INVESTIGATION OF OCULAR CANDIDIASIS IN QUARRY MINING SITE OF ISHIAGU, IVO LOCAL GOVERNMENT AREA OF EBONYI STATE, NIGERIA

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ABSTRACT

Background: Among the various fungal diseases of the eye, candidiasis remains a major one with potentially sight – threatening complications which could result in either partial or complete loss of vision.

Aim: The present study investigated the effects of quarry mining activities in the cause of ocular candidiasis.

Methodology: Standard experimental survey and analysis were conducted on both quarry and non-quarry miners of age limits > 15years. A total of 230 subjects (130 male and 100 female) were tested in this study. Schimer paper was used to collect tears which were subjected to culture, re-culture, characterization, isolation and plasmid profiling.

Results: The result revealed a prevalence of 26.52% of fungal ocular infection among the tested persons. The following isolated Candida species; *C. albican, C. tropicalis, C. stellatoidea* and *C. utilis* and some other fungal agents including *Rhodotorula rubra*, *Fusarium* sp., *Aspergillus* sp. and *Penicillum* sp were significantly ($p<0.05$) higher in the male compared to the female subjects. The age limits; 26-30 and 36- 40 years were mostly affected, while the least affected persons were < 20 years. The results obtained from occupational disposition of the examined subjects revealed highest ocular candidiasis in quarry workers followed by farmers and learned people. There were significant increase ($p<0.05$) in physical features and signs such as pains, hyperemia (redness), lid edema, allergy, keratitis, retinal haemorrhage and ocular discharge in males compared to females. In the susceptibility study with antifungal agents, Amphotericin B revealed 100% efficacy to all the fungal isolates, while fluconazole, ketoconazole and voriconazole showed only 20% resistance to some fungal species which became completely susceptible after plasmid curing.

Conclusion: This study has shown that quarrying activities have the potentials of inducing ocular candidiasis which are often contracted through fungal agents in suspended dust particles.

Keywords: Quarry mining, Ocular infection, Candidiasis, Retinal haemorrhage, Antifungals

INTRODUCTION

Several anthropogenic activities aimed at socio-economic development of man and his communities have been implicated in ocular infections, especially those that cause dust production resulting in microbial aerosols and distortion of air quality (Nwaugo *et al*., 2008). Quarry mining is economically essential because of its daily use for house, road and bridge construction including other construction projects. Environmental/air pollution is highly associated to mining related industries in the world (Ali, 2014; Landrigan *et al*., 2018), more especially in the developing countries such as Nigeria, where regulations guiding mining are not available and/or not followed strictly.

Pollutions as a result of quarrying process may occur during drilling, excavation, blasting of heavy underground stone beds, crushing, removal of dust and other wastes (Zhang *et al*., 2012). Pollution from quarrying may also occur via transportation from excavation to crushing site or from crushing site to the end user (construction site) (Zhang *et al*., 2012). Quarry mining operations affect the quality of air, water and soil as well as noise levels. Mining and quarrying operations frequently involve high degrees of environmental impacts which can extend well beyond the operational areas of the companies involved. These impacts have been associated more with human health problems (Rebell *et al*., 1976). Some of these diseases include Silicolisis (miners diseases), a disabling and often fatal lung diseases caused by breathing dust of very small particles of crystalline Silica (NIOSH, 2007), Pneumonia (black lung diseases) caused by dust generated in drilling mine sand. Rock blasting also generates dusts which can also cause respiratory diseases (Abdollahisharif *et al*., 2016). The quarry mining activities is potentially hazardous to the eye. Among the various fungal diseases of the eye, candidiasis remains a major one with potentially sight – threatening complications which could result in either partial or complete loss of vision. Several species of *Candida* have been implicated in oular candidiasis with *C. albicans* being the most implicated. Other Candida species reported in Ocular candidiasis include *C. tropicalis*, *C. stellatoidea* and *C. utilis* (Thomas, 1994).

Though several studies have been carried out concerning quarry mining activities, there is no documented evidence of ocular microbial infections involving Candida species as etiological agents, especially in Ishiagu Area of Nigeria. Therefore this study investigated the ocular candidiasis in quarry mining areas of Ishiagu, Ivo Local Government Area of Ebonyi State.

MATERIALS AND METHODS

Study Area

Ishiagu is a rural community in Ivo Local Government Area of Ebonyi State, Nigeria where farming is the main stay of the people. Heavy metal lead and zinc (Pb and Zn) mining is the major industrial activity in the area coupled with stone quarrying. Ishiagu lies in the typical Guinea Savannah region of the tropical climate zone of Nigeria and two distinct seasons - rainy and dry seasons. The rainy season months are usually April to September while the dry season lasts from October to February (Nwaugo *et al*., 2008).

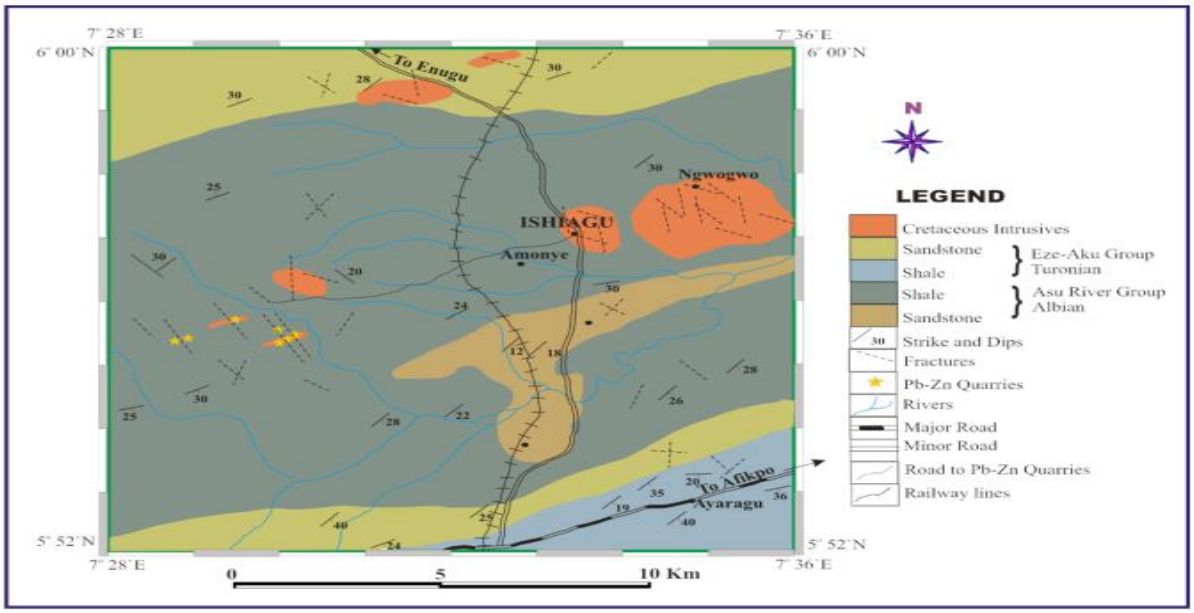

Figure 1: Geologic map of the Ishiagu Area

Study design

A well-structured questionnaire was used to collect data on demography, gender, level of education, occupation, age and other relevant information of the target population. The symptoms experienced by the individuals screened were also recorded.

All participants included in this study were subjected to eye examination using the penlight, ophthalmoscope, slit lamp and other necessary appliances. Out of 230 participants in this study 61 had findings that necessitated further examination to isolate the pathogens responsible for the clinical ocular features such as corneal ulcer, lid edema, conjunctivitis, cotton wool spots and retinal haemorrhage.

Specimen collection

Schirmer's paper was used to collect tear film. The paper was placed at the top of the lower eyelid to collect the tear films from each individual. Specimen was transported in sealed plastic bags and the laboratory analysis was carried out immediately after specimen collection. **Microbial Analysis for Fungi**

Direct mount

Each Schirmer paper was rinsed in physiological saline and 2-3 drops were placed on a glass slide, covered with cover slip and mounted on a microscope. The preparations were viewed under x10 and x40 objectives lenses under low light intensity. Fungal morphology and other characteristics were observed and recorded accordingly.

Inoculation and Incubation

The schirmer's paper was aseptically placed on prepared Sabourund Dextrose Agar in Petri dish. This was incubated at room temperature (25- 28^{0}C) for 3-7 days and observed daily for yeast and fungal growth. Colonies growing outside the inoculated areas were regarded as contaminant.

Pasty growth suspected to be yeast colonies were sub cultured into fresh Potato Dextrose Agar (PDA). Fluffy growth fungal was allowed to develop further and re- sub cultured for pure colony isolation using spot inoculation technique.

Identification and characterization of fungal isolates

The mycological identification and characterization of fungal isolates was based on microscopic and macroscopic examination of the colonies observed. The microscopic examination of the isolate was done using lactophenol cotton blue stain. Features seen on the slides were compared to established characteristics fungal features using the pictorial aids according to Larone (2014) and Swizar *et al.* (2016).

Lactophenol Cotton Blue Stain (LPCB)

Two inoculation needles were sterilized using the Bunsen burner flame and cooled near the flame. With one of the needles, a small portion of the fungal mycelia was placed in 2-3 drops of the lactophenol cotton blue solution on the glass slide. The mycelia were teased out using the other sterile needle. A cover slip was gently used to cover the preparation to avoid air bubbles. The preparation was then observed under x10 and x40 objective lenses of the microscope for characteristics fungal features using the nature and arrangement of the fruiting bodies and mycelia. Where the fungal mycelia were collected with small portion of the agar, a drop of 10% potassium hydroxide (KOH) was added and heated gently over a Bunsen burner flame to dissolve the agar before teasing and covering with cover slip.

Again, the microscopic observations are with x10 and x40 objective tenses under low light intensity.

Gram's Reaction (Hucker's Method)

The pasty cream coloured colonies (yeast) were subjected to Gram stain. A wire loop was flamed to sterilize and was used to place a colony of the isolate on a grease-free glass slide. This was emulsified in a drop of sterile distilled water to give a thin preparation. The smear was passed over the flame of a Bunsen burner to heat fix. The smear was covered with crystal violet solution for 1 minute. The slide was rinsed with clean water. The water was tipped off and covered with Lugol's iodine solution for 30 seconds. Subsequently the slide was rinsed in a slow running tap water for 5 seconds and was decolourized with 70% ethanol. It was washed after 10-15 seconds with clean water and covered with safranin solution for 30 seconds. Again, the slide was rinsed in slowly running water and air-dried.

Finally the smear was examined microscopically with x100 objective after adding the Oil immersion to check the staining reaction, cell arrangement and cell shapes. Gram negative cells stains pale to pink or red while Gram's positive cells stain purple or violet.

Gram Tube Test

This germination tube test is to distinguish *C. albicans* from other *Candida* species. A drop of serum was placed on a grease-free glass slide. A small portion of the yeast colony was emulsified in the serum. The slide was cover with cover slip and inoculated at 37^0C for 30-60minutes. It was then viewed with the x10 and x40 objective lenses of the microscope for the production of germination tube by the yeast cells. The germination tube is actually the development of a pseudo hypha by the cells which looked like a germination of a bean seed.

C albicans produces such tubes while other *Candida* species do not.

Antifungal susceptibility test

The antifungal susceptibility test was carried out with commercially prepared antifungal drugs, using agar well diffusion method (Mosier *et al*., 1977) standardized by National Committee for Clinical and Laboratory Standard (NCCLS, 2004) where 0 – 8mm diameter zone of inhibition was taken to be resistant, 9 – 15mm as intermediate and above 16m sensitive.

The isolated fungal colonies were subcultured on Sabouraud dextrose agar (SDA) individually. Four wells, each 6mm in diameter were cut out of the agar using cork borer and 15mg/ml (Fluconazole), 20 mg/ml (Ketoconazole) and 20mg/ml (voriconazole) and amphotericin B were placed into each well. The plates were incubated at room temperature (25 to 28^0C) for 2 to 5 days. The diameter of zones of inhibition were measured with a transparent ruler and recorded accordingly. Three readings were reported and the average taken.

Antifungal Tablets used: Fluconazole 150mg, Ketoconazole 200mg, Amphotericin B and Voriconazole.

Plasmid curing

Fungal and yeast isolates that showed resistance to the antifungal agents tested were subjected to plasmid curing. This was to ascertain whether the resistance to the drugs was genomic or from a plasmid. The test was done using 0.5% acrydine dye solution. 10ml of the 0.3% acrydine dye was added to 90ml of Sabouroud dextrose broth after sterilization at 121^0C and 15 p.s.i. for 15 minutes in the autoclave. On cooling to room temperature, the acrydine dye was added and inoculated with the test fungal or yeast isolate. This was incubated at room temperature (25 – 28^0C) for 2 -5 days.

The fungal isolates were then re-tested for susceptibility to antifungal agents. The results obtained were compared to the initial susceptibility results. Any isolate that become susceptible after the curing was said to contain plasmid that caused the resistance initially. However, if the resistance continued after curing process, it was not from plasmid but inherent in the cell genome

Data Management and Statistical Analysis

Data was analysed using the statistical packages for social sciences (SPSS), version 17 (spss.inc.chicago il, 2008).

RESULTS

In this study, a total of 230 individuals were screened for ocular fungal infections. Females constituted 100 (43.49%) while the males were 130 (56.52%). Out of the 130 males screened 42 (32.30%) had ocular fungal infections, with 19.00% (19) of the 100 females having the same ocular fungal infections (Table 1). The males were statistically more infected than the females ($P< 0.05$).

Table 1: Prevalence of fungal ocular infections in Ishiagu

Gender	NE	NI	%
Male	130	42	32.30
Female	100	19	19.00
Total	230	61	26.52

NE = Number examined, NI = Number infected

Eight (8) different fungal isolates were observed during the research. These include four species of Candida – *C. albicans, C. tropicalis, C. stellatoida* and *C. utilis*. Other fungi observed were *Rhodotorulla, Fusarium, Penicillium* and *Aspergillus* species. In the males, the most prevalent *Candida* species was *C. albiains* where 23.80% of the infected 42 followed by *C. tropicalis* (6.15%) with *C. utilis* and *C. stellatoidea* having the same percentage of 3.84%. A similar trend was observed in females with *C. albicans* having 9.00%, followed by *C. utilis* and *C. stellatoidea* showing only 2.00% each (Table 2). The other fungal species isolated were *Aspergillus species* and *R. rubra* (4.61%), *Fusarium* species (3.84%) and *Penicillium* species (3.07%) in males. The values observed in females were *Aspergillus* species (4.00%) and the other fungal species giving only 2.00% each.

Table 2: Aetiologic agents of the ocular infections according to gender in the population examined

Organism	Male		Female		Total	
	NE	NI	NE	NI	NE	NI
C. albicans	130	30(23.07)	100	9(9.00)	230	39
C. tropicalis	130	8(6.15)	100	4(4.00)	230	12
C. stellatoidea	130	5(3.84)	100	2(2.00)	230	7
C. utilis	130	5(3.84)	100	2(2.00)	230	7
Others						
R. rubra	130	6(4.61)	100	2(2.00)	230	8
Fusarium sp.	130	5(3.84)	100	2(2.00)	230	7
Aspergillus sp.	130	6(4.61)	100	4(4.00)	230	10
Penicillum sp.	130	4(3.07)	100	2(2.00)	230	6

NE = Number examined, NI = Number infected

Table 3: Prevalence of the fungal species among the infected individuals

Organism	Male		Female		Total	
	NI	NO	NI	NO	NI	NO
C. albicans	42	30(71.42)	19	9(47.36)	61	39
C. *Tropicalis*	42	8(19.04)	19	4(21.05)	61	12
C. stellatoidea	42	5(11.90)	19	2(10.52)	61	7
C. utilis	42	5(11.90)	19	2(10.52)	61	7
Others						
R. rubra	42	6(14.28)	19	2(10.52)	61	8
Fusarium sp.	42	5(11.90)	19	2(10.52)	61	7
Aspergillus sp.	42	6(14.28)	19	4(21.05)	61	10
Penicillum sp.	42	4(9.52)	19	2(10.52)	61	6

NE = Number examined, NO = Number organism

Table 4: Age related influence on the ocular fungal infections in the study area

Age	Male		Female		Total	
	NE	NI	NE	NI	NE	NI
≤15	15	4(26.66)	10	1(10.00)	25	5(20.00)
16-20	16	2(12.50)	10	1(10.00)	26	3(11.53)
21-25	18	4(22.22)	8	1(12.50)	26	5(19.23)
26-30	23	10(43.47)	10	2(20.00)	33	12(36.36)
31-35	14	6(42.85)	14	3(21.42)	28	9(32.14)
36-40	12	5(41.66)	14	4(28.57)	26	9(34.61)
41-45	12	4(33.33)	13	4(30.77)	25	8(32.00)
46-50	10	4(40.00)	15	2(13.33)	25	6(24.00)
>50	10	5(50.00)	6	1(16.66)	16	6(37.5)
Total	130	42(32.30)	100	19(19.00)	230	61(26.52)

NE = Number examined, NI = Number infected

Table 5: Occupational disposition of the total population examined

Occupation	NE	NI	%
Quarry workers	55	26	47.27
Farmers	45	15	33.33
Civil servants	20	3	15.00
Health workers	25	3	12.00
Artisans	40	9	22.50
Learned	30	2	6.66
Others	15	3	20.00
Total	230	61	26.52

NE = Number examined, NI = Number infected

Table 6: Occupational disposition of the infected population

Occupation	NI	%
Quarry workers	26	42.62
Farmers	15	24.59
Civil servants	3	4.91
Health workers	3	4.91
Artisans	9	14.75
Learned	2	3.27
Others	3	4.91
Total	61	100

NI = Number infected

Table 7: Symptoms observed among the infected individuals

Symptom	Male	Female	Total
Asymptomatic	2(4.76)	1(5.26)	3(4.91)
Pains	13(30.95)	6(31.57)	19(31.14)
Hyperemia (Redness)	6(14.28)	4(21.05)	10(16.39)
Allergy (Itching)	9(21.42)	3(15.78)	12(19.67)
Discharge	12(28.57)	5(26.31)	17(27.80)

Table 8: Susceptibility of the isolates to antifungal agents in percentage

	Amphotericin B		Fluconazole		Ketoconazole		Voriconazole	
Microbes	S	R	S	R	S	R	S	R
C. albicans	100	-	100	-	100	-	100	-
C. tropicalis	100	-	100	-	100	-	100	-
C. stellatoidea	100	-	80	20	100	-	100	-
C. utilis	100	-	100	-	80	20	100	-
Others								
R. rubra	100	-	100	20	100	-	100	-
Fusarium sp.	100	-	80	20	100	-	80	20
Aspergillus sp.	100	-	80	-	100	20	100	-
Penicillum sp.	100	-	100	-	80	20	80	-

S = Susceptible, R = resistance

Table 9: Susceptibility of the isolates after plasmid curing

Organism	Fluconazole		Ketoconazole		Voriconazole	
	S	R	S	R	S	R
C. albicans	100	-	100	-	100	-
C. tropicalis	100	-	100	-	100	-
C. stellatoidea	100	-	100	-	100	-
C. utilis	100	-	80	-	100	-
Others						
R. rubra	100	-	100	-	100	-
Fusarium sp.	100	-	100	-	100	-
Aspergillus sp.	100	-	100	-	100	-
Penicillum sp.	100	-	100	-	100	-

S = Susceptible, R = resistance

Table 10: Signs observed among the infected individuals

Signs	Male		Female		Total	
	N	%	N	%	N	%
Corneal ulcer (Keratitis)	8 (40)		12 (60)		20 (32.79)	
Conjunctivitis (Redness)	4 (40)		6 (60)		10 (16.39)	
Lid Edema (Swelling)	9 (60)		6 (40)		15 (24.59)	
Cotton wool spots (Retina)	7 (58.3)		5 (41.67)		12 (19.67)	
Retinal Haemorrhage	1 (25)		3 (75)		4 (6.56)	
Total	29		32		61	

N= Number

Table 11: Clinical Features of Fungal Isolates

Isolates	Corneal ulcer (keratitis) N %	Conjunctivitis (redness) N %	Lid Edema (Swelling) N %	Cotton wool spots (retina) N %	Retinal Haemorrhage (Roth Spots) N %
C. albicans	8 (40.0)	2 (20.0)	4 (26.67)	2 (16.67)	1 (25.0)
C. tropicalis	1 (5.0)	2 (20.0)	3 (20.0)	2 (16.67)	1 (25.0)
C. stellatoidea	0 (0.0)	1 (10.0)	1 (6.67)	1 (8.33)	1 (25.0)
C. utilis	1 (5.0)	1 (10.0)	1 (6.67)	0 (0.00)	1 (25.0)
R. rubra	2 (5.0)	0 (0.00)	1 (6.67)	1 (8.33)	0 (0.00)
Fusarium sp.	4 (20.0)	1 (10.0)	2 (13.33)	2 (16.67)	0 (0.00)
Aspergillus sp.	3 (15.0)	2 (20.0)	1 (6.67)	1 (8.33)	0 (0.00)
Penicillin sp.	1 (5.0)	1 (10.0)	2 (13.33)	3 (25.0)	0 (0.00)
Total	20	10	15	12	4

N= Number

DISCUSSION

In this study which investigated the prevalence of ocular fungal infections in Quarry (Rock) mining area, Ishiagu, Ebonyi State, 61(26.52%) of the 230 individuals screened were infected. In India, 86% prevalence of ocular fungal infections was reported by Umamageswari *et al*. (2013). However, Sridhar *et al*. (2013) reported a prevalence of ocular fungal infections of 23.9% while Pleyer *et al*. (1995) observed 23.7% prevalence. These are close to the 26.52% prevalence observed in this research.

The males had higher prevalence of 32.30% compared to the 19.00% observed in the females. This could be attributed to the male outdoor activities which are likely to expose them to infections. Observations in this research showed that *Candida* species have been severally implicated in ocular fungal infections causing ocular candidiasis. This is in line with the reports of other researchers (Sridhar *et al*., 2013; Deweng *et al*. 2017). *Canddia* including *C. albicans,* and *C. parapsilosis,* observed in this work have been reported by (Ranjith *et al*. 2017). In this work, *Candida albicans* was also observed to be higher than other species causing ocular candidiasis. This observation is agreement with the findings of Sun *et al*. (2007) and Durand, (2013).

The species identified in this study include; *Aspergillus, Fusarium* and *Penicillium*. Deweng *et al*. (2017) and Manoj and Prasannakumar (2002) stated that these fungi along with *Alternaria* and *Acremomium* species could cause ocular mycotic infections including keratatis and Corneal perforations. Rahi *et al*. (1995) and Umamageswari *et al*. (2013) reported that *Aspergillus* and *Fusarium* species were the most common ocular mycotic infections agents in their various studies.

Analysis of the occupational influence on the ocular fungal infections showed that Quarry workers were the most infected (47.27%) followed by the farmers (33.33%) while the least were learned (6.66%) and civil servants (15.00%). The Quarry workers and farmers are those that mostly come in contact with dusts and soil particles in the air. Nwaugo *et al*. (2008) and Ezichi (2011) reported that these particulate matters carry soil microorganisms including bacteria and fungi that could thrive when they settle on favourable surfaces such as the eye. The implication of Quarry activities as the major cause of the ocular mycotic infections in the study area could be further buttressed as the infected Quarry workers constituted 42.62% of the 61 individuals with ocular mycosis in the study population. In the same vine, farmers made up 24.59% of the 61 infected. No other group constituted up to 15%

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Observations concerning age- related prevalence of the ocular mycotic infections indicate that age has significant influence. Males of >50 years, 26-35 years were significantly more infected than those of other ages. On the other hand, women of 31-45 years were more infected than in the other age groups in the female. This agrees with Nwaugo *et al*. (2008) that young men of 26 years and above engage in quarry operations at various levels than the females. Ezichi (2011) also observed that females also engaged in light operations in the downstream aspects of the Quarry industry. This therefore suggests that more males beginning from 26 years age in Quarry industry activities while the females come in later. This therefore indicates that the males are likely to be more exposed to the ocular mycotic infections, which accounts for the higher prevalence of the infections in males. Pleyer *et al*. (1995) reported that ocular pains, itching, blurred vision and discharge are common symptoms in ocular mycotic infections. These authors suggest corneal perforation and keratitis as the cause of the symptoms in mycotic ocular infections. These same symptoms were also observed in this study. Rahi *et al*. (1995) reported that most *Candida* species are susceptible to Amphotericin B but show various susceptible rates to most other antifungal agents. Among the *Candida* spp, *C. albicans* showed no resistance to fluconazole, Ketoconazole and Amphotericin B. Amphotericin B is more costly in the area and not commonly available which could reduce its abuse in the study area, hence its high efficacy against *C. albicans.* Similarly *C. utilis* and *C. stellatoidea* also showed some resistance to fluconazole and ketoconazole respectively. In the same vein *Fusarium* and *Penicillium* species showed 20% resistance each to Ketoconazole and Voriconazole respectively. This indicates that all the pathogens, though mycotic had different susceptibility rates to the antifungal agents tested.

Observations in the results of this study indicate that after the plasmid curing experiments, all the isolates previously resistant to the antifungal agents tested, became susceptible to the antifungal agents. It could therefore be inferred that the resistance previously experienced was plasmid induced. The elution or removal of the plasmids through the activities of acridine dye resulted in the new susceptibility observed at the end of the experiment.

CONCLUSION

This study has shown that quarrying activities have the potentials of inducing ocular candidiasis. The candida species identified include; *C. albican*, *C. tropicalis*, *C. stellatoidea* and *C. utilis* and other fungal agents include; *Rhodotorula rubra*, *Fusarium* sp., *Aspergillus* sp. and *Penicillum sp*. The clinical features of ocular candidiasis include: pains, hyperemia (redness), lid edema, allergy, keratitis, retinal haemorrhage and ocular discharge. The is study revealed that Amphotericin B and azole drugs like fluconazole, ketoconazole and voriconazole could be employed in the treatment of ocular candidiasis. Additionally, early detection of ocular candidiasis and effective eye care services would go a long way to reduce the burden of this impaired vision in quarry mining areas. Based on the findings of this study, there is need for further studies to understand the effective way of tackling the burdens of impaired vision among quarry mining workers.

Competing interest

The authors declare no competing interest.

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