Journal of Water & Health

Journal of Water and Health Vol 21 No 5, 643 doi: 10.2166/wh.2023.015

Periplaneta americana (Blattodea: Blattidae) fungal pathogens in hospital sewer systems: molecular and phylogenetic approaches

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ABSTRACT

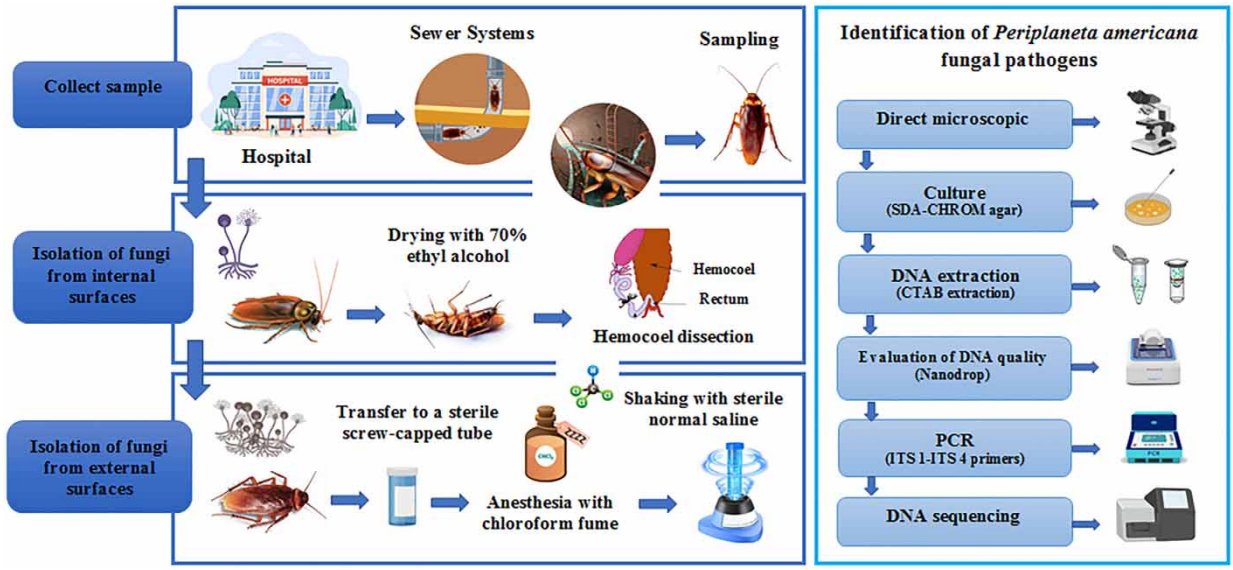
Cockroaches are known as mechanical vectors of some pathogens that can infect humans. The present study aims to rapidly identify *Periplaneta americana* fungal pathogens from sewer systems of public hospitals in Esfahan using the polymerase chain reaction (PCR) technique. A total of 55 *P. americana* cockroaches were randomly collected by direct trapping from sewer systems of seven hospitals and screened for fungal infectious agents using standard morphological methods and the PCR sequencing. From the American cockroach, we isolated 62 yeasts and 31 molds from the surface, hemocoel, and digestive tract of *P. americana*. Based on DNA sequence comparisons and other taxonomic characteristics, they were identified as more than four species of yeast and four species of mold. Yeast species including *Pichia kudriavzevii*, *Candida glabrata*, *Pichia kluyveri*, and *Candida viswanathii*, and molds such as *Aspergillus niger, Penicillium italicum, Mucor plumbeus*, and *Rhizopus oryzae* were isolated repeatedly from the surface, hemocoel, and digestive tract of *P. americana*. Our results show that the use of a combination of morphological, molecular techniques, and phylogenetic analysis can lead to the identification of pathogenic fungal agents in American cockroaches and also knowledge of fungal pathogens-arthropod host relationships.

Key words: fungi, hospitals, Iran, PCR, *Periplaneta americana*, phylogeny

HIGHLIGHTS

- *Periplaneta americana* acts as a potential mechanical vector of medically important fungal pathogens from inside sewer systems to hospitals and conversely.
- *Pichia kudriavzevii*, *Candida glabrata, Pichia kluyveri, Candida viswanathii*, and molds species such as *Aspergillus niger*, *Penicillium italicum, Mucor plumbeus*, and *Rhizopus oryzae* were isolated from the surface, hemocoel, and digestive tract of *P. americana* cockroaches.

GRAPHICAL ABSTRACT



INTRODUCTION

Over 3,500 known cockroach species have survived on the earth for more than 300 million years, almost without change. Four of them generally live in human habitations: the American cockroach (*Periplaneta americana*), the German cockroach (*Blattella germanica*), the brown-banded cockroach (*Supella longipalpa*), and the Oriental cockroach (*Blatta orientalis*) (Peterson & Coats 2001; Tatfeng *et al.* 2005). The American cockroach, Linnaeus, 1758, in reddish-brown and light-colored edges to the body with about 30 mm, is the largest species of common cockroaches (Mirzayans 1986). It is often considered a pest and, despite its name, is native to Africa and the Middle East (AL-Marjani *et al.* 2017).

Infestations of cockroaches, such as the *P. americana*, can occur in homes and institutions with poor hygiene standards (Fakoorziba *et al.* 2010). They play a considerable role in transmitting pathogenic microorganisms by different parts of their bodies (body hairs, appendages, and mouthparts) and secretions (regurgitates and feces) (Vazirianzadeh *et al.* 2009; Hamu *et al.* 2014), as a wide variety of fungi (60 species), protozoa (90 species), helminths (45 species), and bacteria (150 species) have been isolated either from their body surfaces and/or digestive tract (Jalil *et al.* 2012). Cockroaches, in addition to acting as carriers of microorganisms, can also act as reservoir hosts, allowing such microorganisms to multiply (Rosenstreich *et al.* 1997). Most of the microorganisms, which have been excluded from cockroaches, are transmitted to humans via the consumption of contaminated water and/or food (Adenusi *et al.* 2018). On the other hand, cockroaches as potential vectors of hospital-acquired infections should be taken into consideration because they can frequently move between hospital wards, surgical centers, intensive therapy units, and kitchens of hospitals, and their ability to disseminate pathogenic agents is a significant concern for many healthcare professionals (Saichua *et al.* 2008). Several reports from other countries indicate that cockroaches collected from hospitals are carriers of pathogenic fungi such as *Candida* spp., *Aspergillus* spp., or *Penicillium* spp. (Motevali-Haghi *et al.* 2014; Kassiri *et al.* 2018).

Therefore, we attempted to isolate fungal pathogens from the hemocoel, digestive tract, and body surface of *P. americana* cockroach specimens. Although we previously discussed some of the important health and medical molds and yeasts related to American cockroaches using morphological characteristics (Khodabande *et al.* 2020), in the present study, the fungi in the sewage system of hospitals in Esfahan City are discussed from a molecular and phylogenetic point of view.

METHODS

Insect collection and fungi isolation

A total of 55 cockroaches were caught directly, over several days, in the first hours of the night and were placed separately in a sterile screw-capped tube that has been previously described (Chamavit *et al.* 2011). Then, they were transferred to the

Esfahan Health Research Station affiliated with the Faculty of Health, Tehran University of Medical Sciences for identification and further processing. The cockroaches were anesthetized and killed by exposure to chloroform fume, and after binding, 2 ml of sterile normal saline (0.9%) was added to the test tube. After shaking, 1 ml of the washing was cultured on Sabouraud's dextrose agar (Difco) with 0.5% chloramphenicol at 30 °C for 2 weeks (Fotedar *et al.* 1991). To isolate fungi from internal surfaces and to decontaminate external surfaces, cockroaches were washed in 70% ethyl alcohol for 2 min, allowed to dry, and washed twice in sterile normal saline for 2–3 min to remove traces of alcohol. The gut and the hemocoel were dissected under aseptic conditions. The gut and hemocoel were then macerated in 2 ml of sterile normal saline, and the macerates were processed as described earlier (Fotedar & Banerjee 1992). Identification of filamentous fungi and yeasts was based on their macroscopic and microscopic characteristics, including colony color on CHROM agar *Candida* medium (CHROM agar Company, Paris, France), germ tube tests in serum at 37 °C for 2–3 h, and microscopic morphology on Corn-meal agar (Difco) with 1% tween 80 (Kantarcioglu & Yucel 2002; Khodabandeh *et al.* 2020).

Molecular analysis

DNA extraction

Various methods have been described for DNA extraction from fungi. We used a modified CTAB protocol, which is as follows: briefly, a portion of each fungus (4–5 mm^3) was removed and broken down by grinding with glass rods. The ground organism was transferred into a 50-mL tube containing TES buffer (10 mL Tris, 20 mM EDTA, 1% sodium dodecyl sulfate, and 4 mg proteinase K). Each tube was incubated at 45 °C for 45 min and mixed by turning. Then, 3.9 mL NaCl was added, and the samples were blended before adding 1.4 mL CTAB. Subsequently, the samples were incubated for 15 min at 65 °C, cooled in an ice/water bath, and then 10 mL chloroform–isoamyl alcohol was added and kept overnight in an ice/water bath. The upper phase was transferred to another centrifugation tube and spun for 20 min at 4,000 g (5 °C). The aqueous phase was carried to a new tube with isopropanol (10 mL), mixed, and centrifuged for 10 min at 4,000 g and room temperature. The supernatant was thrown away, and the mass was washed with 70% ethanol, dried, and dissolved in Tris-EDTA buffer solution (4.5 mL Tris, 1 mM EDTA, pH set to 8.0 with HCl). Coagulate material was removed by centrifugation, and DNA was concentrated by ethanol precipitation (2.5 vol. of 96% ethanol and 1/10 vol. of 5 M ammonium acetate), dissolved in TE (0.5 mL) (Ahmadi *et al.* 2015) and stored for further processing.

Evaluation of DNA quality and quantity

The quality and quantity of DNA were assessed and predicted using a Nanodrop 2000 (Thermo Scientific, Wilmington, Delaware, USA) by calculating the A260/A280 ratios as described by Sambrook *et al.* (1989). To evaluate the purity of the extracted DNA, absorbance ratios at 260 nm/280 nm (DNA/protein) were measured. A high ratio of greater than 2 shows the purity of DNA.

PCR and DNA sequencing

The extracted DNA was amplified using the ITS1 (5′TCCGTAGGTGAACCTGCGG3′) and ITS4 (5′TCCTCCGCTTATTG ATATGC3′) primers (Ahmadi *et al.* 2016). The polymerase chain reaction (PCR) mixture included 5 μl of extracted DNA, 0.5 μM of each primer, 12.5 μl of premix (Amplicon, Denmark), and adequate distilled water used in a final reaction volume of 25 μl. The PCR cycling program was 96 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, and a final extension step at 72 °C for 10 min in an Applied Biosystem thermocycler. Four microliters of the PCR product were analyzed by electrophoresis on 1.5% agarose gel and were visualized and photographed on a transilluminator (UVtec, Cambridge, UK). To assess the size of the amplicons, a 100-bp ladder was used as a DNA size marker in each gel. Sequencing was done with 25 μl of PCR products using the forward primer employed in PCR. The products were purified and sent to Takapouzist Company (Tehran, Iran) for sequencing. The DNA sequences were put to an advanced analysis using comparison search BLAST (http://www.ncbi.nlm.nih.gov/blast) and phylogenetic estimating by ClustalW (http://www.ebi.ac.uk/clustalw) for each concerned DNA sequence. MEGA7 determined the best-fit model of evolution. The identification method of *P. americana* fungal pathogens is schematized in the Graphical Abstract.

Statistical analyses

Descriptive statistics were applied to test the transport rate, whereas Chi-squared and Fisher's exact test analysis was used to determine significant differences between the parameters. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 55 *P. americana* cockroaches (31 males and 24 females) were included. Morphological and molecular techniques have proved the presence and the type of fungi.

Morphological characteristics for identification of cockroaches' fungal transport

Of 55 cockroaches caught, 49 (89.09%) were found to be carriers of pathogenic fungi. Based on morphological tests, fungal microorganisms isolated from the external and internal surfaces of cockroaches were categorized into eight groups as follows: molds including *Aspergillus niger*, *Penicillium*, *Mucor*, and *Rhizopus*, as well as yeast including *Pichia kudriavzevii*, *Candida glabrata*, *Pichia kluyveri*, and *Candida viswanathii* (Table 1). The most prevalent significant yeast and mold identified were *P. kudriavzevii* and *A. niger*. Statistically, no significant difference was observed in the transport rate and species of fungi.

Sex-related frequency and sites of predilection

The transport rate of important fungi identified relative to cockroach sexes is also shown in Table 1. According to the table, male cockroaches were more common carriers of fungi than females, with a higher transport rate (85.45%). The fungi were more recorded on the internal surface (94.54%) of cockroaches than on the external surface (74.54%). However, no significant difference was seen in the sites of predilection as well as the sex of fungi.

Evaluation of purity of the extracted DNA

The A 260/A 280 ratio of the DNA ranged from 1.85 to 2.11, indicating significant ratios and purity of the isolated DNA and being free from protein contamination.

PCR assay and sequence analyses

According to the results of electrophoresis of PCR products, the tests performed on isolates have yielded 350- to 850-bp bands. Amplicons were successfully sequenced, and their identity was confirmed using BLAST analysis as *P. kudriavzevii*, *C. glabrata*, *P. kluyveri*, *C. viswanathii*, *A. niger*, *Penicillium italicum*, *Mucor plumbeus*, and *Rhizopus oryzae*. Some identified sequences were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/blast) under accession numbers MT797173.1, MT797293.1, MT797385.1, MT797808.1, OP562870.1, and OP536204.1.

Phylogenetic analyses

Figure 1 shows the phylogenetic tree obtained from the ClustalW alignment using MEGA7 software for six of the novel sequences (as indicated by orange arrows) and representative lines from most of the previously described species (as indicated by GenBank accession numbers). A consensus of the two trees based on ITS rDNA means that both novel and previously described mold (*A. niger*) and yeasts associated with *P. americana* do not form a monophyletic group.

The phylogram presented four main groups: one composed of *P. kudriavzevii*, the second composed of *P. kluyveri*, and the third composed of *C. viswanathii*. The fourth was the external grouping, *C. glabrata* (Figure 1(a)). Within the *P. kudriavzevii, P. kluyveri*, and *C. viswanathii*, sister taxa *C. glabrata* was included in the external grouping. Their grouping was well supported by the MEGA7 analysis. The sequences were deposited in GenBank with the accession numbers MT797173.1 for

Table 1 | Prevalence of fungi grouped by sites of infestation and by sexes of the *P. americana*

Fungus	External surface	Internal surface	Male	Female	Total
Aspergillus niger	11 (20)	11 (20)	15 (27.27)	7 (12.72)	22 (40)
Penicillium italicum	3 (5.45)	1 (1.81)	1 (1.81)	3 (5.45)	4 (7.27)
Mucor plumbeus	3 (5.45)	0.00	1 (1.81)	2 (3.63)	3 (5.45)
Rhizopus oryzae	2 (3.63)	0.00	1 (1.81)	1 (1.81)	2 (3.63)
Pichia kudriavzevii	9 (16.36)	14 (25.45)	12 (21.81)	11 (20.00)	23 (41.81)
Candida glabrata	7 (12.72)	10 (18.18)	8 (14.54)	9 (16.36)	17 (30.90)
Pichia kluyveri	5 (9.09)	9 (16.36)	4 (7.27)	10 (18.18)	14 (25.45)
Candida viswanathii	1 (1.81)	7 (12.72)	5 (9.09)	3 (5.45)	8 (14.54)

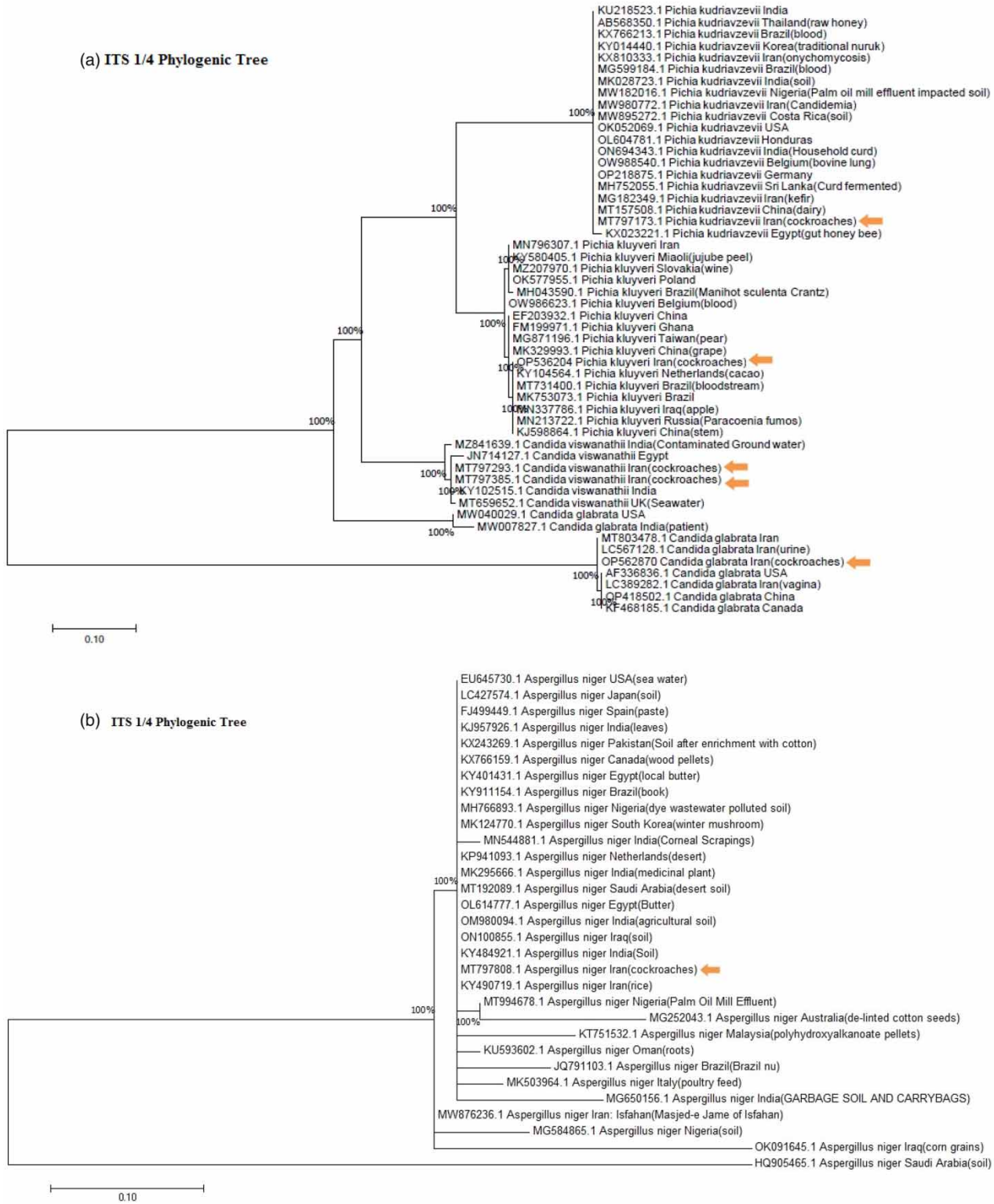


Figure 1 | Dendrograms of *P. kudriavzevii*, *C. viswanathii*, *P. kluyveri*, *C. glabrata* (a), and *Aspergillus niger* (b) strains isolated from the American cockroach (*P. americana*). Trimmed internal transcribed spacer 1/4 (ITS 1/4) was aligned using MEGA7 software. Orange arrows indicate themes identified in this study (GenBank accession numbers MT797173.1, MT797293.1, MT797385.1, MT797808.1, OP562870.1, and OP536204.1. Please refer to the online version of this paper to see this figure in color: http://dx.doi.org/10.2166/wh.2023.015.

P. kudriavzevii, OP536204.1 for *P. kluyveri*, MT797293.1 and MT797385.1 for *C. viswanathii*, and OP562870.1 for *C. glabrata* (Figure 1(a)).

In the phylogram, the sequence of *P. kudriavzevii* (MT797173.1) identified in this study clustered with the *P. kudriavzevii* species previously identified from environmental samples related to gut honey bee and dairy products from China and Egypt as the nearest neighbors. Sequence phylogenetic analysis for the *P. kluyveri* isolate showed that this isolate had the most sequence similarity with *P. kluyveri* isolated from grape, pear, and cocoa samples from China, Taiwan, and the Netherlands. In addition, *C. viswanathii* was similar to GenBank sequences JN714127, KY102515, and MT659652 (isolated from seawater) from Egypt, India, and the United Kingdom. Furthermore, the *C. viswanathii* differed from GenBank accession number MZ841639 and has been previously identified. *C. glabrata* isolate identified in this study had the highest phylogenetic similarity with the known strain of *C. glabrata* in clinical samples such as urine.

The same was observed for isolate MT797808.1 *A. niger*, which grouped with other isolates of *A. niger* from GenBank, also showing the genetic distance from them (Figure 1(b)). Sequence comparisons also indicated that the novel *A. niger* strain is most closely related to previous *A. niger* isolates from rice and soil samples from India and Iran. Three isolates associated with the soil sample have the highest sequence similarity, as shown in Figure 1(b) based on the analysis of ITS-rDNA sequences.

DISCUSSION

Fungal infections often affect immunocompromised and critically ill patients with severe underlying diseases after ingestion, inhalation, or traumatic implantation of spores (Venkatesan *et al.* 2005). Predisposing factors to fungal infections including low age of birth, old age, diabetes mellitus, hemodialysis, neutropenia, AIDS, longer stay in the intensive care unit, respiratory diseases, vascular surgery, presence of CVC, urinary catheter, and prolonged exposure to broad-spectrum antibiotics and antifungal drugs are the most common risk factors for acquiring pathogenic fungi (Seyoum *et al.* 2020).

Although person-to-person contact and the use of medical devices to patients are common methods of transmission for fungal infections, the possible role of vectors such as rodents or insects cannot be excluded (Pai *et al.* 2004). These insects are often present in large numbers in hospital wards. Their omnivorous habit of feeding and indiscriminate deposition of fecal material make them ideal agents for harboring and transmitting microorganisms (Pai *et al.* 2004). Generally, the medical importance of cockroaches is much greater than realized, as they have been shown to carry diverse pathogenic and nonpathogenic bacterial flora, protozoans, helminths, fungi, and viruses (Menasria *et al.* 2014).

The results showed widespread fungal contamination of American cockroaches collected from the surveyed hospitals. In hospital environments, cockroaches could be efficient carriers of nosocomial infections through the spread of pathogenic agents, especially in patients such as those who have undergone surgery and those whose host defenses are suppressed (Fakoorziba *et al.* 2010).

In this study, 49 of 55 (89.09%) cockroaches were carriers of 8 species of medically necessary fungi, including *A. niger*, *P. italicum*, *M. plumbeus*, *R. oryzae*, *P. kudriavzevii*, *C. glabrata*, *P. kluyveri*, and *C. viswanathii*. Interestingly, six of these eight species (*A. niger*, *P. italicum*, *P. kudriavzevii*, *C. glabrata*, *P. kluyveri*, and *C. viswanathii*) were isolated repeatedly on the inner and outer surfaces of *P. americana* throughout the 2 years of the study. The repeated isolation of these fungi suggests that they are closely associated with the *P. americana* collected in this study. Our study revealed that fungi from *Pichia* and *Aspergillus* prevailed over others. Furthermore, the fungi were more recorded on the cockroaches' internal surface (94.54%) than on the external surface (74.54%).

The present study approved that cockroaches were contaminated with fungi of medical importance in hospital sewer systems. Other previous reports have also reported that hospital cockroaches could carry medically important fungi and bacteria (Fakoorziba *et al.* 2010; Kassiri *et al.* 2018; Zahraei-Ramazani *et al.* 2018). In the north of Iran, Motevali-Haghi *et al.* (2014) isolated several fungal strains from American cockroaches, including *Aspergillus*, *Fusarium*, *Penicillium*, and *Geotrichum* spp., as well as *Candida* and *Rhodotrula* spp., on the external body surfaces of cockroaches. Similarly, in the other study, in the west of Iran, which was conducted by Salehzadeh *et al.* (2007) in public hospitals, the isolated pathogenic fungi included *Candida* spp., *Mucor* spp., *A. niger*, *Rhizopus* spp., *Penicillium* spp., and *Aspergillus fumigatus*; among which *Candida* spp. showed the highest prevalence. In other studies reported from India and Brazil, *Candida* and *Aspergillus* species were the most common fungi found on cockroaches, respectively (Lemos *et al.* 2006; Baggio-Deibler *et al.* 2018). These fungi are opportunistic and known for causing several nosocomial sicknesses (Wisplinghoff *et al.* 2004). *Aspergillus* species can

cause severe and life-threatening infections in immunocompromised patients, such as bone marrow transplant recipients and lung disorders (Salehi *et al.* 2020). *Candida* species are opportunistic and symbiotic yeasts in the natural flora of the human body that can colonize the body without causing symptoms, but in the presence of predisposing factors, they can cause a wide range of infectious diseases in humans (Motoa *et al.* 2017).

According to these facts and the results of the present study, more attention should be paid in healthcare settings to the possible role of American cockroaches in the spread of medically essential fungi among human populations. Moreover, various vector control approaches should reduce American cockroach population densities, particularly in hospitals, where immunocompromised people are more likely to be exposed to opportunistic infections (Hussein 2014).

Following the morphological analysis, this study identified the fungal isolates using PCR and sequencing. PCR was performed on the isolates to amplify the ITS regions in rDNA, using ITS1/ITS4 primer pairs; subsequently, the sequences of the amplicons were obtained and analyzed in GenBank. Differences among the ITS rDNA region sequences are known as an accurate and laborious method for classifying fungi. Using molecular techniques and phylogenetic analyses helps us provide essential insights into fungal pathogens in insect samples (Preuner & Lion 2009). In the phylogenetic analysis, the sequences of all five species were grouped with other related species from GenBank but presented a genetic distance from them. These results emphasize that the fungal isolates from American cockroaches can be accurately identified by genetic sequencing of the ITS rDNA region. In this study, the majority of identified American cockroach fungal pathogens sequences were aligned with species previously identified from environmental samples, including dairy products, fruits, water, and soil samples.

Yeast species, during fermentations, produce volatile metabolites that can be categorized into alcohols, acids, esters, ketones, and phenols. These metabolites are helpful to the organism producing them and other organisms (Aogbosiomwan 2013). On the other hand, fungal species can contaminate agricultural produce before, during, and after harvest and during transportation and processing. Components of fruit and fruit-derived beverages contribute to microbial proliferation and are prone to microbial spoilage. As a result, metabolites from microbial fermentation can attract cockroaches, be raised as a health hazard, and spread infection (Aogbosiomwan 2013). For example, Aogbosiomwan (2013) reported using yeasts, including *P. kudriavzevii* and *Candida ethanolica,* to attract cockroaches at locations at the student residential halls at the University of Technology Malaysia. Their results showed that *P. kudriavzevii* has excellent potential as an eco-friendly cockroach attractant, and this attraction could be a result of the metabolites produced.

In the present study, *C. glabrata* was the only yeast aligned with previously identified sequences from clinical samples such as urine. *C. glabrata* is a species often isolated from the bloodstream of patients with fungal infections and is very important due to its resistance to azole antifungal agents (Nedret Koc *et al.* 2002). Several studies have shown that the species most frequently isolated in cockroaches were *C. glabrata*, *C. parapsilosis,* and *C. pseudotropicalis* (Lemos *et al.* 2006). Therefore, the possible role of vectors such as cockroaches in disseminating nosocomial infections caused by fungal species in susceptible hospital individuals cannot be excluded (Fakoorziba *et al.* 2010). Moreover, isolates of *A. niger*, which in some cases are regarded as opportunistic pathogens, were isolated from cockroach samples (Saichua *et al.* 2008), which has also occurred in our report. *A. niger* has a widespread distribution but is generally isolated from the soil (Saichua *et al.* 2008). Based on the analysis of ITS-rDNA sequences, *A. niger* isolates identified in this study had the highest sequence similarity with the three previous isolates associated with the soil sample. It should be stressed that *Aspergillus* spores in the air may cause sinusitis, arthritis, keratitis, and onychomycosis in immunosuppressed and debilitated reservoir hosts (Lugauskas *et al.* 2004). Therefore, it is interesting to note that one of the reasons for the spread of these opportunistic fungi is possibly due to cockroaches dispersing in the hospital environment (Saichua *et al.* 2008).

Considering that medically necessary fungi have been isolated from cockroaches in hospitals in several studies (Fakoorziba *et al.* 2010; Zahraei-Ramazani *et al.* 2018; Salehi *et al.* 2020), this is a warning signal to alert and prevent fungal infections in hospitals. Therefore, eradicating cockroaches from hospital areas is necessary to control patients' nosocomial fungal and bacterial infections.

Over the last few decades, new molecular tools have dramatically improved the resolution of fungal systematics, and there have been enormous advances in this field (Enkerli & Widmer 2010). Nonetheless, despite the importance of molecular methods in identifying fungal isolates, morphological characteristics are primarily used in clinical laboratories (Khodabandeh *et al.* 2020). Molecular techniques have significant advantages in terms of sensitivity, turnaround time, large-scale throughput, and rapid availability of results (Preuner & Lion 2009).

Although direct examination and culture preparation are the gold standards for diagnosing fungal agents, these methods have disadvantages such as experienced laboratory personnel requirements, unreliable when the organism has a low

count, or inability to differentiate accurately (Kozel & Wickes 2014). Hence, cultivation-independent approaches are now preferred for species identification in environmental samples such as insect corpses because they are frequently more efficient than cultivation-based approaches and avoid time-consuming culture steps. Furthermore, they enable the investigation of difficult species to isolate and cultivate which are morphologically challenging to identify (Enkerli & Widmer 2010). Cultivation-independent analyses have been developed for species identification in various insect samples (Castrillo *et al.* 2007; Enkerli & Widmer 2010). Currently, cultivation-independent quantitative detection tools, mainly PCR assays, have increasingly influenced ecological research on insects and, in combination with other disciplines, have contributed to the progress made during the last decade (Castrillo *et al.* 2007).

Furthermore, by using these techniques, we became aware of the previously underestimated role that covert infections may play in insect populations. Therefore, more emphasis has been placed on the use of molecular methods in the detection of fungal agents in insects (Hendolin *et al.* 2000). In many countries, including Iran, large numbers of cockroaches, especially members of the American cockroach family, which are often found in and around hospital wards, can be considered a potential threat (Zahraei-Ramazani *et al.* 2018). Considering that, in our study and other studies conducted in Iran (Motevali-Haghi *et al.* 2014; Kassiri *et al.* 2018; Zahraei-Ramazani *et al.* 2018), American cockroaches are mentioned as one of the significant sources of fungal contamination, it is suggested to conduct more studies on the relationship between cockroaches and pathogenic fungi. Finally, the limitations of this study were the small number of cockroaches studied and the impossibility of sampling in other seasons of the year. Meanwhile, in the case of PCR, no were selected for sequence determination due to financial limitations.

CONCLUSION

In this study, we used a combination of morphological, molecular techniques, and phylogenetic analysis to reveal the presence of pathogenic molds and yeasts in American cockroaches collected from sewer systems of hospitals in Esfahan, Iran. The PCR assay is a fast, low-cost, and reliable tool for screening the microbial agents involved in American cockroaches. Molecular and phylogenetic approaches will help us better understand the interactions between fungal pathogens and their arthropod reservoir hosts.

ACKNOWLEDGEMENTS

The authors appreciate the cooperation of the employees of the Esfahan Health Research Station, affiliated with the Faculty of Health, Tehran University of Medical Sciences, in conducting field and laboratory operations such as collecting, diagnosing, and dissecting, as well as isolating fungi from American cockroaches.

FUNDING INFORMATION

This study was financially supported (numbering project: 17230507942003) by the Islamic Azad University Research Council, Falavarjan Branch.

DATA DEPOSITION

The identified sequences have been deposited in the NCBI database (http://www.ncbi.nlm.nih.gov/blast) under accession numbers MT797173.1, MT797293.1, MT797385.1, MT797808.1, OP562870.1, and OP536204.1.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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First received 13 January 2023; accepted in revised form 25 March 2023. Available online 11 April 2023