Isolation frequency of medically important fungi and fluconazole resistant of Candida sp from hospital cockroaches.

CURRENT STATUS: UNDER REVIEW

Antimicrobial Resistance and Infection Control

Doreen Anna Mloka dmloka@yahoo.com
Muhimbili University of Health and Allied Sciences School of Pharmacy
Corresponding Author
ORCiD: 0000-0002-7300-1958

George M Bwire
Muhimbili University of Health and Allied Sciences

Kennedy Mwambete
Muhimbili University of Health and Allied Sciences

DOI:
10.21203/rs.2.24339/v1

SUBJECT AREAS
Applied & Industrial Microbiology General Microbiology

KEYWORDS
Cockroaches, fungi, fluconazole resistance
Abstract

Background
Cockroaches are common pests in homes and hospitals. They are known to cause allergic reactions in certain individuals and have found to be potential vectors for various bacterial and parasitic pathogens. This study assessed the potential of hospital cockroaches to act as vectors of medically important fungal pathogens on their external surfaces.

Methods
Cockroaches were captured from the main Intensive care unit (ICU), burn unit, adult surgical wards, pediatric oncology wards, intern hostel kitchen and the central kitchen of a national referral teaching hospital in Tanzania. Normal saline washings from the external surface of cockroaches were cultured on standard mycological media to facilitate isolation and identification of medically important molds and yeasts. Susceptibility of Candida sp isolates to fluconazole was tested using the CLSI M27-A3 microdilution method.

Results
At total of 72 cockroaches were captured from various sites of the hospital between February and March 2015. All cockroaches captured were shown to carry medically importance fungi. A total of 956 medically important fungi were isolated, 57.9% were Candida sp., 23.2% Aspergillus sp., 3.1% Cladosporium sp., 1.8% Rhizopus sp., 1.2% Geotrichum sp., 0.9% Pencillum sp., 0.7% Alternaria sp, 0.6% Fusarium sp, 0.3% Mucor sp and 10.1% others. Aspergillus fumigatus (50.0%) was the most commonly isolated followed by Aspergillus niger (15.8%) among the Aspergillus isolates. Over 16.3% of the Candida isolates not intrinsically resistant to fluconazole showed resistance to this drug. Resistance was most frequently found in Candida pseudotropicalis (23.8%) and Candida glabarata (20.0%) and least in isolates of Candida albicans (6.3%).
Conclusion

The external surfaces of cockroaches from this hospital may act as reservoirs of medically important opportunistic fungi exhibiting resistance to fluconazole.

Background

Cockroaches belong to the order Orthoptera or Dictyoptera and the families Blattidae or Blattelidae. They are common pests of domestic dwellings, hospitals and industrial areas. They tend to live hide in the dark cracks and crevices of kitchens, toilets and food stores; as these are ideal environments in terms of temperature, humidity and sources of nutrition for them to flourish. Kitchens and toilets are areas that are often contaminated with infectious microorganisms including bacteria, viruses, protozoa and fungi, as result it may not be unexpected that cockroaches may be reservoirs of infectious microorganisms. There have been a numbers of studies have suggested that hospital cockroaches may be potential carriers of infectious microorganisms including drug resistant bacteria (1-8). Nevertheless there are limited studies that have looked in role of cockroaches as vectors and reservoirs of opportunistic and HAI associated with fungal pathogens (9) Hospital care associated infections (HAI) are a major public health concern worldwide because of the morbidity, mortality and cost associated with them (10-11). The WHO estimates that the prevalence of HAIs varies between 5.7% and 20.0% in low- and middle-income countries (12). Although elimination of HAIs is impossible, regular surveillance and effective infection control prevention (ICP) programs can help reduce its incidence. However, its implementation in developing countries is a challenge. Many developing country hospitals often lack adequate staff, equipment and supplies to effectively implement effective ICP measures. Basic infection control practices such as hand hygiene, are often not adhered too to the lack of clean running water (13). Moreover, the inability to regularly monitor and evaluate HAIs in these settings, may result in the delayed detection of outbreaks.
Subsequently increasing the morbidity, mortality, and cost associated with these types of infections (14–16).

Despite these impediments, it is imperative that health care institutions, especially in resource-limited settings, strive to identify sources and implement preventative measures to the incidence of HAIs in their environments. Hospital environments provide the perfect environment in terms of temperature, humidity, and sources of nutrition for them to flourish. In Tanzania, there is a limited amount of published data on prevalence of HAI and even less so data on the non-fomite vectors of fungal HAIs (17–20). To our knowledge, this is the first report of a study performed in a Tanzanian hospital, investigating the role of cockroaches as vectors and reservoirs of HAI-associated fungal infections.

METHODS

Methods

Settings

The study to determine the prevalence of medically important fungi on the surface of hospital cockroaches was conducted at a national referral teaching hospital located in Dar es Salaam, Tanzania. The facility is a 1,500 bed hospital, attending 1,000 to 1,200 outpatients per day, admitting 1,000 to 1,200 inpatients per week. The hospital has 25 departments and 106 units.

Study Design

An experimental laboratory-based study was conducted. Hospital cockroaches were trapped, over a period of two months between February and March 2017. Cockroach adhesive Sticky paper (Bbac®) was placed overnight on the floors of wards of the burn unit, intensive care unit, surgical department, neonatal unit, the hospital main kitchen, pediatric oncology, and the students intern hostel kitchen at MNH. Trapped live
cockroaches were collected using sterile forceps and placed in sterile capped test tubes for transportation to the laboratory.

**Fungal Isolation, Culture and identification**

Sterile normal saline (10mls) was added to each test tube and the live cockroaches were thoroughly shaken for 2 minutes. Cockroach washings (5mls) was then aseptically removed to sterile tube for six set of 10 serial dilutions ranging from 1/10 to 1/10^6 of cockroach washings were then serially diluted in sterile water and 1 ml duplicate aliquots of the washings were cultured on Sabouraud’s dextrose agar (*Ponadisa*) containing 0.5% chloramphenicol and gentamicin 0.05%. Plates were incubated at 30°C for 3 weeks and examined daily for viable counts. Filamentous fungi and yeast colonies were identified by using their microscopic and macroscopic characteristics, such as topography, texture, pigmentation, mycelium type, hyphae form, spore type and type of reproductive structure. Yeast were further identified by the germ tube test, the presence of chlamydo conidia on Corn meal plus Tween 80 agar (*Oxoid, United Kingdom*) and color of colonies on CHROMagar™ Candida.

**Antifungal susceptibility profile of Candida Isolates**

Susceptibility to fluconazole on 60 randomly selected Candida sp Isolates was tested using the CLSI M27-A3 microdilution method (21). Reference grade fluconazole was obtained powder from Sigma Aldrich, Germany. Antifungal susceptibility testing were performed in microdilution plates in which 0.1 mL of fluconazole 3X concentrate was used. The inocula were prepared from overnight (24-48 h) cultures in Sabouraud dextrose agar. The inoculum suspension was prepared using a spectrophotometer to produce by a 0.5 Mac Farland Standard at 530nm wavelength to produce standard inoculum of approximately 1 x 10^6 cells per ml. The stock fluconazole solution was diluted in RPMI-
1640 buffered with morpholinepropanesulfonic acid (MOPS). On the day of testing, microdilution plates containing 100 µL of RPMI-1640 and serial dilutions of fluconazole were inoculated with 100 µL of diluted culture, resulting in $0.5 \times 10^3$ to $2.5 \times 10^3$ cells/mL in each well. The plates were incubated at 35°C for 24-48 h, and the MIC endpoint was determined and interpreted according to CSLI M27-A3 guidelines. *C. parapsilosis* ATCC 22019 served as the quality control isolate.

**Data processing and analysis**

Laboratory data were entered and analysed using SPSS version 20. Descriptive statistics was presented as percentages in tables and figures.

**Results**

A total of 72 cockroaches were captured from the different wards of the tertiary teaching hospital as shown in Figure 1. The majority of cockroaches were trapped from the Main hospital kitchen (15), followed by the Intern kitchen (14) Burn unit (9) and Neonatal unit (11), no cockroaches were captured from the intensive care unit.

The highest total fungal viable counts were found in cockroaches from the main hospital kitchen ($3.5 \times 10^5$cfu/ml) followed by the surgical wards ($2.8 \times 10^5$cfu/ml) and pediatric oncology wards ($2.0 \times 10^5$cfu/ml) respectively as seen in Figure 2.

A total of 956 medically important fungi were isolated. The majority of isolates were Candida sp. Overall 554 isolates were of Candida sp., 222, Aspergillus sp., 30 Cladosporium sp., 17 Rhizopus sp., 11 Geotrichum sp., 9 Pencillium sp., 7 *Alternaria* sp, 6 Fusarium sp, 3 Mucor sp and 97 others as seen in figure 3.

Medically important Candida Sp. Including *Candida albicans* were isolated and identified (23%) as can be seen in Figure 4.

In addition to Candida sp., isolates of opportunistic pathogenic Aspergillus sp like
Aspergillus fumigatus (50%) were also identified as can be seen in Figure 5. Among the 108 randomly selected Candida isolates, 16.3% showed resistance to fluconazole. The majority of the resistant isolates found in isolates of *C. parapsilosis* and *C. glabarata* as shown in Table 1.

**Discussion**

Fungal HAIs infections continue to be a major cause of morbidity and mortality despite the current developments in terms of diagnosis and therapeutic options. This study is the first description of the fungal profile of the external surfaces of hospital cockroaches in a Tanzanian tertiary teaching hospital. The study demonstrated that hospital cockroaches represent a potential reservoir of fungal pathogens associated with HAIs. The presence of 7 medically important fungal genera including Candida, Aspergillus, Cladosporium, Mucor, Fusarium, Rhizopus and Pencillum on the outer surfaces of hospital cockroaches are similar to results of other studies conducted in Iran, Iraq and Brazil (4-6). This high fungal carriage rate (100%) is quite significant in terms of its implication with regards to the transmission of fungal pathogens associated with HAIs. Especially considering the habits of these insect, that tend to move freely over various hospital arears. The fact these insects and when feeding, regurgitate food from their crop and may defecate on hospital surfaces, suggests that these insects may have a role to play in the spread of infectious fungal agents. The finding that a high percentage of tested cockroaches were contaminated with Candida sp isolates (57.9%) and Aspergillus sp (23.2%) respectively seems further proof that these insects may vectors for the spread of nosocomial fungal agents. Particularly as considering that the presence of *C. pseudotropicalis*, *C. albicans*, *C. parapsilosis* *C. glabarata* and *C. rugosa*, known fungal pathogens. That are increasingly being reported as agents of life threatening and drug resistant HAIs associated with high treatment costs, morbidity and mortality (22). *C. pseudotropicalis*, *C. albicans*,
C. parapsilosis and C. glabarata, are known to be important causes of blood stream infection in neonates, transplant recipients, granulocytopenic patients and surgical patients (22-27). While C. rugosa is now more and more being recognized as an emerging pathogen of blood stream infections of individuals who are immunocompromised (28). The isolation of Aspergillus fumigatus and A. niger further supports the finding that hospital cockroaches may be potential reservoirs of major fungal pathogens associated with life threatening HAIs infections of immunocompromised individuals. These isolates are known to be major causes of the invasive fungal infections particularly in transplant and Acquired immunodeficiency syndrome (AIDS) patients (25-26). The presence of other known causes of opportunistic and nosocomial fungal infections species such as Cladosporium, Mucor, Fusarium, Rhizopus and Pencillum, further cements the concept that cockroaches in hospitals may be major public health concern and confirm the findings of other studies conducted on domestic and hospital cockroaches (1-9). What the study findings indicate is that there needs to be more studies to be conducted to ascertain if medically important fungi on the surfaces of hospital cockroaches are etiological agents of HAIs including of newer fungal pathogens immunocompromised patients such as Talaromyces marneffei (29).

The majority of cockroaches were trapped from the Main hospital kitchen and none cockroaches were captured from the main intensive care unit of this hospital. This indicates that infection prevention and control measures are being to adhered in sensitive hospital areas. However there needs to be concentrated efforts for effective fumigation practices in the food handling areas. Especially considering the fact, that cockroaches carrying drug resistant pathogens may wander from any part of the hospital through the hospital sewage systems unnoticed. Moreover, hospital environmental control teams therefore needs to do a thorough assessment of the resistance of the hospital cockroaches.
to common pesticides before on deciding what chemicals to use. This is view of recent study findings that have shown that cockroaches in domestic and hospital settings are becoming resistant to common pesticides (30).

Fluconazole has been the corner stone therapeutic option for many African countries in treating fungal infections particularly for vaginal and oral candidiasis. This study found that 16.3% of randomly selected Candida isolates exhibited resistance to fluconazole. This is higher than has been previously reported in Tanzania were it shown that resistance to fluconazole was only 6.8% (30). This finding maybe a warning sign for the continued empirical use fluconazole for prophylaxis and or treatment of systemic or localized Candida infections in African and Tanzanian health care setting as resistance to fluconazole is on the rise (31). The presence of fluconazole resistant Candida sp isolate on the outer surfaces of cockroaches thus clearly suggests that cockroaches can act as vectors to transmit drug resistant fungal strains in hospital settings, especially within hospitals that lack of effective pest control measures.

A limitation of this study, is that it only determined only the prevalence of fungal isolates on the surfaces of hospital cockroaches. The study did not use molecular tools characterize the isolates and compare them with isolates of confirmed causes of HAIs infections at this hospital. Moreover, the antifungal susceptibility testing was only done yeasts and using only one drug fluconazole.

**Conclusion**

Hospital cockroaches’ outer surfaces may be an important reservoir of potentially drug resistant fungal pathogens associated hospital acquired infections. There is a need for hospital to assess their pest control measures to ensure they are effective against cockroaches. We recommend that similar studies be conducted to involve other potential infectious pathogen vectors that are commonly found in hospital environments such as
flies and rats.

Abbreviations

AIDS - Acquired immunodeficiency syndrome

ATCC - American Type Culture Collection

CLSI - Clinical & Laboratory Standards Institute

HAIs - Hospital acquired infections

WHO - World Health Organization

Declarations

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Availability of data and Materials**

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

**Competing Interests**

The authors declare no competing interest.

**Funding**

This was a non-funded project; the investing author used their own funds to support the data collection and analysis

**Author Contribution**

DM participated in fungal identification, antifungal susceptibility testing and drafted the manuscript. GB participated in antifungal susceptibility testing. KD conceived the study and drafted the manuscript. All authors read and approved the final manuscript.
Acknowledgments

The author wishes to thank the management of leadership of Muhimbili National Hospital and Muhimbili Orthopedic Institute for their support and Mr Ezeikiel M. Marandu for his technical assistance.

References

1. Donkor E. Review Nosocomial Pathogens: An In-Depth Analysis of the Vectorial Potential of Cockroaches. Trop. Med. Infect. Dis. 2019, 4, 14; doi:10.3390/tropicalmed4010014

2. Moges F, Eshetie S, Endris M, Huruy K, Muluye D. Multidrug Resistant Strains in Gondar Town, Ethiopia. BioMed Research International Volume 2016, Article ID 2825056

3. Tilahun B. Worku B, Tachbele E, Terefe S, Kloos H and Legesse W. High load of multidrug resistant nosocomial neonatal pathogens carried by cockroaches in a neonatal intensive care unit at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. Antimicrobial Resistance and Infection Control 2012, 1:12

4. Motevali Haghi SF, Aghili SH, Gholami SH, Salmanian B, Nikokar SH, Khangolzadeh M. Isolation of medically important fungi from cockroaches trapped at hospitals of Sari, Iran. Bull. Env. Pharmacol. Life Sci., Vol 3 [Special Issue V] 2014: 29-3

5. Lemos AA, Lemos JA, Prado MA, Pimenta FC, Gir E, Silva HM, Silva MR. Cockroaches as carriers of fungi of medical importance. Mycoses. 2006 Jan; 49(1):23-5.

6. Salehzadeha A, Tavacolb P, Mahjubc H. Bacterial, fungal and parasitic contamination of cockroaches in public hospitals of Hamadan, Iran; J Vect Borne Dis 44, June 2007, pp. 105-110

7. Geravi H, HajatFotedar R, Shriniwas UB, Verma A. Cockroaches (Blattella germanica) as carriers of microorganisms of medical importance in hospitals. Epidemiol Infect
8. Kassiri H and Kazemi S. Cockroaches [Periplaneta americana (L.), Dictyoptera; Blattidae] as Carriers of Bacterial Pathogens, Khorramshahr County, Iran. Jundishapur J Microbiol. 2012;5 (1):320-322. DOI: 10.5812/kowsar.20083645.2434

9. Fotedar R and Banerjee U. Nosocomial Fungal Infections: Study of the possible Role of Cockroaches (Blattella germanica) as Vectors. Acta Tropica 1992; 50: 339-343

10. A Nair, WJ Steinberg, T Habib, H Saeed & JE Raubenheimer (2018) Prevalence of healthcare-associated infection at a tertiary hospital in the Northern Cape Province, South Africa, South African Family Practice, 60:5, 162-167, DOI: 10.1080/20786190.2018.1487211

11. Allegranzi B, Bagheri-Nejad S, Combescure C, Graafmans W, Attar H, Donaldson L, Pittet D. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet 2011, 377:228–241.

12. Health care-associated infections- FACT SHEET, http://www.who.int/gpsc/country_work/gpsc_ccisc_fact_sheet_en.pdf, accessed December 19th 2019

13. Chawla S, Gupta S, Onchiri F, Habermann E, Kushner A, Stewart T. Water availability at hospitals in low- and middle-income countries: implications for improving access to safe surgical care. Journal of Surgical Research; September 2016, Volume 205, Issue 1, Pages 169–178.

14. Loftus MJ, Guitartb C, Tartarib E, Stewardsona AJ, Amerc F, Rodriguesd F, Fong Leee Y, Mehtarf S, Sitholef BL and Pittet D. Hand hygiene in low- and middle-income countries. International Journal of Infectious Diseases 86 (2019) 25–30

15. Gichuhi A, Kamau S, Nyangena E, Otieno-Ayayo Z. Health Care Workers Adherence to Infection Prevention Practices and Control Measures: A Case of a Level Four District
Hospital in Kenya. American Journal of Nursing Science 2015; 4(2): 39-44

16. Lowman W. Active surveillance of hospital-acquired infections in South Africa: Implementation, impact and challenges; SAMJ, S. Afr. med. j. vol.106 n.5 Cape Town May. 2016http://dx.doi.org/10.7196/samj.2016.v106i5.10783

17. Eriksen HM, Chugulu S, Kondo S, Lingaas E. (2003) Surgical-site infections at Kilimanjaro Christian Medical Center. Journal of Hospital Infection55: 14-20.

18. Fehr J, Hatz C, Soka I, Kibatala P, Urassa H, Smith T, Mshinda H, Frei R, Widmer A. (2006) Risk factors for surgical site infections in a Tanzanian district hospital: A challenge for the traditional national nosocomial infections surveillance system index. Infection Control and Hospital Epidemiology27: 1401-1404.

19. Akoko, LO, Mwanga AH, Fredrick F, Mbembati NM. Risk Factors of Surgical Site Infection at Muhimbili National Hospital, Dar es Salaam, Tanzania. Vol 17, No 3 (2012)

20. Gosling R, Mbatia R, Savage A, Mulligan J, and Reyburn H. Prevalence of hospital-acquired infections in a tertiary referral hospital in northern Tanzania. Ann Trop Med Parasitol. 2003 Jan; 97(1):69-73.

21. CLSI: Reference method for broth dilution antifungal susceptibility testing of yeasts: 3rd M27-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

22. Orasch C, Marchetti O, Garbino J, Schrenze J, Zimmer S, et al; Candida species distribution and antifungal susceptibility testing according to European Committee on Antimicrobial Susceptibility Testing and new vs. old Clinical and Laboratory Standards Institute clinical breakpoints: a 6-year prospective candidaemia survey from the fungal infection network of Switzerland; Clin Microbiol Infect 2014; 20: 698–705.

23. Fridkin S. The Changing Face of Fungal Infections in Health Care Settings. Clinical
Infectious Diseases 2005;41:1455–60

24. Singh N. Trends in the Epidemiology of Opportunistic Fungal Infections: Predisposing Factors and the Impact of Antimicrobial Use Practices. Clinical Infectious Diseases 2001;33:1692–6

25. Pfaller MA, Diekema DJ, Colombo AL, Kibbler C. Ng Kp, Gibbs DL, Newell VA. Candida rugosa, an Emerging Fungal Pathogen with Resistance to Azoles: Geographic and Temporal Trends from the ARTEMIS DISK Antifungal Surveillance Program. Journal of Clinical Microbiology, Oct. 2006, p. 3578–358

26. Trofa D, Ga´cser A, Nosanchuk J. Candida parapsilosis, an Emerging Fungal Pathogen. Clinical Microbiology Reviews, Oct. 2008, p. 606–625.

27. Pfaller MA and Diekema DJ. Rare and Emerging Opportunistic Fungal Pathogens: Concern for Resistance beyond Candida albicans and Aspergillus fumigatus. J. Clin. Microbiol. 2004, 42(10):4419.

28. Vuichard D, Weisser M, Orasch C, Frei R, Heim D, Passweg JR and Widmer AF. Weekly use of fluconazole as prophylaxis in haematological patients at risk for invasive; BMC Infectious Diseases 2014, 14:573

29. Gorai S, Saha M, Madhab V, Mitra S. Talaromycosis (Penicilliosis): A Rare, Opportunistic Systemic Fungal Infection. Indian J Dermatol. 2019;64(4):331–333. doi:10.4103/ijd.IJD_70_17

30. Hamza OJ, Matee MI, Moshi MJ, Simon EN, Mugusi F, Mikx FH, Helderman WH, Rijs AJ, van der Ven AJ, Verweij PE. Species distribution and in vitro antifungal susceptibility of oral yeast isolates from Tanzanian HIV-infected patients with primary and recurrent oropharyngeal candidiasis. BMC Microbiol. 2008 Aug 12;8:135. doi: 10.1186/1471-2180-8-135.

31. Africa CWJ and Abrantes PMdS. Candida antifungal drug resistance in sub-Saharan
Table

Table 1. Candida ssp. isolates resistance to Fluconazole

| Species        | Number of Isolates | Susceptible (S) | Susceptible dose dependent (SDD) | R-Resistant Percentage Resistant isolates (%) |
|----------------|-------------------|-----------------|----------------------------------|-----------------------------------------------|
| C. pseudotropicalis | 42                | 18              | 14                               | 10                                           |
| C. albicans     | 32                | 22              | 8                                | 2                                            |
| C. rugosa       | 8                 | 6               | 1                                | 1                                            |
| C. parapsilosis | 16                | 5               | 2                                | 3                                            |
| C. glabrata     | 10                | 8               | 1                                | 2                                            |

Key: Candida isolates except for C. glabrata; S-Susceptible if MIC was ≤2µg/ml; SDD- Susceptible dose dependent if MIC was 4µg/ml and R-Resistant if MIC was ≥8µg/ml

For C. glabrata; SDD- Susceptible dose dependent if MIC was ≤32µg/ml and R-Resistant if MIC was ≥64µg/ml respectively (21).

Figures
Figure 1
Cockroach Distribution in various areas of the hospital

Figure 2
Fungal Load on Cockroach Surfaces

Fungal Load cfu/ml
Figure 3

Fungal Isolates
**Candida spp., Isolation Frequency**

![Candida spp. Isolation Frequency Graph](image)

- **Figure 4**

  Candida sp. isolates on the external surfaces of cockroaches

**Aspergillus spp., Isolation Frequency**

- **Figure 5**

  Aspergillus sp. Isolation frequency on the external surfaces of cockroaches
