PREVALENCE OF NON-DERMATOPHYTIC MOLDS ASSOCIATED WITH CUTANEOUS MYCOSES IN CATTLE IN ABIA AND IMO STATES, NIGERIA

Keywords: Cattle, Non-dermatophytic molds, Cutanenous mycoses, Abia, Imo, fungi

1. INTRODUCTION

Cattle is a major protein source in Nigeria and cattle rearing is one of the major occupation practiced by the fulanis in the northern part of Nigeria. They breed and nuture these animals till when the mature it is then sold to business men in other parts of the country.These business men supply to cattle markets within the States, were it is then butchered and sold to smaller retailers who will then sell to the masses. As this is a large business chain that is used to earn a source of livelihood, it can also be processed and used as a source of meat to prepare different delicasies within the country[1]. In the cause of breeding these animal, the fulani herdsmen move them from one geographical location to another within the bushes in search of grazing lands. This exposure therefore predisposes them to a lot of challenges .

Cutaneous mycosis in cattle is a worldwide zoonotic infection which are usually responsible for economic losses in the farm[2].It is a highly contagious skin infection all over the world especially in the tropics (Africa, India)[3] and it is known to be caused by a dermatophyte *Trichophyton verrucossum*[4-5-6].

However, recent studies have shown that non-dermatophytic molds (NDMs); *Aspergillus* species, *Penicillum* species, *Fusarium* species, *Cladosporium* species, *Alternaria* species are fast replacing the dermatophytes in causing cutaneous mycoses[7-8-9].These non-dermatophytic molds were known to be mere environmental contaminants[10]. They are also known to be good secretors of enzymes such as amylase, protease, cellulase, keratinase, lipases which can also act as a virulent factor[11].

Due to poor personal hygiene by the animal handlers, they also are at higher risks to this infection since they are at close contact with these animals[12-13]. It can also be transmitted from animal to animal[12].

Therefore, considering the indispensable nature of cattle in Nigeria and the world at large, the highly contagious nature of cutaneous mycoses among animals communities has being highly reported as a

public health challenge in Nigeria and the world at large, including its prolonged duration of treatment and mismanagement[14]. Therefore, it becomes evident that this study identifies these non-dermatophytic molds commonly associated with cutaneous mycoses in cattle in Abia and Imo States, Nigeria.

2. MATERIAL AND METHODS

Study Area

This study was carried out in the three major cattle markets in Abia and Imo States Southeastern, Nigeria respectively. Applying the systematic random sampling method[15], since it is a migrate population a total of 2255 cattle were encountered from the six cattle markets in both States, diving by the desired sample size which is 5, the total number of cattle skin sampled was 451. Samples were collected from both infected and asymptomatic animals. Only matured cows were found in the markets and mainly male cows were seen, since the females are usually left in the farms for procreation.

Ethical Permit

This ethical permit is based on the compliance of the ethical standard of Imo and Abia State in Nigeria on cattle market , the Ethical permit No; NAR/Vet/XX of the Ministry of Agriculture and Natural Resources Owerri, Imo state dated 13th March ,2017 and that of the Ministry of Agriculture Umuahia, Abia state Ref. No; DVS/01/RCH/01/18, dated 4th June,2018, was also issued before the commencement of the screening of the cattles in both States.

Method of Sample Collection

Samples of skin scrapings were collected from different parts of the cattle body especially areas suspected infected. These areas were cleaned with 70% disposable alcohol swab pad and allowed to evaporate. A sterile tooth brush was used to scrape the surface of the required areas of the cattle skin. These skin samples were collected in a sterile paper packets and placed in a sterile bottles. Each collected sample was labeled based on location /zone where it was collected and date of collection. The sample containers were wrapped with aluminum foil and taken to the microbiology laboratory Imo State University, Owerri, Nigeria and this were processed within two hours.

Microscopic examination of samples

Some of the skin samples was examined by direct microscopy as described by Chesbrough[16], which were prepared with 10% KOH while the remaining were then cultured on the selected media.

Culture technique

Each sample was cultured directly on the plates of sabouraud dextrose agar (SDA) by spread plate method. The sterile brushes which was used for sample collection, was spread on the plates containing 20mg chloramphenicol each to inhibit bacteria growth. This was later kept in the incubators at 37℃ for 1-2 weeks.This was done on duplicate plate.

Discrete pure colonies of each isolates were inoculated on Sabouraud dextrose agar slants and incubated at 37ºC for 1-2 weeks. The slants containing the pure cultures were stored in the refrigerator until required for further studies.

Identification of isolates

The identification was based on growth rate, colonial and microscopic morphology. The isolates were identified on plates using standard methods as in Cheesbrough (2010). The microscopic observations from slide culture of the isolates were compared with standard mycology atlas for identification [17]. Molecular analysis of the isolates was also carried on sixteen (16) isolates using sequencing the BigDye Terminator kit on a 3510 ABI sequencer by Inaqaba Biotechnological, Pretoria South Africa.

Pathogenecity test

Twenty mice were bought from the department of Biochemisry Imo State PolythenicUmuagwo and checked for skin infection. The mice skin were cleaed with sterile water, fed for two weeks and allowed to acclimatize before introducing ten isolates that were recovered from cattle with lesion on them. The rats were paired and the cages were labeled with the name of the isolate for this investigation. A fragment of the isolate were diluted in 5ml of distilled water containing beads to break the fungal strands after which it was then rubbed on the skin of the mice with a sterile stick[18].

Method of analysis of data

The data from this study were analysed statistically using multiple comparison in percentages and analysis of variance (ANOVA) as in Martins[19].

3. RESULTS AND DISCUSSION

Out of 2255 cattle encountered, 451 cattle skin were sampled of which 53(11.7%) had lesions which is an indication of fungal infections . The samples analysed showed positive results on KOH and culture. Out of 451 cattle skin , 223 were sampled from Abia state of which 24(10.8%) had lesions while 228 were sampled from Imo state of which 29(12.7%) had lesion (Table 1). Among the sampled population all 451 (100%) were matured male cows ready to sell. Out of 31 skin sample sampled from the head non had lesion (0%), 58 were sampled from the leg and 10 (18.9%) had lesion, 15 from ear and 3(6%) had lesion, 16 from neck and 0(0%)

had lesion, 8 were sampled from the groin and 6 (11.3%) had lesion, 285 were samped from the abdomen and 21(40%) had lesion while 38 were sampled from the tail region and 7(13.2%) had lesion (Table 2). The amplified ITS fragments of the fungal isolates visualized under the blue transilluminator was shown in figure 1 (Lane 1- 11). Isolates from lanes 1,2, 4, 6-11 represents ITS bands at 600bp while lane 3 represents 500bp. L represents the 100bp molecular ladder. Figure 2 shows results obtained from ITS sequence from the isolate which produced an exact match during the megablast search for highly similar sequences from the NCBI non reductant nucleotide (nr/nt) database. The ITS of the isolates showed 99-100% similarity to other species. The evolutionary distances which was computed using the Jukes-cantor method were in agreement with the phylogenetic placement of ITS of C18 within the *Penicillum*sp. and revealed a closely relatedness to *Penicillumcitrinum* (MH990629) other than any other *Penicillum*sp., C9 and C10 revealed a closely relatedness to *Aspergillus fumigatus* (MK461083), C14 and C16 to *Aspergillus terreus*(MK418744), C6 and C13 revealed a closely relatedness to *Aspergillus welwitschiae* (MG576117), C7, C11, C12 is related to *Aspergillus aculeatus* (MK461093), C3 is closely related to *Aspergillus flavus* (Mk 299130), C2 and C19 is related to *Fusarium succisae*(Mk 418691), C17 is closely related to *Aspergillus sydowii*(Mk 396475), C23 is related to *Talaromyceskendriikii*(kko 98055), C 25 closely related to *Curvulariakusanol*(MG975624), C15 is related to *Cladosporium tenuissimum* (MK 357638), C22 is related to *Pestalotiopsismicrospora* (MK 224482), C24 was also found to be related to *Fusarium solani*(MH517359), CI to *Fusarium lichenicola*(KH921661) and C20 was found closely related to *Fusarium oxysporum*(KM203578).

The DNA sequencing identified a total of 16 non dermatophytic molds species belonging to 8 genera; *Apergillus*genera , *Talaromyces*genera, *Curvularia* genera, *Cladosporium* genera, *Pestalotiopsis* genera, *Fusarium* genera, *Penicillum*genera and *Absidia* genera (Table 3) were mostly isolated from the samples and the all occurred at different frequencies. In Abia State, out of 223 cattle skin sampled a total of 12 non-dermatophytic molds species were recovered which belongs to *Penicillium citrinum* (4%), *Aspergillus fumigatus* (4.7%), *Aspergillus terreus* (2%), *Aspergillus welwitschiae* (15%), *Aspergillus flavus*(11.1%), *Aspergillus aculeatus* (7.3%), *Aspergillus sydowii* (3.6%), *Fusarium solani* (2.2%), *Cladosporium tenuissimum*(4.5%), *Fusarium linchenicola* (19.4%), *Fusarium succisae*(12.5%) and *Absidia* specie (14%). Based on the result, *Fusarium lichenicola* was the most frequently isolated followed by *Aspergillus welwitchiae*, *Absidia* specie while the least isolated were *Aspergillus terreus* and *Fusarium solani.* Among these isolates, ten were recovered from the 24 samples that had lesion within the State and they include *Penicillium citrinum* (5%), *Aspergillus fumigatus* (2%), *Aspergilliuswelwitschiae* (21.3%), *Aspergillius aculeatus* (5%), *Aspergillus flavus* (5%), *Aspergillus sydowii* (3.3%), *Cladosporium tenuissinum* (7%), *Fusarium lichenicola*(13.1%), *Fusarium succisae* (20%) and *Absidia*specie (20%) (Table 4, 5). Based on the results, *Aspergillus welwitschiae*, *Absidia*specie and *Fusarium succisae* were the most prevalent non-dermatophytic mold recovered. In Imo State, out of 228 cattle skin sampled a total of 16 non-dermatophytic molds were recovered and the include *Penicillium citrinum* (1.5%), *Aspergillius fumigatus* (2.1%), *Aspergillus terreus* (4%), *Aspergillus welwitschiae* (12%), *Aspergillus flavus* (8%), *Aspergillus aculeatus* (11%), *Aspergillus sydowii*(7.1%), *Talaromyceskendrickii* (0.3%),

Curvulariakusanol (1.5%), *Cladosporium tenuissinum* (5.5%), *Pestalotiopsismicrospora* (0.3%), *Fusarium solani*(4.6%), *Fusarium linchenicola* (16%), *Fusarium succisae* (10.4%), *Fusarium oxysporum* (4.6%) and *Absidia* specie (11.3%). The result showed that *Fusarium linchenicola* was the most frequently isolated, followed by *Aspergillus welwitschiae* and *Absidia* specie while the least isolated were *Talaromyceskendrickii* and *Pestalotiopsismicrospora*. Of these non-dermatophytic molds recovered, ten were among those recovered from the 28 that had lesion within the State and the include *Aspergillus welwitschiae* (28.2%), *Aspergillus aculeatus* (15.5%), *Aspergillus flavus* (1.4%), *Aspergillus sydowii*(8.5%0, *Talaromyceskendrickii* (1.4%), *Cladosporium tenuissimum* (5.6%), *Pestalotiopsismicrospora* (1.4%), *Fusarium linchenicola* (14.1%), *Fusarium succisae*(9.9%) and *Absidia* specie (14.1%) (Table 4, 5). Out of the twelve isolates recovered from skins that had lesions from both States, ten were subjected to pathogengcity test, only three *Aspergillus welwitschiae*, *Cladosporium tenuissinum* and *Absidia* specie were able to elicit clinical lesions suggestive of cutaneous mycoses.

Based on the analysis shown in this study, it has revealed a high prevalence of non-dermatophytic molds associated with cattle skin (including those with lesion and those without lesion) in Abia and Imo States, Nigeria. Infection was mostly recorded from the abdomen than the other anatomical sites. This could be attributed to the fact that these animals lay and roll on the soil with their abdomen than other parts of their body, thereby predisposing their glabrous skin to contaminated soil. These animals, often lay side by side to each other, thereby coming in contact with other infected animals in the market. They can also incure trauma on their glabrous skin due to the stacking effect they suffer on transit in the trucks while coming down to the East from the North or it can be as a result of trauma incurred in the bushes during grazing, this paves way for these non-dermatophytic molds to gain access through the skin. This agrees with work by Emenuga[20]. In their work they stated that lesions were mainly observed on the animals (goats and sheep) glabrous skin. Balogun[21] in their work also reported that lesions were seen more on the limbs (18.7%) of horses than other parts of the body were samples were collected (tail, head and abdomen), when they analyzed the prevalence and distribution of dermatophytes among domestic horses in Kwara State, Nigeria.

It is also important to note that only matured male cows were encountered in this study and as such age and sex variables were eliminated. This was attributed to the fact that only ready to sell cows (matured cow) are found in the markets. For the sex, according to the veterinary doctors, the females cattle are hardly sold out by the farmers as they are usually left in the farms for procreation. The sellers are also business men who buy commodities that they can sell and make profit. According to them buyers do prefer male cows than they female cows. This they attributed to the fact that the male cows are easy to prepare than the female cows so they rarely purchase them. Among the non-dermatophytic molds isolated from this study, *Aspergillus* species 338(43.6%) were the most frequently isolated followed by *Fusarium* species 269(34.7%) then *Absidia* specie 100(12.9%), *Cladosporium* specie38(4.9%), *Penicillium* specie 23(3.0%), *Curvularia* specie 5(0.6%). *Pestalotiopsis* specie 1(0.1%) and *Talaromyces* specie 1(0.1%) from both States. This can be attributed to the fact that most of these isolates have been reported to be major soil and vegetation inhabitants[22-23-24]. Since

these cows lay mostly on these soils there is a high tendency of these molds colonizing the animal skin and also considering that they are usually taken into the bushes for grazing, there is a high tendency of picking some from the grasses . Similar works have revealed our findings although not in cattle, Igor[25] isolated *Aspergillus* species as the most prevalent followed by *Penicillum* sp., *Alternaria* sp., and *Fusarium* specie. when he examined mycological skin swabs from dog skin. El-said[26] isolated saprophytes in the frequency order of *Aspergillus*, *Penicillum*, *Alternaria* species when he sampled hair from sheep and goat in Libya. Paixao[27] in his study also isolated *Aspergillus* specie (37.9%) as the highest saprobe followed by *Penicilliums*pecie (21.4%). *Cladosporium* specie (8.7%), *Fusarium* species (7.8%), *Curvularia* specie (3.9%) when he surveyed saprophytic fungi from dogs and cats in the city of Fortaleza, Brazil and also Ponnusamy[28] in his work recovered *Curvularia* specie from skin infection in goat in Cauvery delta region of Tamil Nadu, India.

In this present study *Aspergillus* spp., *Fusarium* spp., *Absidia* spp., *Cladosporium* spp. and *Penicillum* spp. are the most frequently isolated non-dermatophytic mold in this study; this is not suprising as these molds have been reported to be highly ubiquitous in nature[29- 30-31-32-33]. A number of skin infections in man and animal have also been linked to them especially in immunocompromised host and onychomycosis[34-35-36-37].

In this study, there were no significant difference between the non-dermatophytic molds recovered from both States although they were recovered at different frequencies. This is understandable since these animals are being produced in the same area in the Northern part of the country. Four non-dermatophytic molds recovered from Imo State were not recovered from Abia State which includes *Curvulariakusanol*5(1.5%), *Pestalotiopsismicrosporum*1(0.1%), *Talaromyceskendrinkii*1(0.3%) and *Fusarium oxysporum*15(4.6%). Judging by their low frequency of occurrence there is a high possibility that these molds were picked up by these cows on transit or within the cattle market environment.

In this study, it was also observed that similar non-dermatophytic molds recovered from asymptomatic cows were also recovered from those cows with lesions. This strengthens the notion that these non-dermatophytic molds naturally colonize the cattle skin but if by coincidence the immunity of the host is lowered, they can take advantage by causing cutaneous infection. Also the possibility of some of these molds being categorized as true pathogens cannot be underestimated. This is also in accordance with other published works by Jain[38], their work analyzed the current status of *Fusarium* infection in animal and human and concluded that this filamentous fungus is widely distributed in the soil and on plants and can cause superficial infections especially on compromise host. Suzana[39] in their study also agrees that *Clasdosporium*spp. are opportunistic pathogens that are capable of causing cutaneous infections. In another study by Bakheshwain[40]andDider Pin[2]also observed that most mold floras are known to cause superficial or cutaneous infections due to its ability to utilize keratin .

Among the twelve non-dermatophytic molds isolated from cattle with lesion in both States, 10 of them were subjected to pathogenecity test and only *Aspergillus welwitschiae*, *Cladosporium tenuissinum* and *Absidia* specie were able to elicit alopecia, nodule and discolouration on healthy albino mice skin respectively. These observations are clinical symptoms suggestive of cutaneous mycoses. This observation therefore estabilishes that the non-dermatophytic molds observed as mere opportunistic pathogens may actually be true pathogens. Previous works have also implicated the non-dermatophytic molds in causing cutaneous skin infections, Subha[41] in their work revealed that loss of fur is an indication of lesion production. Marcelo[24] also demonstrated that *Cladosporium* species are capable of causing localized superficial infections. Also considering that these molds are known to be good producers of industrial enzymes such as keratinase, protease, lipase, amylase[42-43-44]. This can obviously explain their capability of degrading skin tissues as these enzymes can also act as virulent factors to cutaneous mycoses[45].

Table 1: Total population of cattle sampled including cattle with lesion in Abia and Imo states, Nigeria

States	Total population	Population with lesion(%)
ABIA	223	24
IMO	228	29
TOTAL	451	53

Table 2: Distribution of samples collected based on anatomical sites including sites infected with lesions

Anatomical sites	Total samples collected from anatomical sites	total samples collected from anatomical sites with lesion	% occurrence of anatomical sites with lesions
HEAD	31	0	0.0

LEG	58	10	18.9
EAR	15	3	6.0
NECK	16	0	0.0
GROIN	8	6	11.3
ABDOMEN	285	27	51.0
TAIL	38	7	13.2
TOTAL	451	53	

Table 3: Frequency of occurrence of Non- dermatophytic mold genera isolated from Abia and Imo states Nigeria

Genera	**Total occurrence**	**Percentage occurrence (%)**
Aspergillus species	338	43.6
Fusarium species	269	34.7
Penicillum species	23	3.0
Cladosporium species	38	4.9
Curvularia species	5	0.6
Pestalotiopsis species	1	0.1
Talaromyces species	1	0.1
Absidia species	100	12.9
TOTAL	775	

Table 4: Frequency of occurrence of non-dermatophytic molds isolated from cattle skin(symptomatic and asymptomatic) in Abia and Imo states Nigeria

Species of non-dermatophytic Molds	Number of occurrence Abia	Imo	Total occurrence(%)
Penicillumcitrinum	18	5	23 (3.0)
Aspergillus fumigatus	21	7	28 (3.6)
Aspergillus terreus	8	13	21(2.7)
Aspergillus welwitschiae	67	38	105 (13.5)
Aspergillus flavus	50	26	76 (10.0)
Aspergillus aculeatus	3336		69 (9.0)
Aspergillus sydowii	16	23	39 (5.0)
Talaromyceskendrickii	0	1	1(0.1)
Curvulariakusanol	0	5	5(0.6)
Cladosporium tenuissinum	20	18	38 (4.9)
Pestalotiopsismicrospora	0	1	1 (0.1)
Fusarium solani	10	15	25 (3.2)
Fusarium linchenicola	87	52	139 (17.9)
Fusarium succisae	56	34	90 (12.0)
Fusarium oxysporum	0	15	15 (2.0)
Absidia specie	63	37	100 (12.9)
TOTAL	449	326	775

Table 5 : frequency of occurrence of non-dermatophytic molds isolated from cattle skin with lesion (symtomatic) in abia and imo states NIGERIA

SPECIES OF NON-DERMATOPHYTIC MOLDS	FREQUENCY OF OCCURRENCE (%)	
ABIA STATE	**IMO STATE**	
Penicillumcitrinum	3 (5.0)	0
Aspergillus fumigatus	1 (2.0)	0
Aspergillus welwitschiae	13 (21.3)	20 (28.2)
Aspergillus aculeatus 3 (5.0)	11 (15.5)	
Aspergillus flavus	3 (5.0)	1 (1.4)
*Aspergillus sydowii*2 (3.3)	6 (8.5)	
Cladosporium tenuissinum	4 (7.0)	4 (5.6)
Talaromyceskendrickii 0	1 (1.4)	
Pestalotiopsismicrospora 0	1 (1.4)	
*Fusarium linchenicola*8 (13.1)	10 (14.1)	
*Fusarium succisae*12 (20.0)	7 (9.9)	
Absidia specie	12 (20.0)	10 (14.1)
TOTAL	61	71

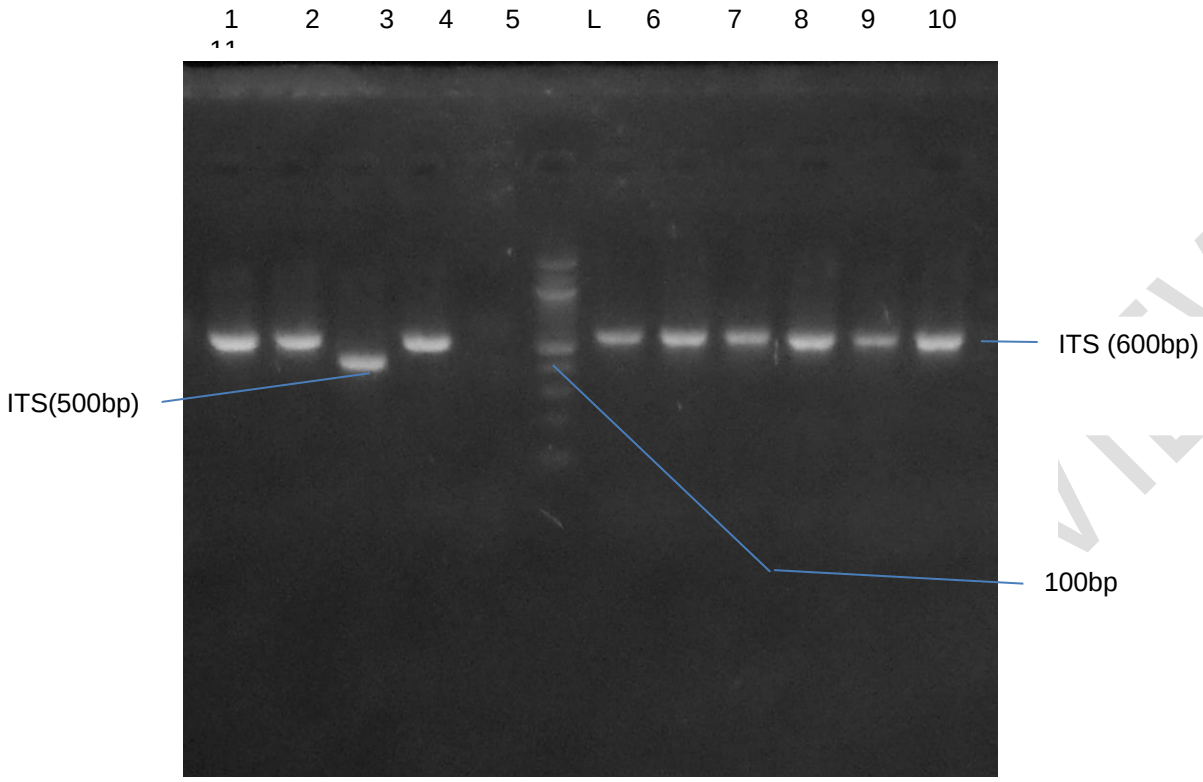

Figure 1.: Agarose gel electrophoresis showing the amplified ITS fragment of the fungal isolates. Lanes 1-4, 6-11 represent the ITS bands at 400bp and 600bp while lane L represents the 100bp molecular ladder.

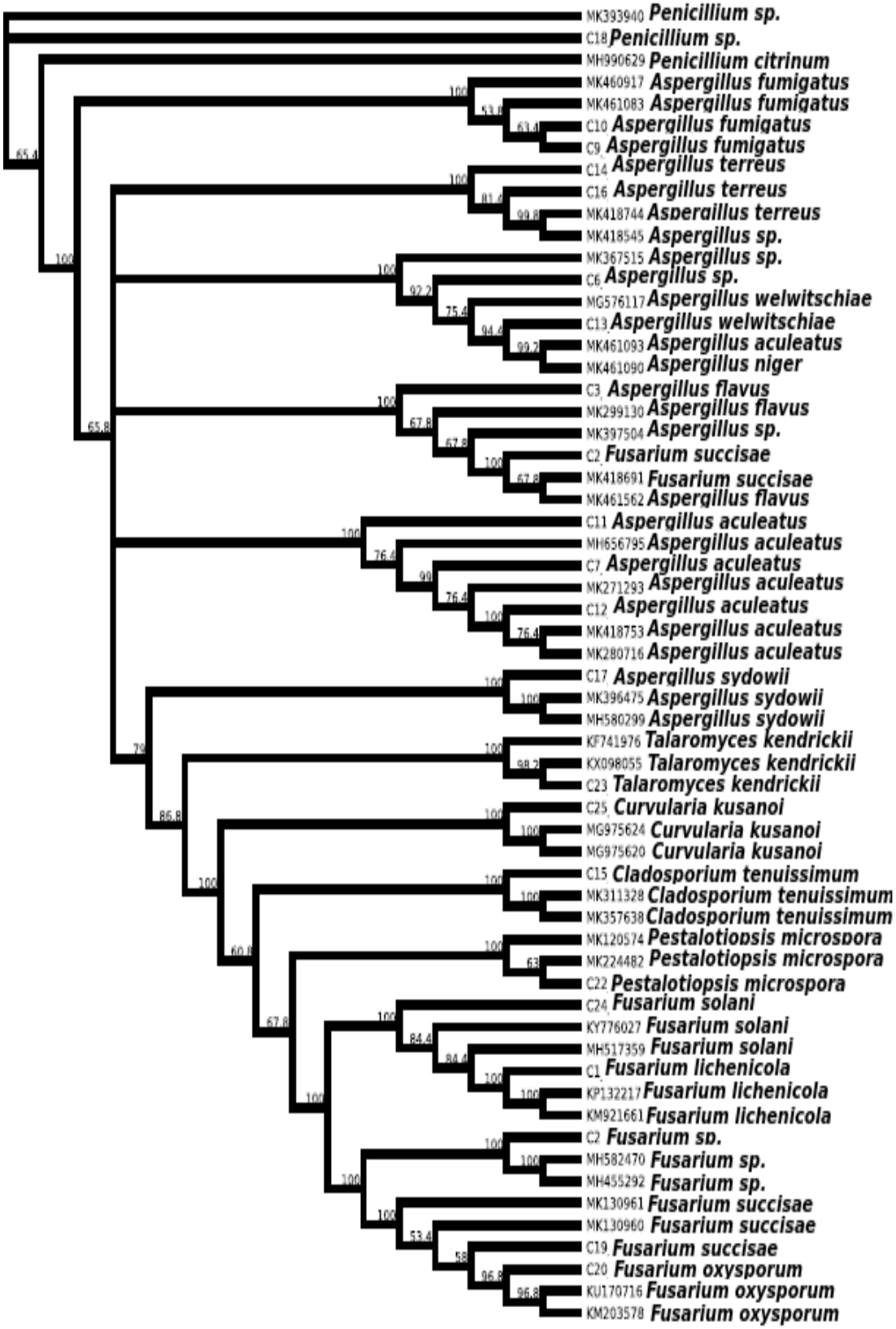
MK393940 Penicillium sp.
C18 Penicillium sp.
MH990629 Penicillium citrinum
MK460917 Aspergillus fumigatus
MK461083 Aspergillus fumigatus
C10 Aspergillus fumigatus
C9 Aspergillus fumigatus
C14 Aspergillus terreus
C16 Aspergillus terreus
MK418744 Aspergillus terreus
MK418545 Aspergillus sp.
MK367515 Aspergillus sp.
C6 Aspergillus sp.
MG576117 Aspergillus welwitschiae
C13 Aspergillus welwitschiae
MK461093 Aspergillus aculeatus
MK461090 Aspergillus niger
C3 Aspergillus flavus
MK299130 Aspergillus flavus
MK397504 Aspergillus sp.
C2 Fusarium succisae
MK418691 Fusarium succisae
MK461562 Aspergillus flavus
C11 Aspergillus aculeatus
MH656795 Aspergillus aculeatus
C7 Aspergillus aculeatus
MK271293 Aspergillus aculeatus
C12 Aspergillus aculeatus
MK418753 Aspergillus aculeatus
MK280716 Aspergillus aculeatus
C17 Aspergillus sydowii
MK396475 Aspergillus sydowii
MH580299 Aspergillus sydowii
KF741976 Talaromyces kendrickii
KX098055 Talaromyces kendrickii
C23 Talaromyces kendrickii
C25 Curvularia kusanoi
MG975624 Curvularia kusanoi
MG975620 Curvularia kusanoi
C15 Cladosporium tenuissimum
MK311328 Cladosporium tenuissimum
MK357638 Cladosporium tenuissimum
MK120574 Pestalotiopsis microspora
MK224482 Pestalotiopsis microspora
C22 Pestalotiopsis microspora
C24 Fusarium solani
KY776027 Fusarium solani
MH517359 Fusarium solani
C1 Fusarium lichenicola
KP132217 Fusarium lichenicola
KM921661 Fusarium lichenicola
C2 Fusarium sp.
MH582470 Fusarium sp.
MH455292 Fusarium sp.
MK130961 Fusarium succisae
MK130960 Fusarium succisae
C19 Fusarium succisae
C20 Fusarium oxysporum
KU170716 Fusarium oxysporum
KM203578 Fusarium oxysporum

Figure 2: **The Phylogenetic tree from the internal transcriber spacer (ITS) obtained from the isolate in this study**

4. CONCLUSION

This study also indicates that irrespective of the well-established advantages of the cattle fur, it acts as a potential receptacle to these molds and as such makes it easier for the molds to gain access into the skin once it is traumatized . It cannot also be overemphasized that this association between these non-dermatophytic molds and the animal might encourage a parasitic relationship between them which might invariably affect the size of the cow. This present study also revealed that movement of cattle from one geographical area to another might encourage the dispersal and picking up of new non-dermatophytic molds by these animals and nomads. This study therefore suggests that government should encourage farmers to keep these animals in well constructed barns whereby their forages can be brought to them instead of moving them about. It is also important that they should be kept in columns in trucks while transporting them to reduce the tendency of incurring trauma on their skins and moreso regular checks by veterinary doctors should be ensured as this will go a long way in controlling the colonization of the animal skin by these non-dermatophytic molds. There is also need for those taking care of these animals to observe precautionary measures like improving on their personal hygiene, wearing of protective kits while taking care of these animals as some of these fungal spores can easily be picked up ignorantly from them since majority of the cows are asymptomatic carriers. Pathogenecity tests carried out also showed a high possibility of some of these non-dermatophytic molds infecting human skin. Further research will be carried out to screen some of these non-dermatophytic molds for enzyme production.

ETHICAL APPROVAL

Based on the compliance of the ethical standard of Imo and Abia State in Nigeria , the Ethical permit No; NAR/Vet/XX of the Ministry of Agriculture and Natural Resources Owerri, Imo state dated 13th March ,2017 and that of the Ministry of Agriculture Umuahia, Abia state Ref. No; DVS/01/RCH/01/18, dated 4th June,2018, was also issued before the commencement of the screening of the cattles in both States.

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APPENDIX

Examples of some lesions on different parts of cattle skin encountered during this research work