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

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Research

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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 HAI s varies between 5.7% and 20.0% in low- and middle-income countries (12). Although elimination of HAI s 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 HAI s 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 HAI s 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 of published data on prevalence of HAI and even less so data on the non-fomite vectors of fungal HAI s (17–20). To our knowledge, this is the first report of a study performed in a Tanzanian hospital, investigation 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 te 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 (Bbacb®) 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 x 10^3 to 2.5 x 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 X10^5 cfu/ml) followed by the surgical wards (2.8 X10^5 cfu/ml) and pediatric oncology wards (2.0 X10^5 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 Pencillium 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 areas. 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 immunocomprised (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 a 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 that 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.

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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.

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References


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Table

Table 1. Candida ssp. isolates resistance to Fluconazole
<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Isolates</th>
<th>Susceptible (S)</th>
<th>Susceptible dose dependent (SDD)</th>
<th>R-Resistant</th>
<th>Percentage Resistant isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. pseudotropicalis</td>
<td>42</td>
<td>18</td>
<td>14</td>
<td>10</td>
<td>23.8</td>
</tr>
<tr>
<td>C. albicans</td>
<td>32</td>
<td>22</td>
<td>8</td>
<td>2</td>
<td>6.3</td>
</tr>
<tr>
<td>C. rugosa</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>16</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>18.8</td>
</tr>
<tr>
<td>C. glabarata</td>
<td>10</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Key:  Candida isolates except for C. glabarata; 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. glabarata; 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

Number of cockroaches trapped per site

<table>
<thead>
<tr>
<th>Ward</th>
<th>Cockroaches Trapped</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU</td>
<td>9</td>
</tr>
<tr>
<td>Burn Unit</td>
<td>15</td>
</tr>
<tr>
<td>Main Kitchen</td>
<td>14</td>
</tr>
<tr>
<td>Intern Hostel Kitchen</td>
<td>7</td>
</tr>
<tr>
<td>Surgical Wards</td>
<td>5</td>
</tr>
<tr>
<td>Pediatric Oncology</td>
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</tbody>
</table>

Fungal Load cfu/ml

<table>
<thead>
<tr>
<th>Hospital Site</th>
<th>Fungal Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU</td>
<td>0</td>
</tr>
<tr>
<td>Burn Unit</td>
<td>1.09</td>
</tr>
<tr>
<td>Main Kitchen</td>
<td>3.51</td>
</tr>
<tr>
<td>Intern Hostel Kitchen</td>
<td>1.11</td>
</tr>
<tr>
<td>Surgical Wards</td>
<td>2.8</td>
</tr>
<tr>
<td>MOI</td>
<td>2.02</td>
</tr>
</tbody>
</table>
Fungal Isolates from Cockroach Surfaces

Figure 3

Fungal Isolates
Figure 4

Candida sp. isolates on the external surfaces of cockroaches
Figure 5

Aspergillus sp. Isolation frequency on the external surfaces of cockroaches