, Vandecasteele SJ, Wilmer A, et al. Invasive aspergillosis in critically ill patients without malignancy. Am J Respir Crit Care Med 2004;170:621–5.
[12] Garnacho-Montero J, Amaya-Villar R, Ortiz-Leyba C, et al. Isolation of *Aspergillus* spp. from the respiratory tract in critically ill patients: risk factors, clinical presentation and outcome. Crit Care 2005;9:R191–9.
[13] De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 2008;46:1813–21.
[14] Bulpa P. Dive a, Sibille Y: Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease. Eur Respir J 2007;30:782–800.
[15] Vandewoude KH, Blot SI, Depuydt P, et al. Clinical relevance of *Aspergillus* isolation from respiratory tract samples in critically ill patients. Crit Care 2006;10:R31.
[16] Zaman K, Rudramurthy SM, Das A, et al. Molecular diagnosis of rhino-orbito-cerebral mucormycosis from fresh tissue samples. J Med Microbiol 2017;66:1124–9.
[17] D'Haese J, Theunissen K, Vermeulen E, et al. Detection of galactomannan in bronchoalveolar lavage fluid samples of patients at risk for invasive pulmonary aspergillosis: analytical and clinical validity. J Clin Microbiol 2012;50:1258–63.
[18] Pergam SA. Fungal pneumonia in patients with hematologic malignancies and hematopoietic cell transplantation. Clin Chest Med 2017;38:279–94.
[19] Eriksson M, Lemstrom K, Suojaranta-Ylinen R, et al. Control of early *Aspergillus* mortality after lung transplantation: outcome and risk factors. Transplant Proc 2010;42:4459–64.
[20] Garnacho-Montero J, Olaechea P, Alvarez-Lerma F, et al. Epidemiology, diagnosis and treatment of fungal respiratory infections in the critically ill patient. Rev Esp Quimioter 2013;26:173–88.
[21] Montagna MT, Caggiano G, Lovero G, et al. Epidemiology of invasive fungal infections in the intensive care unit: results of a multicenter Italian survey (AURORA Project). Infection 2013;41:645–53.
[22] Blot SI, Taccone FS, Van den Abeele AM, et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. Am J Respir Crit Care Med 2012;186:56–64.
[23] Nucci F, Nouer SA, Capone D, et al. Fusariosis. Semin Respir Crit Care Med 2015;36:706–14.
[24] Rodriguez-Tudela JL, Berenguer J, Guarro J, et al. Epidemiology and outcome of *Scedosporium prolificans* infection, a review of 162 cases. Med Mycol 2009;47:359–70.
[25] Chakrabarti A, Singh R. Mucormycosis in India: unique features. Mycoses 2014;57(Suppl. 3):85–90.
[26] Baddley JW, Stephens JM, Ji X, et al. Aspergillosis in Intensive Care Unit (ICU) patients: epidemiology and economic outcomes. BMC Infect Dis 2013;13:29.

[27] Tortorano AM, Dho G, Prigitano A, et al. Invasive fungal infections in the intensive care unit: a multicentre, prospective, observational study in Italy (2006-2008). Mycoses 2012;55:73–9.
[28] Shah MM, Hsiao EI, Kirsch CM, et al. Invasive pulmonary aspergillosis and influenza co-infection in immunocompetent hosts: case reports and review of the literature. Diagn Microbiol Infect Dis 2018;91:147–52.
[29] van de Veerdonk FL, Kolwijck E, Lestrade PP, et al. Influenza-associated aspergillosis in critically Ill patients. Am J Respir Crit Care Med 2017;196:524–7.
[30] Wauters J, Baar I, Meersseman P, et al. Invasive pulmonary aspergillosis is a frequent complication of critically ill H1N1 patients: a retrospective study. Intensive Care Med 2012;38:1761–8.
[31] Hatipoglu N, Hatipoglu H. Combination antifungal therapy for invasive fungal infections in children and adults. Expert Rev Anti Infect Ther 2013;11:523–35.
[32] Khasawneh F, Mohamad T, Moughrabieh MK, et al. Isolation of *Aspergillus* in critically ill patients: a potential marker of poor outcome. J Crit Care 2006; 21:322–7.
[33] Agarwal R, Aggarwal AN, Gupta D, et al. Etiology and outcomes of pulmonary and extrapulmonary acute lung injury/ARDS in a respiratory ICU in North India. Chest 2006;130:724–9.