Repurposing FDA approved drugs against the human fungal pathogen, *Candida albicans*

Kevin Kim, Leeor Zilbermintz and Mikhail Martchenko*

**Abstract**

**Background:** The high cost and prolonged timeline of new drug discovery and development are major roadblocks to creating therapies for infectious diseases. *Candida albicans* is an opportunistic fungal pathogen that is the most common cause of fatal fungal infections in humans and costs $2–4 billion dollars to treat in the US alone.

**Methods:** To accelerate drug discovery, we screened a library of 1581 existing FDA approved drugs, as well as drugs approved abroad, for inhibitors of *C. albicans*. The screen was done on YPD yeast growth media as well as on the serum plate assay developed in this study.

**Results:** We discovered that fifteen drugs, all of which were originally approved for treating various infectious and non-infectious diseases, were able to kill *Candida albicans*. Additionally, one of those drugs, Octodrine, displays wide-spectrum anti-microbial activity. Compared to other selected anti-*Candida* drugs, Octodrine was shown to be one of the most effective drugs in killing serum-grown *Candida albicans* without significantly affecting the survival of host macrophages and skin cells.

**Conclusions:** This approach is useful for the discovery of economically viable new therapies against infectious diseases.

**Keywords:** Antifungal, Drug-discovery, Off-label drug use, Small molecules

**Background**

While almost all of us possess *Candida albicans* in our oral cavity, gastrointestinal tracts, genitourinary tracts, and on skin as a relatively harmless commensal organism, *C. albicans* is a major systemic fungal pathogen in humans [1]. *Candida* evades and escapes from the host’s innate immunity, causing irritating and recurrent infections that can range from thrush in immunocompetent colonized hosts, to life-threatening systemic infections in immunocompromised individuals such as patients with HIV, or those receiving immunesuppressing cancer chemotherapy and corticosteroids. Surprisingly, only 10 to 20% of individuals who develop bloodstream *Candida* infections are seriously immunocompromised. A large majority of patients develop *Candida* infections because they have become more susceptible while hospitalized due to the use of broad-spectrum antibiotics, surgery, and intravenous catheters. As a result, infections from *C. albicans* ranks as the fourth most common hospital-acquired infection in the United States. The cost of treating bloodstream *Candida* infections is $2–4 billion per year in the US alone [2]. In the US, annual incidence of systemic candidiasis is approximately 70,000 cases per year, which results in the death rate of about 30 to 40%, even after treatment with antifungal therapy [3].

The situation is especially grave in cancer patients. The incidence of *Candida* infection in all cancer patients is very high, ranging from 40 to 88% [4, 5]. The mortality rate among the *Candida* infected cancer patients reaches an alarmingly high 75% amongst the Filipino and Pacific Islanders [5]. Early antifungal treatment, although poorly tolerated by the host, is mandatory to improve the survival of cancer patients. Unfortunately, 30% of *Candida* isolates are resistant to all antifungal treatments [6].

Host serum plays a prominent role in the pathogenicity of *C. albicans*. On the one hand, serum promotes morphological switching of *Candida* from yeast to hyphal forms, which is necessary for its evasion of phagocytosis and organ colonization [7]. On the other hand, serum is known to
inhibit the activity of known antifungal drugs [8]. Taken together, these laboratory observations explain in part the clinical mortality observed during Candida blood infections, even when patients are treated with antifungals [5].

Several clinical and laboratory data suggests that currently available antifungal therapies are mostly ineffective in treating Candida infections [9]. Despite extensive research dedicated to the development of new therapeutic strategies, there are only a limited number of available drugs to fight against invasive fungal infections. Indeed, only four molecular classes targeting three distinct fungal metabolic pathways are currently used in clinical practice to treat systemic fungal infections. These include: fluoropyrimidine analogs, polyenes, azoles, and echinocandins [9, 10]. However, the efficacy of some of these drugs is severely limited because of their unacceptable toxicity, poor activity in blood, or the emergence of resistance; thereby underscoring an urgent necessity for new antifungal agents. Several other classes, such as morpholines and allylamines are only used as topical agents due to either their poor efficacy, or severe adverse effects when administered systemically [9, 10].

Unfortunately, the development of an entirely new drug is a long and expensive process. New drugs have to undergo an arduous approval process by the FDA in order to establish safety of the drug for human consumption [11]. We propose that the repurposing of existing FDA-approved drugs as antifungal agents may decrease the time and effort of bringing drugs with novel antifungal activity from the bench to the bedside. Recently, another group investigated the ability of FDA-approved drugs to inhibit C. albicans biofilm formation by screening the Prestwick Library, a commercially available chemical library of 1200 drugs [12]. However, C. albicans biofilm formation is just one of many pathogenesis strategies, such as yeast-to-hyphal phenotypic switching, white-opaque phenotypic switching, ability to adhere to mammalian tissues, and secretion of aspartyl proteinases [1, 7]. The goal of this study is to identify drugs capable of killing blood-borne Candida albicans, and we use serum as an in vitro surrogate of host blood. To this end, we have tested the anti-Candida activity of drugs from the Johns Hopkins Clinical Compound Library (JHCCL) [13] on Candida grown on serum-containing media. This library consists of drug-compounds that are FDA-approved with a diverse range of functions, mechanisms of action and well-characterized pharmacological and toxicological properties.

**Methods**

**Candida albicans and bacterial strains**

Strain SN250 is the wild type reference strain of C. albicans. It serves as the reference strain for our genetic knockout library screen. It is derived from the wild type strain SC5314, a human clinical isolate recovered from a patient with generalized candidiasis [14]. SN250 was used for drug screening experiments. The bacterial strains consisted of Bacillus cereus strain 10987 and Escherichia coli strain C600. The genetic screen for mutant sensitivity to Octodrine was tested with three C. albicans libraries that were previously created in [15–17].

**Media and growth conditions**

C. albicans strains were cultured in liquid YPD medium at 30 °C overnight. E. coli and B. cereus were cultured in liquid LB medium at 37 °C and 30 °C, respectively, shaking overnight. A novel method to incorporate fetal bovine serum to agar was devised. Fetal bovine serum was preheated in a water bath set at 65 °C. We found that isothermal conditions of the two mixtures eliminated the formation of foam upon coalescence. The agar solution for the serum mixture consisted of 16 g agar, which was then brought up to 300 ml with nanopure water. The agar solution was then autoclaved at 120 °C for 45 min. The agar and serum mixtures were then amalgamated while in their isothermal states.

**Chemicals**

An FDA-approved drug library comprising of 1500 drugs was purchased from Johns Hopkins, titled, Johns Hopkins Clinical Compound Library (JHCCL) version 1.0. The drugs arrived as 10 mM stock solutions in sealed microtiter plates and were made using DMSO or water as solvents. Drugs were arrayed in 96-well plates and screened at a stock concentration of 10 mM. Drugs were from Fisher and Sigma and were of the highest purity available. The library was stored at –20 °C until use. Prior to use, the library of drugs was thawed at room temperature. Drugs of interest were isolated and reproduced from prepared 10 mM solutions. Antimycin A, Captan, Chlorquinaldol, Clotrimazole, Disulfiram, Fluvasatin, Mycophenolic Acid, Methylbenzethonium Chloride, Miconazole, Nitroxoline, Octodrine, Pirithione Zinc, Fluconazole, and Octanoic Acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nifuroxime was purchased from MP Biochemicals (Solon, OH, USA). All drugs were prepared to 10 mM using DMSO as the solvent. DMSO was purchased from Amresco” (Solon, OH, USA). Fetal bovine serum (Triple Membrane 0.1 μm filtered) was purchased from GeneMate BioExpress (Kaysville, UT, USA).

**Screening assay**

The sensitivity of C. albicans to drugs was assessed by a chemical screen. The absorbance at optical density at 600 nm (OD₆₀₀) of yeast and bacterial overnight cultures were determined for each of our experiments. The absorbance values were then converted to cells/ml using McFarland’s scale. Twenty five million Candida cells were added to all 10 cm petri dishes; 600 million bacterial cells were added to all 10 cm bacterial petri dishes.
Five microlitre of each drug was placed directly on the agar surface using a multichannel pipette and slight contact of the tip to the agar made to leave an impression to facilitate later analysis. Drugs that were replicated were done on petri dishes, following the same protocol in at least five independent experiments. For the replication studies, only a single drug was placed per plate. The plates were incubated at either 25 or 37 °C for 24 h. The drugs-of-interest were selected on their ability to produce a distinct zone of inhibition of fungal growth greater than the zone made by DMSO alone and at the same time that is comparable to, if not greater than the positive control, Fluconazole. The zones of inhibition were quantified by measuring their diameters in mm, as recommended by the Clinical Laboratory Standards Institute (CLSI) procedures outlined in the manual M44-A2 [18, 19]. In addition, we utilized the software ImageJ [20] to digitally quantify the magnitude of every zone of inhibition.

*E. coli* and *B. cereus* were cultured overnight in liquid LB media at 37 and 30 °C respectively. The sensitivity of *E. coli* and *B. cereus* to Octodrine was assessed by spreading 6 × 10^8 cells of their respective overnight cultures onto petri dishes containing solid LB media. 5 μl of neat Octodrine was placed directly on the agar surfaces. The plates with *E. coli* were incubated at 37 °C, while plates with *B. cereus* were incubated at 30 °C for 24 h.

**Determination of minimal effective drug concentrations by drug diffusion susceptibility testing**

Plates inoculated with *C. albicans* were prepared using the protocol described above. To elucidate the ability of the varying concentrations of drugs to form zones of inhibition, two-fold serial drug dilution experiments were performed. To perform the first two-fold dilution, one part of the 10 mM stock solution was mixed with one part DMSO. Each subsequent dilution was done with aliquots from the prior dilution mixed with equal parts of DMSO. 5 μl of each drug dilution was spotted onto a lawn grown on YPD plate, as well as 5 μl of DMSO as a negative control. Dilutions beyond 0.07813 mM that were still forming a significant zone of inhibition were further diluted on a separate plate. Drug-treated plates were then incubated at either 25 or 37 °C for 24 h. Two-fold serial dilutions of Octodrine were done on YPD and Serum plates with undiluted Octodrine as the most concentrated solution.

**Genotypic mutant screening against Octodrine**

Each *C. albicans* knockout strain from one of the three *C. albicans* libraries [15–17] was cultured in individual wells of 96 well plate in 100 μl of YPD media overnight at 30 °C. 5 μl of each Candida knockout overnight culture was spotted onto a YPD solid plate using a multichannel pipette. The cells were then left to absorb into the YPD plates for 1 h at 25 °C. Using a multichannel pipette, 5 μl of Octodrine 10 mM was spotted directly on top of the *C. albicans* cells. The plates were then placed in an incubator set at 30 °C and left overnight. Analysis of plates consisted of isolating any strains that exhibited resistance to 5 μl of 10 mM Octodrine. Resistance was noted by the ability of the Candida cells and the subsequent drug-treated spot to lack a zone of inhibition.

**Mammalian cell culture, drug treatment, and survival assay**

RAW264.7 mouse macrophage cells (ATCC number TIB-71) and human melanoma C32 cells (ATCC number CRL-1585) were maintained in DMEM (Sigma-Aldrich) supplemented with 10 % FBS (Bioexpress) and 100 μg/mL penicillin and 100 μg/mL streptomycin. Mammalian cells (10,000 per well) were seeded in 96-well plates (100 μl/ well) 24 h before the assay. During the assay, 1 μl of 10 mM drug was added to 100 μl of cell-containing media. Two-fold serial dilutions of the media were performed. RAW264.7 and C32 cells were treated with drugs for 24 and 48 h respectively, and determination of cell viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed as described in [21]. Each data point shown in the figure for MTT assays represents the average of results from at least two wells in each of at least two separate experiments. Cell viability is shown as the percentage of survivors obtained relative to untreated cells grown in media only (100 %).

**Image capture and image processing**

All images were taken with an 8MP iSight camera with an aperture size of F2.4 and touch-to-focus capabilities. Images were standardized with a universal template to allow for direct comparison between images. Images are stock and unaltered by any graphics editing software.

**Results**

**Moderate ability of Fluconazole to kill serum-grown Candida albicans at 37 °C**

It has been reported that host serum markedly inhibits growth of the human fungal pathogen, *Candida albicans* [22–24]. We have confirmed these observations by showing that *Candida* wild type strain SN250 [15] is unable to grow in liquid 100 % Fetal Bovine Serum at either 25 or 37 °C (data not shown). However, during the course of our experiments, we have observed that *Candida* is able to grow and establish a lawn on solid serum plates containing 62.5 % v/v FBS and 37.5 % v/v agar solution, at both temperatures (Fig. 1a).

We investigated whether Fluconazole, the widely used anti-*Candida* drug, was able to kill *C. albicans* on solid serum plates. While Fluconazole was shown to be an
effective inhibitor of Candida growth on standard solid YPD plates, (Fig. 1a), we observed that Fluconazole moderately inhibits the growth of Candida on serum plates (Fig. 1a and Additional file 1: Table S1). It should be noted, however that Fluconazole formed prominent zone of inhibition on the solid YPD plates. In addition, we observed that Fluconazole exhibited diminished effectiveness at forming zone of inhibition at 37 °C compared to 25 °C (Fig. 1a and Additional file 1: Table S1). We decided to assess the drug-pathogen interactions at 25 °C in addition to body temperature because there is a serious problem of Candida growth in catheters where it may be exposed to serum at room temperatures [25].

Screening of inhibitors of Candida albicans lawn formation
In light of the moderate effectiveness of Fluconazole to kill C. albicans in serum at the physiologically relevant temperature (37 °C), and in search for alternative antifungal drugs, we decided to screen the library of chemicals
Candida albicans

We identified inhibitors of Candida growth formation by performing a primary screen on the 1581 drugs belonging to the JHCCL. We plated Candida on solid YPD and serum plates, and placed 5 μl of each 10 mM drug on top of the fungal lawn. We tested the effect of exposure to each of the drugs from the library on their ability to inhibit Candida growth, as well as their ability to form a zone of inhibition within the fungal lawn. Based on this screen, we identified 15 drugs that inhibit Candida growth on solely YPD or on both YPD and serum plates in at least five independent experiments at two temperatures, 25 °C and 37 °C (Fig. 1a). We classified the 15 hits into three different classes to facilitate interpretation of our results. They were: 6 hits from 32 known antifungals, 5 hits from 359 antimicrobials/antiseptics, and 4 hits from 1190 other multifunctional drugs (Fig. 1b). While these drugs were chosen in these screens for their ability to inhibit C. albicans lawn formation, the actual levels of inhibition varied from weak to strong inhibition (Fig. 1a and Additional file 1: Table S1).

All 6 selected antifungal drugs, Antimycin A, Captan, Clotrimazole, Fluconazole, Miconazole, and Pyrithione Zinc showed strong inhibition of Candida growth on YPD plates at 25 °C and 37 °C (Fig. 1a and Additional file 1: Table S1). However, with the exception of the pesticide Captan, all of the antifungals exhibited weak inhibition of Candida growth on serum plates (Fig. 1a and Additional file 1: Table S1).

Out of the five antimicrobial/antiseptic drugs selected by our screen, Chlorquinaldol and Methyl-benzethonium chloride displayed the strongest inhibition of C. albicans growth on serum plates. Nifuroxime, Nitroxoline, and Octanoic acid showed weaker inhibition of Candida growth on serum plates (Fig. 1a and Additional file 1: Table S1).

Interestingly, from a drug-repurposing point of view, 4 drugs from 1190 of the other multifunctional drugs, showed anti-Candida activity on YPD plates (Fig. 1a and Additional file 1: Table S1). Of those, Fluvastatin and Mycophenolic acid showed very strong inhibition of YPD grown Candida, but failed to inhibit its growth on serum plates (Fig. 1a and Additional file 1: Table S1). The other 2 drugs, Disulfiram and Octodrine showed a consistent moderate-to-weak anti-Candida activity on YPD and serum plates (Fig. 1a and Additional file 1: Table S1).

Diffusion susceptibility testing of the hits obtained from the screen

The 15 hits from the primary screen consisting of antifungals, antimicrobials, and miscellaneous drugs were tested in diffusion susceptibility assays in order to determine their potency against C. albicans lawns. These confirmatory screenings were performed over a range of drug concentrations, where 5 μl of drugs within the range of 10 mM to 0.3 μM were applied on YPD-grown Candida cells and plates were incubated for 24 h. YPD plates were chosen as the testing media since some of the drug hits were ineffective in inhibiting fungal growth on serum plates. The efficacy of each drug was evaluated by estimating the inhibitory concentration at which the drug formed a zone of inhibition on a fungal lawn of YPD plates.

We found that with the exception of Fluconazole and Captan, all antifungals were able to inhibit Candida growth in the μM drug range (Fig. 2 and Additional file 1: Table S2). All five antimicrobial and antiseptic drugs were only able to inhibit fungal growth in the mM drug concentrations (Fig. 2 and Additional file 1: Table S2). Surprisingly, among the drugs approved for non-infectious disease treatments, Fluvastatin was able to inhibit C. albicans growth in the μM drug concentration, while the rest of the drugs were only inhibitory in the mM drug concentrations.

Broad-spectrum antimicrobial properties of Octodrine

Although nine antimicrobial and non-antimicrobial drugs we discovered have not been approved by FDA to treat fungal infections, eight of them were found previously to kill Candida albicans [26–39]. Octodrine, the drug previously used as a decongestant [40, 41], showed an ability to inhibit serum-grown C. albicans when applied at 10 mM 5 μl drop (Fig. 1). Furthermore, Octodrine is the only drug that has not been tested to kill fungi previously. We wanted to investigate whether the application of an even higher concentration of Octodrine would augment its antifungal properties. To test this, we applied 5 μl of neat, undiluted Octodrine, which is produced in a liquid form, as well as its two-fold serial dilutions on the serum and YPD grown Candida lawn. We discovered that the undiluted amount of Octodrine was effective in eliminating the fungal growth (Figs. 3 and 4 and Additional file 1: Tables S3 and S4). We also observed that Octodrine is more effective in inhibiting serum grown Candida compared to YPD grown Candida, as the inhibitory concentrations of Octodrine were more pronounced on serum than on YPD plates (Fig. 3, Additional file 1: Table S3).

In order to identify Candida proteins and signaling pathways that mediate the lethality of Octodrine, we screened three C. albicans knockout libraries, collectively consisting of 908 mutant strains lacking one of the previously demonstrated virulence genes, for any alterations in sensitivity to 10 mM Octodrine. These libraries
consisted of 647 mutant strains lacking one of the essential virulence genes [15], 96 cell wall protein mutants [42], and 165 transcription factors mutants [43]. We found that all Candida mutants were as sensitive to 5 μl of 10 mM Octodrine as the wild type strain (Additional file 1: Figure S1).

We hypothesized that Octodrine may kill microorganisms by targeting their non-protein cellular components. To investigate this we tested the sensitivities of Escherichia coli strain C600 and Bacillus cereus strain 10987, gram-negative and gram-positive bacteria, respectively, and frequent disease-causing bacteria, to Octodrine. These bacterial strains were chosen because they are wild type strains with known genotypes and genomes [44, 45]. We observed that 5 μl of neat Octodrine formed a prominent zone of inhibition in both E. coli and B. cereus lawns (Fig. 4 and Additional file 1: Table S4), which suggests that Octodrine possesses wide spectrum antimicrobial properties.

Since Octodrine was one of the most prominent drugs that killed C. albicans in serum, we tested the sensitivity of mouse macrophage cell line RAW264.7 to Octodrine, and compared it to the rest of selected drugs. Since Octodrine stock was dissolved in DMSO, we had to test the sensitivity of the cell line to this solvent alone. Cell viability was determined by MTT assay (Methods) and was calculated as the percentage of surviving cells in various chemical concentrations relative to cells treated with media alone. We observed that the sensitivity of mouse macrophages to Octodrine was close to the sensitivity to the DMSO alone (Table 1). This strongly suggests that Octodrine concentrations that kill Candida albicans in serum (Fig. 1a) do not affect the survival of host phagocytes. The sensitivity of RAW264.7 cells to DMSO has previously been reported [46], and is consistent with the cellular sensitivity seen here. We tested the sensitivity of RAW264.7 macrophage like cells because macrophages are first line of defense against Candida, and knowing the sensitivity of those cells to antifungal is an important question. We confirmed these observations by testing sensitivity of human skin melanoma cell line, C32 (Table 1). With the exception of Octodrine, Floconazole, Nifuroxime, and Fluvastatin, all other selected drugs adversely affected the survival of host cells, which suggests that they may have undesirable side effects when used in blood. We chose this cell line because of the relevance of skin to Candida infections.

Discussion
This study was designed to test FDA and foreign-approved small molecules drugs for their antifungal properties, with the objective of reducing the cost and time necessary to develop much needed anti-Candida albicans therapies. This library consists of an FDA-approved, off-patent collection of 1581 small molecules (10 mM) that are used as drugs for a variety of diseases, including infectious, neurodegenerative, psychiatric, cardiovascular diseases and cancer. Such an approach would rapidly expedite the drug discovery and development process since the general pharmacology, toxicology, and pharmacokinetic properties of all these drugs are already well established. This would facilitate the further analysis of the novel functionalities of the established molecules because the structure, chemical
**Fig. 3** Agar Octodrine diffusion susceptibility assay of *Candida albicans* SN250. Plates were treated with various concentrations of Octodrine after spreading of liquid *Candida* culture and left to incubate overnight in either 25 or 37 °C. The equal volume of negative control, DMSO, was also included. The size of the zone of inhibition on each image is shown with the same scale (mm), with standard deviation values are shown in Additional file 1 Table S3.

**Fig. 4** The sensitivities of *Candida albicans*, *Escherichia coli*, and *Bacillus cereus* to neat Octodrine. Plates were treated with 5 μl of undiluted Octodrine after spreading of 200 μl of liquid *Candida* (25 x 10⁶ cells) or bacterial cultures (600 x 10⁶ cells) on 10 cm petri dish and left to incubate overnight in either 37 °C for *C. albicans* and *E. coli* or in 30 °C for *B. cereus*. The size of the zone of inhibition on each image is shown with the same scale (mm), with standard deviation values are shown in Additional file 1 Table S4.
in vivo Candida albicans activity was mild [26]. Candida components that support Candida C. albicans properties. Overall, all drugs discovered in our C. albicans mutants showed Candida growth. In addition, shows that these drugs could be Minimal cytotoxic concentration (MCC) calculations for Candida biofilm formation, while we Candida growth. (Medley & James Laboratories) [40, 41], was a drug that had not been previously established as an anti-fungal. We observed that although its anti-Candida activity was mild on YPD, it displayed one of the best Candida growth inhibition on serum compared to other drugs. It also has a potential to be one of the safest of the discovered drugs because it did not affect the sensitivity of mammalian cells significantly. The fact that no C. albicans mutants showed a decrease in sensitivity to Octodrine argues against the potential emergence of Octodrine-resistant Candida strains, and favors the usage of this drug as a new antifungal treatment against Candida. In addition, we showed that Octodrine is capable of killing Gram-positive as well as Gram-negative bacteria, making it a desirable broad-spectrum antimicrobial countermeasure, that probably kills microbes by targeting their non-protein components. Octodrine was previously shown to agonize Estrogen properties, and biological functions of almost all members of this library are known.

We have developed a serum-based assay to address the limitations of currently used YPD media, whose components do not represent in vivo components that support the growth of Candida. The moderate-to-mild effectiveness of Fluconazole and other FDA approved antifungal drugs on our serum assay is comparable to the effectiveness of Octodrine in killing serum-grown C. albicans.

In our study, fifteen out of 1581 drugs displayed anti-Candida properties. Overall, all drugs discovered in our study could be separated into three structural categories: five-membered heterocyclic drugs, such as azoles and oxoles, six-membered heterocyclic compounds (pyridines), and other structures (Fig. 5). The fact that we selected six drugs that were previously approved by FDA to treat fungal infections biologically validates our approach. The six antifungal drugs obtained as hits from the screen comprised three different chemical classes: azoles (Fluconazole, Captan, Clotrimazole, and Miconazole), pyridine (Pyritrione Zinc), and other structures (Antimycin A). Additionally, we found five antimicrobial/antiseptic drugs to be effective at inhibiting C. albicans lawn formation in the screen. These drugs include general antiseptics and antibacterial antibiotics, and comprise three different chemical classes: oxoles (Nifuroxime), pyridines (Nitroxoline and Chlorquinaldole), and other structures (Octanoic acid and Benzethonium Chloride) (Fig. 5). The fact that these five other drugs that were approved by FDA to treat other non-fungal infectious diseases were also observed to kill Candida albicans shows that these drugs could be repurposed to be broad-spectrum anti-microbial drugs.

Lastly, from the point of view of drug repurposing, the most interesting class of drugs would be the one with demonstrated activity against a variety of diseases, but with no known or characterized antifungal activity to date. From the screen, we found four drugs belonging to this class to be effective in preventing Candida growth. These drugs have been designed for several indications, including immune-suppression (Mycophenolic acid), deterrent of alcohol consumption (Disulfiram), antihyperlipidemic (Fluvastatin), and decongestant (Octodrine). These drugs comprise three different chemical classes:azole (Fluvastatin), oxole (Mycophenolic acid), and other structures (Disulfiram and Octodrine) (Fig. 5). These four drugs that were approved to treat non-infectious diseases showed antifungal properties and thus, could be repurposed as new antifungal drugs.

Recently, another group investigated the ability of FDA-approved drugs to inhibit C. albicans biofilm formation [12] by screening the Prestwick Library, a commercially available chemical library of 1200 drugs. Interestingly, the authors of that paper discovered several antifungal drugs in common to our study: Miconazole, Clotrimazole, and Methylbenzonium chloride. The difference between the two studies is that Siles et al. [12] screened for drugs capable of inhibiting Candida biofilm formation, while we looked for FDA approved drugs capable of inhibiting the growth of non-biofilm C. albicans growth. In addition, with the exception of Octodrine, every one out of fifteen discovered drugs in our study had been previously tested for the ability to kill Candida albicans [26–39].

In this study, Octodrine, which was previously used as a decongestant and registered under the name of Vaporpac (Medley & James Laboratories) [40, 41], was a drug that had not been previously established as an anti-fungal. We observed that although its anti-Candida activity was mild on YPD, it displayed one of the best Candida growth inhibition on serum compared to other drugs. It also has a potential to be one of the safest of the discovered drugs because it did not affect the sensitivity of mammalian cells significantly. The fact that no C. albicans mutants showed a decrease in sensitivity to Octodrine argues against the potential emergence of Octodrine-resistant Candida strains, and favors the usage of this drug as a new antifungal treatment against Candida. In addition, we showed that Octodrine is capable of killing Gram-positive as well as Gram-negative bacteria, making it a desirable broad-spectrum antimicrobial countermeasure, that probably kills microbes by targeting their non-protein components. Octodrine was previously shown to agonize Estrogen

| Drug                        | MCC (µM) RAW264.7 | MCC (µM) C32 |
|-----------------------------|-------------------|--------------|
| Fluconazole                 | 100.00            | 100.00       |
| Antimycin                   | 3.13              | 0.098        |
| Captan                      | 12.50             | 12.50        |
| Chlorquinaldol              | 0.20              | 6.25         |
| Clotrimazole                | 6.25              | 12.50        |
| Disulfiram                  | 12.50             | 12.50        |
| Fluvastatin                 | 50.00             | 6.25         |
| Methylbenzethonium Chloride | 6.25              | 1.56         |
| Miconazole                  | 6.25              | 1.56         |
| Mycophenolic Acid           | 0.78              | 1.56         |
| Nifuroxime                  | 25.00             | 25.00        |
| Nitrooxine                  | 6.25              | 6.25         |
| Octanoic Acid               | 0.78              | 6.25         |
| Octodrine                   | 25.00             | 25.00        |
| Pyritrione Zinc             | 0.78              | 1.56         |
| DMSO                        | 50.00             | 50.00        |

The solvent DMSO is used as a negative control. Mammalian cells were treated with drugs at concentrations shown. The drugs were serially-diluted, and the MCC is defined as the first concentration of the drug, which is able to lower cell viability below 100 %. Cell viability was determined by MTT assay (Methods) and is shown as the percentage of survivors relative to cells treated with media alone.
Fig. 5 Chemical structures of 15 drugs shown to have anti-Candida albicans activity in our screen. All drugs were categorized into 3 classes: 5-membered heterocyclic (Azoles and Oxoles) and 6-membered heterocyclic (Pyridines) compounds, as well as drugs of other structures. The FDA approved applications are shown in green. Substructures are indicated in red.
Receptor Alpha with the potency of 21 μM [47]. Thus, any in vivo antimicrobial activity of Octodrine in humans would have to be in the nM range to avoid this side effect. As the pharmacokinetics of Octodrine had been previously established in numerous animal models [48], this approach is useful for the discovery of economically viable new therapies against infectious diseases.

Conclusions
In summary, we have screened the Johns Hopkins Clinical Compound Library, a commercially available chemical library of FDA approved 1581 drugs, for the identification of bioactive drugs against C. albicans growth. Our results provide the comprehensive survey of the inhibition of Candida growth by existing drugs, one of which hadn’t been previously reported to have antifungal properties. From a drug repurposing point of view, the identification of drugs with no known antifungal activity and which demonstrated excellent activity against C. albicans growth in serum opens up a valuable new avenue for the rapid development of antifungal agents, which are urgently needed.

Additional file

Additional file 1: Quantification of the zone of inhibition post-treatment of various drugs on C. albicans strain SN250. Quantification of the zone of inhibition post-treatment of various serially diluted drugs on C. albicans strain SN250. Serial dilution experiments of Octodrine on serum and YPD plates. Quantification of the zone of inhibition of Octodrine in neat form against Candida albicans, Escherichia coli and Bacillus cereus. Elucidation of mechanism of Candida albicans: Eludication of Candida albicans sensitivity to Octodrine.

Competing interests
The authors declare that they have no competing interests.

Author’s contributions
KK, and M.M. designed research; KK, LZ, and MM performed research; KK, LZ, and MM analyzed data; and KK, LZ, and MM wrote the paper. All authors read and approved the final manuscript.

Acknowledgment
We would like to acknowledge Dr. David Sullivan, John Hopkins School of Medicine, for providing us with JHCCL and for his advices. We would also like to acknowledge Fungal Genetics Stock Center for providing us with Candida albicans knockout libraries. We acknowledge Ralph M. Parson’s Foundation awarded to KGI (PI Dr. Steven Casper).

Received: 3 March 2015 Accepted: 27 May 2015
Published online: 09 June 2015

References
1. Gow NA, van de Veerendonk FL, Brown AJ, Netea MG. Candida albicans morphogenesis and host defence: discriminating invasion from colonization. Nat Rev Microbiol. 2012;10(2):112–22. doi:10.1038/nrmicro2711.
2. Wilson LS, Reyes CM, Stoilman M, Speckman J, Allen K, Beney J. The direct cost and incidence of systemic fungal infections. Value Health. 2002;5(1):26–34. doi:10.1192/jnci.108.2.13693780701219089.
3. Perlroth J, Choi B, Spellberg B. Nosocomial fungal infections: epidemiology, diagnosis, and treatment. Med Mycol. 2007;45(4):321–46. doi:10.1080/13693780701219089.
4. DINuble MJ, Hille D, Sable CA, Kartsonis NA. Invasive candidiasis in cancer patients: observations from a randomized clinical trial. J Infect. 2005;50(5):443–9. doi:10.1016/j.jinf.2005.01.016.
5. Fujitani S, Ricardo-Dukelow M, Kamiya T, Sullivan L, Low L. Ethnicity and other possible risk factors for candidemia at tertiary care university hospitals in Hawaii. Infect Control Hosp Epidemiol. 2006;27(11):1261–3. doi:10.1089/02760330600958831.
6. Fisher JF, Sabel JD, Kaufmann CA, Newman CA. Candida urinary tract infections—treatment. Clin Infect Dis. 2011;54:567–66. doi:10.1093/cid/cir112.
7. Berman J, Sudbery PE. Candida Albicans: a molecular revolution built on lessons from budding yeast. Nat Rev Genet. 2002;3(12):918–30. doi:10.1038/nrg948.
8. Paderu P, Garcia-Effron G, Balachov S, Delmas G, Park S, Perlin DS. Serum differentially alters the antifungal properties of echinocandin drugs. Antimicrob Agents Chemother. 2007;51(8):2253–6. doi:10.1128/AAC.01336-06.
9. Vandeputte P, Ferrari S, Coste AT. Antifungal resistance and new strategies to control fungal infections. Int J Microbiol. 2012;2012:713687. doi:10.1155/2012/713687.
10. Oddi FC, Brown AJ, Gow NA. Antifungal agents: mechanisms of action. Trends Microbiol. 2003;11(8):272–9.
11. Behmam REWJ. FDA regulations for drug development. Science. 2010;329:33.
12. Siles SA, Srinivasan A, Pierce CG, Lopez-Ribot JL, Ramosubramanian AK. High-Throughput Screening of a Collection of Known Pharmacologically Active Small Compounds for Identification of Candida albicans Biofilm Inhibitors. Antimicrob Agents Chemother. 2013;57(8):3681–7. doi:10.1128/ AAC.00860-13.
13. Chong CR, Chen X, Shi L, Liu JG, Sullivan Jr DJ. A clinical drug library screen identifies astemizole as an antimalarial agent. Nat Chem Biol. 2006;2(8):415–6. doi:10.1038/nchembio896.
14. Gillum AM, Taey YF, Kirsch DR. Isolation of the Candida albicans gene for orotidine-5-phosphate decarboxylase by complementation of S. cerevisiae ura3 and E. coli pyrF mutations. MGG. 1984;198(1):179–82.
15. Noble SM, French S, Kohn LA, Chen V, Johnson AD. Systematic screens of a Candida albicans homozygous deletion library decouple morphogenetic switching and pathogenicity. Nat Genet. 2010;42(2):590–8. doi:10.1038/ng.505.
16. Rauceo JM, Blankenship JR, Fanning S, Hammaker JF, Deneault JS, Smith FJ. Regulation of the Candida albicans cell wall damage response by transcription factor Sko1 and PAS kinase Pck1. Mol Biol Cell. 2008;19(7):2741–51. doi:10.1091/mbc.E08-02-0191.
17. Homann OR, Dea J, Noble SM, Johnson AD. A phenotypic profile of the Candida albicans regulatory network. PLoS Genet. 2005;1(1), e1000073. doi:10.1371/journal.pgen.1000073.
18. institute CalS, M44-A2. Method for antifungal disk diffusion susceptibility testing of yeasts; approved guideline. 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
19. Arendrup MC, Park S, Brown S, Pfaffer M, Perlins DS. Evaluation of CLSI M44-A2 disk diffusion and associated breakpoint testing of caspofungin and micafungin using a well-characterized panel of wild-type and fks hot spot mutant Candida isolates. Antimicrob Agents Chemother. 2011;55(5):1891–5. doi:10.1128/ AAC.01373-10.
20. ImageJ. http://imagej.nih.gov/ij/. Accessed 28 April 2015.
21. Lu Q, Wei W, Kowalski PE, Chang AC, Cohen SN. EST-based genome-wide gene inactivation identifies ARAP3 as a host protein affecting cellular susceptibility to anthrax toxin. Proc Natl Acad Sci U S A. 2004;101(49):17246–51. doi:10.1073/pnas.0407794101.
22. Hendry AT, Bakerspigel A. Factors affecting serum inhibited growth of Candida albicans and Cryptococcus neoformans. Parasitologia. 1969;7(3):219–29.
23. Elin RJ, Wolff SM. Effect of pH and iron concentration on growth of Candida albicans in human serum. J Infect Dis. 1973;127(6):705–8.
24. King RD, Khan HA, Foye JC, Greenberg JH, Jones HE. Transferrin, iron, and dermatophytes I. Serum dermatophyte inhibitory component definitively identified as unsaturated transferrin. J Lab Clin Med. 1975;86(6):204–12.
25. Andes D, Nett J, Oscher P, Albrecht R, Marchillo K, Pitula A. Development and characterization of an in vivo central venous catheter Candida albicans biofilm model. Infect Immun. 2004;72(10):6023–31. doi:10.1128/ AI.72.10.6023-6031.2004.
26. Kor EJ, Olson VL, Roveicot LJ, McClary DO. An alternate respiratory pathway in Candida albicans. Antonie Van Leeuwenhoek. 1976;42(1–2):33–48.
27. Gale GR, Smith AB, Atkins LM, Walker Jr EM, Gadsden RH. Pharmacology of captan: biochemical effects with special reference to macromolecular synthesis. Toxicol Appl Pharmacol. 1971;18(2):426–41.

28. Lovgren T, Salmela I. In vitro sensitivity of Trichomonas vaginalis and Candida albicans to chemotherapeutic agents. Acta Pathol Microbiol Scand. 1978;86B(3):155–8.

29. Wachtler B, Wilson D, Hube B. Candida albicans adhesion to and invasion and damage of vaginal epithelial cells: stage-specific inhibition by clotrimazole and bifonazole. Antimicrob Agents Chemother. 2011;55(9):4436–9. doi:10.1128/AAC.0144-11.

30. Shukla S, Sauna ZE, Prasad R, Ambudkar SV. Disulfiram is a potent modulator of multidrug transporter Cd1p of Candida albicans. Biochem Biophys Res Commun. 2004;322(2):520–5. doi:10.1016/j.bbrc.2004.07.151.

31. Molepo J, Musenge E. Clade-related phenotypic switching among fluconazole resistant Candida albicans isolates. SADJ. 2012;67(7):326–8.

32. Nash JD, Burgess DS, Talbert RL. Effect of fluvastatin and pravastatin, HMG-CoA reductase inhibitors, on fluconazole activity against Candida albicans. J Med Microbiol. 2002;51(2):105–9.

33. Ichikawa T, Yano Y, Fujita Y, Kashiwabara T, Nagao K. The enhancement effect of three sugar alcohols on the fungicidal effect of benzethonium chloride toward Candida albicans. J Dent. 2008;36(11):965–8. doi:10.1016/j.jdent.2008.07.013.

34. Vandenbosch D, Bink A, Govaert G, Cammue BP, Nelis HJ, Thevissen K. Phytosphingosine-1-phosphate is a signaling molecule involved in miconazole resistance in sessile Candida albicans cells. Antimicrob Agents Chemother. 2012;56(5):2290–4. doi:10.1128/AAC.05106-11.

35. Kohler GA, Gong X, Bentink S, Theiss S, Pagani GM, Agabian N, et al. The functional basis of mycophenolic acid resistance in Candida albicans IWP dehydrogenase. J Biol Chem. 2005;280(12):11295–302. doi:10.1074/jbc.M409847200.

36. Grossman LI. Evaluation of antifungal agents for endodontic use. J Dent Res. 1967;46(1):215–7.

37. Hernandez Molina JM, Llosa J, Ventosa A. In vitro activity of nitroxoline against clinical isolates of Candida species. Mycoses. 1991;34(7–8):323–5.

38. Omura Y, O’Young B, Jones M, Pallos A, Duvvi H, Shimotsuura Y. Caprylic acid in the effective treatment of intractable medical problems of frequent urination, incontinence, chronic upper respiratory infection, root canalled tooth infection, ALS, etc., caused by asbestos & mixed infections of Candida albicans, Helicobacter pylori & cytomegalovirus with or without other microorganisms & mercury. Acupunct Electrother Res. 2011;36(1–2):19–64.

39. De Prijck K, De Smet N, Honraet K, Christiaen S, Coenye T, Schacht E, et al. Inhibition of Candida albicans biofilm formation by antimycotics released from modified polydimethyl siloxane. Mycopathologia. 2010;169(3):167–74. doi:10.1007/s11046-009-9242-4.

40. Druglead-Octodrine. http://www.putubio.com/octodrine-cas-no-543-82-8/. Accessed 28 April 2015.

41. PubChem-Octodrine. http://pubchem.ncbi.nlm.nih.gov/compound/10982. Accessed 28 April 2015.

42. Norice CT, Smith Jr FJ, Solis N, Filler SG, Mitchell AP. Requirement for Candida albicans Sun41 in biofilm formation and virulence. Eukaryot Cell. 2007;6(11):2046–55. doi:10.1128/EC.00314-07.

43. Noble CJ, Mitchell AP. Regulation of cell-surface genes and biofilm formation by the C. albicans transcription factor Bcr1p. Curr Biol. 2005;15(12):1150–5. doi:10.1016/j.cub.2005.05.047.

44. Hanahan D. Studies on transformation of Escherichia coli with plasmids. J Mol Biol. 1983;166(4):557–80.

45. Rasko DA, Ravell J, Olsen OA, Helgason E, Cór RZ, Jiang L, et al. The genome sequence of Bacillus cereus ATCC 10987 reveals metabolic adaptations and a large plasmid related to Bacillus anthracis pXO1. Nucleic Acids Res. 2004;32(3):977–88. doi:10.1093/nar/gkh258.

46. Jeong SY, Marchenko M, Cohen SN. Calpain-dependent cytoskeletal re-arrangement exploited for anthrax toxin endocytosis. Proc Natl Acad Sci U S A. 2013;110(42):E4007–15. doi:10.1073/pnas.1316852110.

47. PubChem-BioAssay. http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=743097. Accessed 28 April 2015.

48. Fellows EJ. The pharmacology of 2-amino-6-methylheptane. J Pharmacol Exp Ther. 1947;90(4):351–8.