 doi:10.20944/preprints202106.0603.v1

REVIEW ARTICLE ABOUT SUPERBUG FUNGUS *C. auris.*

Kashmina Hira Khan, Sabiqa Hussain, Taqdees Malik*, Erum Mazhar

Department of Microbiology, Jinnah University for Women, Karachi Pakistan

Corresponding author:
Dr. Taqdees Malik
Department of Microbiology
Jinnah University for Women
Karachi
Pakistan
taqdees_13@hotmail.com
dr.taqdeesmalik@juw.edu.pk

ABSTRACT

The newly emerging nosocomial pathogen *Candida auris* is linked with persistent hospital-acquired infections and abrupt outbreaks across six continents. Genotypic analysis is indicative of the appearance of independent distinct clades of this particular fungus in different geographic locations simultaneously. Intrusive deep seated infections in addition to colonization have been diagnosed primarily in hospitalized patients and have drawn a lot of attention because of different antifungal susceptibility profiles and transmission despite strict preventive measures. Problems with the accurate identification of *C. auris using* phenotypic and molecular approaches has raised concerns about the detection of relevant levels of the problem. Candida family associated infections are a serious causative agent of mortality and morbidity in immune-compromised individuals. *Candida auris* are also known as superbug fungus that spreads rapidly all over the world. In 2009, shortly after the first case, various strains across the six continents have been recognized as nosocomial pathogens. Simultaneous and independent *C. auris* outbreaks appear to be of great concern for the healthcare settings as well as scientific community. Additionally, microbiological misidentification and multidrug resistance, rarely noticed for other non-albicans Candida species, lead to problems in obliteration and frequent treatment failures for *C. auris* infections. This review article aims to provide a comprehensive and up to date report on the global *C. auris* outbreaks, considering clinical along with microbiological characteristics, virulence mechanisms and susceptibility to antifungals, as well as the effectiveness of available preventive and therapeutic implementations.

KEYWORDS: *Candida auris*, Superbug fungus, biofilm, *Candida albicans*, nosocomial infection, Candidiasis.

INTRODUCTION

Candida auris reportedly was first isolated from 70 years old female inpatient's ear (auris means ear in Latin) at a public hospital of Japan in 2009, identified as potential cause of nosocomial infections by 2011 across six continents >30 countries particularly in individuals with long-term hospital stay with underlying diseases, prior

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

surgical history, immune-compromised patients and people at extremes of their ages. Records show that it has high mortality rate with distinct properties such as, multidrug-resistant, difficulty in phenotypic analysis using conventional microbiological methods, diverging antifungal profiling and extreme virulence which consequently compel health care facilitators and scientific community to enlist *C. auris* as life threatening nosocomial pathogen, often using terms as "stealthy pandemic" and "superbug fungus" showing it's potential to be next pandemic in addition to causing fetal infections in masses.(5)

Various forms of invasive candidiasis and candidemia have been a serious concern over last few decades. C. *albicans* is the most frequently reported species in the Candida clade but surprisingly non-albicans in contrast to albicans are found to be associated with higher rate of death along with antifungal resistance. (1) Prophylactic antifungal drug fluconazole is considered to be associated with emergence of non-albican infections which are proving to be more lethal than those caused by albicans. Out of those *C .auris* is most novel one which in recent times can be categorized as most challenging one with limited treatment options available being multidrug resistant, giving false positive results with conventional phenotypic laboratory and molecular methods, it's often misidentified with *C. hemolunii, C. duobushaemulonii, C. famata, C. lusitaniae, C. sake, C. catenulata, Rhodotorula glutinis, C. tropicalis, Saccharomyces cerevisiae, C. albicans, C. parapsilosis, C. guilliermondii, R. rubra* and *C.galbarata* using traditional diagnosis methodologies.(23) Most cases identified to be caused by C. *auris* were bloodstream infections having 30-60% average mortality rate, yet it is less virulent than *C. albican*.(8)

Presently, C. *auris* is classified into 4 different strains on the basis of biological and biochemical properties, namely, South American strain, South African strain, East Asian strain and South Asian strain. Cases reported in other countries were found to be genetically similar to either of these strains. East Asian strain is found not to be linked with bloodstream infections.(10) A US conducted study shows genetic diversity between isolates obtained from same patient, health-care settings indicating continued and local transmission, all strains show distinctive single nucleotide polymorphisms (SNPs). While exact reason for emergence of *C. auris* remains unknown, many factors including climate change, increased use of antifungals are among suspected modes of transmission. The tendency of *C. auris* to survive in hospital environments indicates its ability to form biofilm i.e. a cell growth pattern in which cells are clustered in micro-colonies protected by a matrix of glucans. Subsequently, *C. auris* can easily survive inert surfaces in addition multidrug resistance can also be seen. Typically, cells enclosed within matrix are termed as sessile and those outside are called planktonic cells which are so protected as to become difficult to remove even with most advanced disinfecting techniques. (16)

Biofilm- forming cells isolated from the patients wound plus catheter tips suggests the presence of *C. auris* as biofilm in patients and sessile cells categorically show resistivity towards several antifungal. Thus biofilm can be an important factor of virulence for *C. auris*. (**Error! Reference source not found.**, 25)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

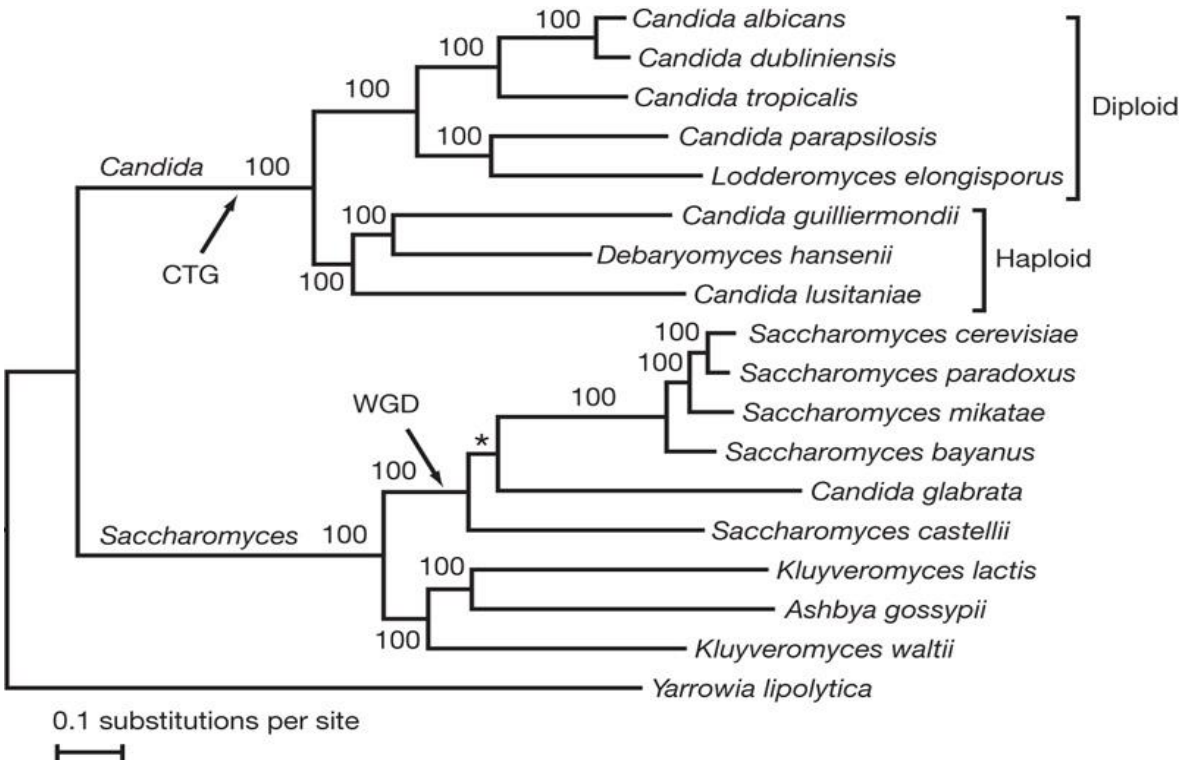

FIGURE 01: Phylogenetic Tree of sequenced Candida and Saccharomyces Clade species

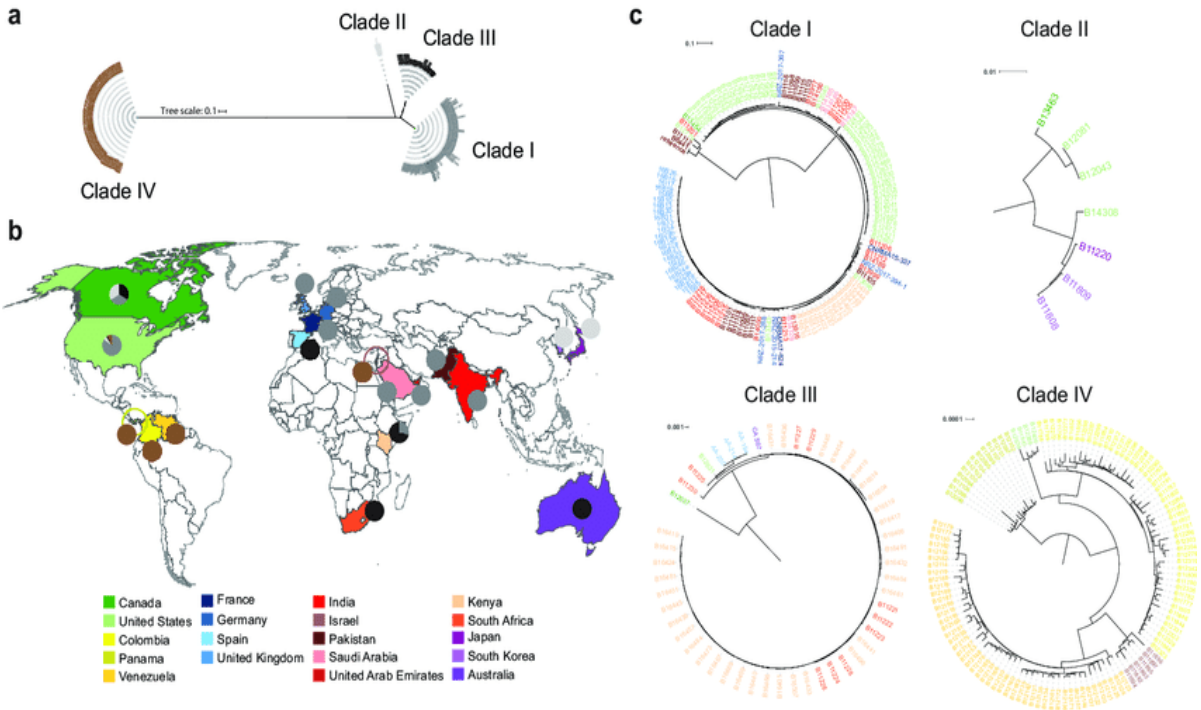

FIGURE 02: Global distribution of *Candida auris* clades.

In Pakistan first case was reported in September, 2014 at a private hospital of Karachi. It was identified as *Saccharomyces cerevisiae* initially but due to its unusual pattern of fungal susceptibility so for proper identification the yeast isolated were retested at

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

the Centers for Disease Control and Prevention (CDC), in Atlanta, USA and was ultimately identified as C. *auris*. Subject came to lime light after simultaneous outbreak in India, South Africa and Venezuela. (4, 29, 20)

Only three hundred forty-nine cases of *C. auris* were reported in the European Union from January 2018 to May 2019 with 63.6% cases of colonization and 24.1% of funganemia. The collaborative survey of The European Centers for Disease Prevention and Control stated that in between 2013 to 2017 many European countries did not have diagnostic facilities or capacity. Improvements were seen in facilities and awareness among masses by the year 2018 yet around seven EU/EEA countries without reference laboratories which lead to misdiagnosis or at times no diagnosis. Standard biochemical tests or commercially available tests misidentified *C. auris* as other species of yeast (e.g. *C. parapsilosis*, *C. famata*, *C. lusitaniae*, *C. guilliermondii*, *and C. haemolunii*) for precise species-level identification more advanced techniques are required including MALDI-TOF, DNA sequencing sometimes both. (40)

This pattern cause's delayed diagnosis which potentially leads to a long pause in treatment this is a fatal factor considering the easy and speedy transmission from healthcare personnel, colonized patients and contaminated surfaces which cannot achieve complete *C. auris* eradication. A lack of consensus on susceptibility breakpoints was seen as the results of minimal inhibitory concentration (MIC) and outcome of their clinical manifestations was not understood completely. Therefore it was very important to have accurate diagnosis to bridge this gap in order to have implementations to prevent infection, strategies to manage and to reduce costs and complications related to outbreak falling on immunocompromised patients and hospitalized patients who currently have underlying disorders in specific. (40)

C. *auris* SAMPLING

Advanced sampling methods should be adapted to evaluate presence of *C. auris*. For this purpose, samples should be taken from most consistent colonization sites i.e. bilateral axillae and groin, as colonization of *C. auris* has been recorded on several bodily sites for instance, ear canals, wounds, urinary tract, vagina, nares, oropharynx, catheter exit sites, rectum etc. So these sites should also be taken into consideration. However, samples should be collected using rayon tip swab or nylon-flocked swabs with composite swab samples from mentioned sites. (40)

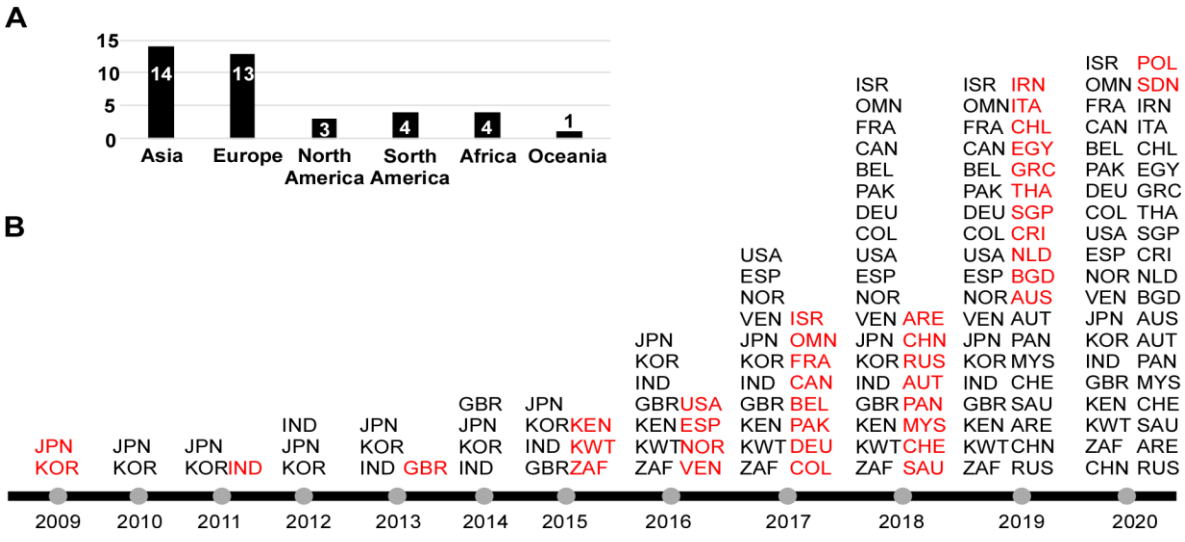

Figure 03: (A) The number of countries on each continent that have reported *C. auris* infection or colonization.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

EMERGENCE

Japan 2009, was the year of discovery towards the emerging infection Candida auris. In this year C. auris first isolated from a 70 year old patient's discharge of ear canal. Across the five continents *Candida auris* is being identified as hospital associated infectious agent. The number of infectious cases is being reported frequently.[1] *Candida auris* is assumed to be present before 2009 as a human pathogen as it has been found to be misidentified a number of times with another specie of Candida genus, *Candida haemuloni*. The US, Centers for Disease Control and Prevention (CDC) enquired about the international surveillance system SENTRY, and during 2009 to 2015 discovered four isolates of *Candida auris* which were considered to be *Candida haemuloni* but were eventually not.(2)
According to a study in South Korea, fifteen isolates were identified as *C. haemuloni* in 2009 and later *C. auris* was discovered to be culprit. The isolates came from the patient's ear canal, which were infected with chronic otitis. In India, between 2009-2011, around twelve isolates of *C. auris* were recognized according to few reports. (1) In 2012 it reported to emerge in Venezuela and later in 2013 in Colombia.(3) In September 2014, *Candida auris* first case was diagnosed in Pakistan.(4) In the United States, *C. auris* infection begun to spread in 2015 and in 2017 around 77 cases were reported. While in 2016 the first case of *C. auris* was reported in Royal Brampton Hospital, London. (2)
According to the CDC's 2019 survey report, *Candida auris* had been isolated as well as identified in more than 30 countries Worldwide. It has emerged across the world except Antarctica turning to be known as "Stealthy Pandemic." (1, 2)

GENOMIC ANALYSIS AND EPIDEMIOLOGY

Since its first isolation from external ear canal, *C. auris* was also isolated from a number of different body parts. Colonization followed by infection was noticed more frequently in immune-compromised patients or inpatients of critical care units, affecting mainly patients at extremes of their ages though many reported cases were from Pediatric population. Isolates were obtained from different countries including US, South Korea, India, Pakistan, Spain, Oman, Israel, Kuwait, Germany, Canada, United Kingdom, Colombia and Norway.(26,21,16) A number of comparative studies were carried out on phenotypic and genotypic basis with the help of isolates from Brazil, Malaysia and Kenya to show demographic variations. *C. auris* has nearly 12.5 Mb haploid genome with GC content approximately 45%. Genomic analysis reveals presence of 6500 to 8500 protein-coding sequences which have the capability to code for virulence factors, for instance, genes for biofilm formation. (11, 14, 27)
Additionally, protein kinases and many transporter genes were also identified which suggestively have core role in acquiring drug resistance. (12, **Error! Reference source not found.**)
Despite striking divergence of genome, C. *auris* has been found closely linked with C. *lusitaniae* and C. *haemuloni*. With thousands of differences in single nucleotide polymorphism (SNP), great variation have been noticed in geographically different clades. In India clonal isolates were not differing on genomic basis however, the collected isolates belonged to different geographic regions. (14, 18)
Moreover, (WGS) Whole genome sequence study of isolated strains from US show similarity by less than 60 SNPs with South Asian clade and fewer than 150 SNPs with South American clade. C. *auris* was found to be misidentified as an ancient isolate of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

South Korean fungemia patient tracing back in 1996. In addition, it was found to be misidentified as another old isolate which was isolated a year prior to its discovery i.e. in 2008 at Pakistan. (17)

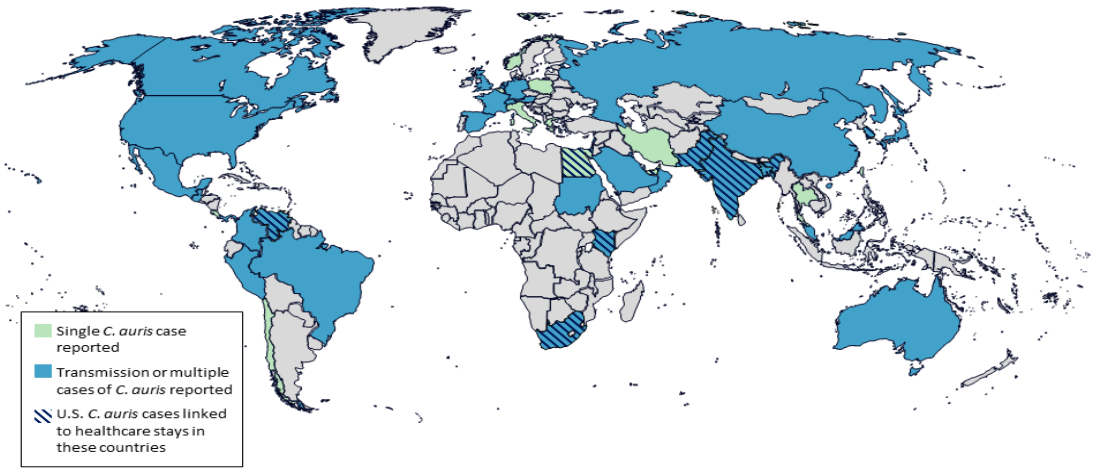

Figure 04: Occurrence frequency of *C. auris* around the globe.

MOLECULAR METHODS OF IDENTIFICATION

Molecular detection of *C. auris* is possible by cultured and non-cultured methods. Culture based molecular techniques are dependent on yeast growth factors with 10% NaCl concentration and 40°C temperature. For nourishing *C. auris* and other Candida species dulcitol acts as a source of Carbon. Internal transcribed regions (ITS) or 28S-rDNA or MALDI-TOF can provide a clear picture of this yeast. Molecular methods are relatively costly, time consuming and technically challenging that is the reason that they cannot be a regular choice for diagnosis. Culture independent techniques for example PCR, RT-PCR, TaqMan PCR, SYBR Green qPCR, LAMP and T2MR are found to be 90% accurate in diagnosis within a limit of 1-10CFU/reaction. These methods save time because they cut the turnaround time from days to hours and are done using large yeast panels which make them cost effective and less difficult to carry out. (40, 41, 43, 48)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

Test	Sample Taken From	Targeted Gene	Reference
PCR and RT-SYBR Green qPCR	Colony	5.8S-ITS2-28S-rDNA	39
RT-SYBR Green qPCR	Swab	5.8S-ITS2-28S	40
RT-TaqMan PCR	Environment and Swab	ITS2 of rDNA	42
Polymerase Chain Reaction	Colony	ITS1-5.8S-ITS2	43
Duplex Polymerase Chain Reaction	Colony	GPI protein-encoding genes	44
Tetraplex Polymerase Chain Reaction	Colony	26s-rDNA	45
Multiplex end-point PCR	Colony	ITS1-5.8S-ITS2	46
Multiplex YEAST PANEL PCR	Spiked Serum and Colony	26s-rDNA	47
GPS MONODOSE dtec qPCR kit	Colony	Species-specific probes and primers	48
T2MR System	Swabs	Species-specific probes and primers	49
LAMP	Environmental samples, swab and Colony	Encoding genes of ferredoxin oxidoreductase	50

Table 01: Molecular methods summarized.

Biochemical Diagnosis

Most commercially available biochemical assays misidentify *C. auris*, those misidentifications are mentioned in a list prepared and revised by the CDC. According to the listing BD Phoenix mistakenly identifies *C. auris* as *C. catenulata*, Candida spp, *C. famata*, RapID Yeast Plus as *C. parapsilosis*, Candida spp., Vitek 2YST identifies it as *C. haemolunii*, *C. duobushaemolunii*, Candia spp., Rhodotorula *glutinis* (red coloration absent), API 20C diagnoses it as *Saccharomyces kluyveri*, *Candida sake*, , *Saccharomyces cerevisiae* and as Candida spp. *C. haemulonii*, MicroScan, MicroScan Walkaway, MicroScan AutoScan identifies as *C. lusitaniae*, *C. parapsilosis*, Candia spp. R. *rubra.* These misidentification arise because *C. auris* is either not in database or has an overlapping profile. Practical identification algorithms can be found on CDC website for above mentioned techniques. All assays mentioned here are having slow turnaround time for the enrichment culture which can be a

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

limiting factor. Moreover, these methods does not possess precision in diagnosis as well making them laborious with false or no results. (47, 49, 50)

Phenotypic Diagnosis

On chromogenic agar (Candida medium) the *C. auris* appears with pink to beige colonies and thrives well at 42°C whereas it shows diverging pattern for growth at elevated temperature, no growth is seen in presence of 0.01% cycloheximide. (27) It may occur in pair, groups or singly, cells can be oval or elongated in shape. Hyphal or pseudohyphal forms are absent except in rudimentary forms occasionally , degree of carbon uptake varied accordingly to the analytical profile index (API) in isolates obtained from South Africa as well as from India, but isolates of South Korean or Japanese region exhibit N-acetylglucosamine assimilation. A comparative in vivo model designed using invertebrate Galleria mellonella and isolate obtained from United Kingdom shows difference in pathogenicity pattern of *Candida auris* in contrast to other Candida species, this analysis showed that isolates of *C. auris* are capable of showing difference of behavior with some showing aggregate formation and others lacking this property.(13,18,20) Isolates which were non-aggregate forming in nature were found to be more pathogenic than those which were aggregate forming, to a certain degree this was comparable to *C. albicans*. This property has no link with hyphal or pseudohyphal formation which *C. auris* generally lack. Another group study compared the virulence factors of *C. auris* and *C. albicans*. Experiments showed that out of 16 strains of *C. auris* were screened, in which 6 had phospholipase activity while 9 were capable of proteinase activity in a strain- dependent manner. One *C. auris* isolate was seen to have a phospholipase activity comparable to that of *C .albicans*. Strong relationship of *C. auris* with ICUs, CVCs, and long-term urinary catheters provides evidence of biofilm formation. Although, biofilm development was observed in non-aggregate-forming strains as well as in aggregation producing strains to some extent, *C. auris* did not show any biofilm formation in one of the in vivo models which is contrary to the behavior of *C. pseudohaemulonii* and *C. haemulonii*. Biomass of *C. auris* was more than *C. glabrata* and less than *C. albicans*. (12, 19)

CLINICAL CHARACTERS, RISK AND OUTCOME

Clinical presentation in most of the cases found not to be specific and it was noticed to be very difficult to distinguish *C. auris* infections from other systematic infections. Most of the isolates were obtained from bloodstream, invasive devices, catheter tips, deep-seated wounds. *C. auris* was identified to cause different infections, including, UTI, bloodstream infections, infective surgical wounds, skin abscesses, bone infections, meningitis and myocarditis. Non-sterile site isolation such as genital sites, lungs, soft tissues, urinary tract shows colonization pattern rather than infection, which is why *C. auris* can transmit from non-sterile sites as well. (25)

Rudramurthy et al. conducted a subgroup analysis and comparative investigation comparing clinical features of the non auris and *C. auris* cases in 27 intensive care units in India to evaluate the risks related to *Candida auris*. Risk factors were same for *C. auris* and non-auris Candida spp. such as previously being exposed to broad-spectrum antibiotics also including antifungal drugs, vascular and gastrointestinal surgery, continued use of a central venous catheter, diabetes mellitus, urinary

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

catheterization, chronic kidney disease, chemotherapy, post- surgical drain placement, blood transfusions, hemodialysis, total nutrition admixture, state of being immunosuppressive and neutropenia, and prolong ICU stay. *C .auris* infections were more frequently reported from patients who were using immunosuppressive agents, for instance, BMT, chemotherapy, hematological malignancies, donor-derived implantation and in patients with primary or acquired poor immune systems.(21)
C. auris in-hospital mortality was determined approximately as 30% to 72%, adult population was found to be more frequently affected with huge number of cases from critically ill or immune-compromised patients. Pediatric population was reported to be affected only in South America and Asia with a better outcome. (4, 12)

INFECTION PREVENTION AND CONTROL

The continued epidemics and rare cases of *C. auris* highlights the importance to take proper preventive precautions. Recent studies depicts difficulty in eradicating *C. auris* infection because of colonization which enables long-term infections and surface adherence by the organism. Preventive measures for outbreaks should be based on early detection of sporadic cases, reservoir identification with instant notification. Instructions were formulated by many international organizations including CDC, Public Health England (PHE-UK), the Center for Opportunistic Tropical and Hospital Infections (COTHI-South Africa) and ECDC, developed guidelines for patient isolation, reservoir prevention, and disinfection of equipment along with environment associated with patient contact. These steps are designed mainly on strategies formulated for confining steps for non-auris multi-drug-resistant pathogens. (13, 19, 15, **Error! Reference source not found.**)
However the actual method of transmission should be determined, prior cases imply that transmission of *C. auris* has primarily involved contact with reservoirs and conveyance from the healthcare settings. Hand to hand transfer and surface contamination have been linked to the ongoing epidemics. (26)
Hypothetically role played by health workers can be a potential cause because in four hospitals in Columbia *C. auris* isolates were obtained from several objects of hospital settings, including mobile phones of patients are healthcare workers, bed-rails, hand controlling knobs of hospital beds, surprisingly large number of positive results were driven from places which were less frequently in contact with patients and more frequently in contact with healthcare workers (hospital equipment, trays, furniture). However, other evidences for presence of *C. auris* were obtained from surfaces like door knobs, clothing cabinets, floor, and alcohol gel dispenser. Conclusively, once *C. auris* enters inside hospital setting, it can be found as major environmental contaminant causing recurring cases by colonization .(18)
C. auris may live on a variety of wet as well as dry surfaces, including plastic, where the superbug fungus can inhabit for 14 days. C. auris is relatively resistant to metallic surface-active cleaners and quaternary compound disinfectants. Disinfectants which are having sporoidical activity or those with hydrogen peroxide formulations are effective in disinfecting surfaces and equipment of healthcare, causing a reduction in colony-forming units (CFU) of *Candida auris*. Cleansers (detergents) based on chlorine, UV rays, and H_2O_2 vapors show their potential ecological decontaminating properties once patient gets discharge. However, despite of disinfecting practices, the occurrence of *C. auris* in a hospital settings is indicative of an involvement of the interaction between biotic and abiotic components even after long exposure of disinfectants. To cut chord of transmission, managements advise sticking to peripheral

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

and central catheter therapy sets, urinary catheter therapy sets and tracheostomy sets. Management recommends keeping to peripheral and central catheter care bundles, urinary tract catheter care bundles, and tracheostomy sites care units to sever the transmission chord. If possible, one time use of other invasive devices or catheters may solve this continuous candida infection, persistent candidemia and enhance the clinical outcome. (14, 27) Patients showing colonization pattern or those with proven or suspected infection of *C. auris* must be secluded with strict preventive measures unless microbiological testing and diagnostic results made available. It is necessary to test the influx of patients from facilities with confirmed *Candida auris* isolation. The groin and armpits, samples of urine and stool, nose, perineal, throat and rectal swab are the preferred sites for screening. While wounds, cannula entrance sites, excretory secretions and endotracheal secretions are the other high risk sites which should be considered.

Virulent Factors

Host cell adherence, stress defiant, thermotolerant, germination, proteinase production and evasion of immune system contribute to the pathogenesis of Candida species. These species of Candida genus are tend to be the chief causers nosocomial infection.(33) The virulent traits considered of *Candida auris* are frequently compared with those of well-studied member of Candida genus, *Candida albicans*. The genome of this nosocomial agent *(C. auris)* encodes many visual virulence factors found in *C. albicans*. Familiar genes are involved in antifungal resistance, phenotypic transformations and biofilm formation. Recent studies of virulence and transcriptome have revealed that how these sustained mechanisms of virulence in fungi contributes to *Candida auris* to become an emerging nosocomial infectious agent.(5)

Adherence & Biofilm Formation:

Microbial colonization, survival and virulence all depend upon the attachment to host cell. Furthermore, microbe-microbe adherence promotes development of microbial biofilms that occurs to be an essential virulent characteristic of Candida species, which confers increased antimicrobial resistance.

Various orthologs of *Candida albicans* adhesins involved in the development of biofilm and virulence are encoded by the genome of *Candida auris*. (5) Alike the C. albicans, the ability of biofilm development in C. auris benefits it to show high resistance towards the antifungal agents.(33) *C. auris* has the ability to cling to plastics furthermore readily produce biofilm. *Candida auris* strains of Clade II, responsible primarily for acute otitis media, has lost a large portion of subtelomeric areas that are rich in putative adhesins, while the other Clades I, III and IV strains have retained them.

Microbial and host microbial adhesions are aided by many GPI- anchored cell proteins. In *Candida albicans*, ALS4 belongs to the well-reviewed adhesin gene family, ALS. And during the filamentous growth in *C. auris* its orthologs is expressed differentially. Some other putative adhesins along with GPI- anchored cell wall genes were increased during *Candida auris* biofilm development in vitro versus Planktonic cells (CSA1, PGA52, HYR3, PGA26 and IFF4).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

Some other GPI-anchored cell wall genes as well as putative adhesins were increased during Candida auris biofilm development in vitro compared to planktonic cells (CSA1, IFF4, HYR3, PGA26, and PGA52).
In *Candida albicans*, the orthologs of ALS3, contributes to adhesin along with invasion which persuades endocytosis of host cell of C. albicans hyphae were also detected by an anti-Als3p on the cell surface of *Candida auris*. ALS1 AND ALS5, two family members of the ALS family have been identified in development of biofilms. In the biofilm formation in C. auris each adhesin plays an important role. (5)
The property of development of biofilm has been reported to be closely associated with the strain/isolates kind and their phenotypic behavior, indicating strain dependent in the *Candida auris.* (32)

Lytic Enzymes:

Essential lytic enzymes which facilitate as virulent factors in human pathogenic fungi include lipases, phospholipases, hemolysins and secreted aspartyl proteases (SAPs). In Candida albicans these lytic enzymes aids the organism to invade host cell and destruct tissue. A number of well-studied Candida albicans lytic enzymes have orthologs in Candida auris genome such as SAPs, lipases and phospholipases. Many studies have shown lytic activity for Candida auris but not much is known that how these lytic enzymes are expressed as compared to their Candida albicans orthologs. While comparing the filamentous cells of Candida auris with yeast cells, two secreted aspartyl protease orthologs were transcriptionally induced, but their exact roles as virulent factors remain unknown. More molecular study is needed to identify the role of these lytic enzymes in C. auris' pathogenesis. (5)

Stress Resistance and Persistence:

Nosocomial pathogen Candida auris has been found to have one of the most intriguing characteristic associated with its emergence such that it is capable enough to survive over abiotic surfaces of health care settings despite of strict cleaning approaches. For up to two and four weeks after contamination, viable strains of auris can be recognized by esterase activity or can be resurrected from the plastic surfaces.
Candida auris in contrast to *Candida albicans*, its metabolism favor's mechanism of respiration, supported by an abundance of sugar transporter along with glycolytic gene expression throughout the yeast development, as well as Citric acid cycle protein enrichment and the lipid profile of C. auris show high levels of ergo sterol and structural lipids which could affect the stress as well as antifungal resistance.
The genes associated with iron metabolism and iron transport in Candida auris were found to be upregulated in biofilm and filamentous cells respectively.
Stress activated protein kinase Hog1 has been recently discovered to play an evolutionary conserved role in *Candida auris* stress resistance, virulence and homeostasis of cell wall. This stress activated protein kinase (Hog1) has identified to be 87% identical to that in *Candida albicans.* (5)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

CURRENT CHALLENGES LINKED TO *C. auris*

There are some challenges faced in identifying that the infection is caused by Candida auris. Challenges include are:

No Specific Clinical Presentation:

Infection caused by Candida auris are invasive and nonspecific therefore very difficult to differentiate between various systemic infections.(1) No specific age group has been identified to be infected, all age groups are susceptible to *Candida auris*. (32) The reported cases of Candida auris infection has been associated with various clinical presentations such as otitis, blood stream infections, meningitis, skin abscesses and urinary tract infections. The CDC states that in case of a suspected bacterial infection if fever and chills fails to improve after treatment with antibiotics then the following are generally considered to be symptoms of an invasive infection of Candida auris.(1)

High Mortal Rate:

Increased number of mortality rate has been observed in *C. auris* infection. The rate has found to vary according to different surveys. Such as the mortality rate has reported to be more than 50% in Far East, Asia, and in United States (Invasive Candida infection), 35.3% in Colombia, and 41.1% in Spain. The infection are lethal unless they are detected in early stage and treated quickly. But as it is difficult to diagnose and its ability to establish resistance to antifungal antibiotics the rate of mortality is increasing. (1)

Misdiagnosed with Other Candida species:

The detection of this *Candida auris* isolate is fraught with difficulties. Over the last few decades, detection techniques for Candida species have advanced steadily, ranging from traditional biochemical methods to nucleic acid based methods. In contrast to other Candida species, it is difficult to distinguish *Candida auris* from cultures. (6)

The sample used for diagnosis of *Candida auris* infections is a culture of fungus in the blood, or body secretions from the infected area, similar like for other Candida infections.(7) Traditional methods used for *Candida auris* isolation and detection can be difficult as when using the conventional biochemical methods *C. auris* is frequently muddled up with other species of the Candida genus (including, *C. haemuloni*, *C. duobushaemolonii), Saccharomyces cerevisiae* or hypopigmented *Rhodotorula glutinis*.(8) The phenotypic methods which often misidentify Candida auris with other species are VITEK 2 YST, Micro Scan, BD phoenix yeast identification system and API 20C (9)

Till now the Candida species were differentiated on the basis of cultural as well as microscopic characteristics. In the case of *Candida auris*, it has observed on Sabouraud dextrose agar (SDA) to give smooth creamy whitish colonies while on CHROM agar gives pink to beige colonies.[1, 10] The pink to beige colony appearance of *Candida auris* it causes difficult to discriminate whether its *Candida auris* or *Candida glabrata*. (31) Under microscopy it may appear as ovoid cells or ellipsoidal

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

in shape. (9, 10) It grows well on 42˚C which is known as unique temperature as the Candida species haemuloni, with which auris is mostly misidentified fails to grow well on 42˚C. (10, 11) Several tests are also used to differentiate this superbug fungus from the other species like by chlamydospore formation in corn, germ tube production, fermentation tests and sugar assimilation.(9)

Molecular techniques have played an essential role to confirm that traditional biochemical tests often misdiagnose *Candida auris* infection in patients. The accepted molecular methods for diagnosis of *Candida auris*, recommended by Centers for Disease Control and Prevention includes MALDI-TOF, matrix assisted laser desorption ionization- time of flight. Other approaches used to spot out and differentiate from closely related species are PCR, polymerase chain reaction, amplified fragment length polymorphism fingerprinting, or sequencing. (1)

Multidrug Resistance:

One of the reasons due to which Candida auris has become a life threatening, troubling and challenging organism to be diagnosed and treated is due to its ability of being resistant to multiple antifungal drugs.[8] Resistance is common in C. auris, one of the reasons could be the discovery of multiple transporter genes as well as protein kinases which aids in drug resistance. (32) The organism has decreased susceptibility to polyenes, echinocandins and azoles. According to in vitro study, it been observed that Candida auris isolates have shown resistance of about 3-37% against voriconazole, 13-35% against amphotericin B, and more than 90% isolates against fluconazole. (1) In the identified C. auris isolates it was observed that almost 40% isolates have shown resistance towards more than two classes of antifungal drugs, therefore now known as Multidrug Resistant (MDR). While around 4% of C. auris isolates shown been resistant towards all antifungal medication groups. (31)

AWARENESS OF NOSOCOMIAL INFECTION

Approximately 30 countries, means few countries have reported the Candida infection which indicates that the infection remains to be unexplored or unreported in many countries. There is no clue whether the infection exists or not in those countries were remains to be undetected. As this infection tends to be newly emerging around the world attributed with invasive infections, its lack of awareness can lead to transmission that goes unnoticed. In this case, the workers of health care units should watch out for this deadly infection and avoid an outbreak, laboratory examination procedures should be modified and shall execute control expedient.(1)

CONCLUSION

Candida auris is labeled as a highly infectious emerging public threat. The cases are being reported progressively in each country of the World. It is an organism which has phylogenetic relationship with other species of Candida genus. Conventional biochemical testing have a hard time detecting it. Nonspecific clinical presentation, increased mortal rate, misidentification and multidrug resistance are the main challenges for *Candida auris* infection. Accurate detection is only possible by molecular approaches. But the official treatment recommended for the infection

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 June 2021 doi:10.20944/preprints202106.0603.v1

remains unknown. Therefore it is necessary to apply measures to raise awareness, prevent and control the infection to reduce the infection rate.

Acronyms Used In This Article

ARE, United Arab Emirates
AUT, Austria;
AUS, Australia;
BGD, Bangladesh;
BEL, Belgium;
CAN, Canada;
CHL, Chile;
COL, Colombia
CHE, Switzerland;
CHN, China;
CRI, Costa Rica;
DEU, Germany;
ESP, Spain;
EGY, Egypt;
FRA, France;
GRC, Greece;
GBR, United Kingdom;
IND, India;
IRN, Iran;
ITA, Italy
ISR, Israel;
JPN, Japan;
KOR, Korea (South);
KEN, Kenya;
KWT, Kuwait;
MYS, Malaysia;
NOR, Norway;
NLD, the Netherlands;
OMN, Oman;
PAN, Panama;
PAK, Pakistan;
POL, Poland;
RUS, Russia;
SDN, Sudan
SGP, Singapore;
SAU, Saudi Arabia;
THA, Thailand;
VEN, Venezuela;
USA, United States of America;
ZAF, South Africa.

Figure 03

LAMP; Loop-mediated Isothermal amplification
qPCR; Quantitative Polymerase Chain Reaction.
T2MR System; T2 Magnetic Resonance System

Table 01

REFERENCES

1. Ademe, M., & Girma, F. (2020). Candida auris: From multidrug resistance to pan-resistant strains. Infection and Drug Resistance, 13, 1287.
2. Rhodes, J., & Fisher, M. C. (2019). Global epidemiology of emerging Candida auris. *Current opinion in microbiology*, *52*, 84-89.
3. Chakrabarti, A., & Sood, P. (2021). On the emergence, spread and resistance of Candida auris: host, pathogen and environmental tipping points. Journal of Medical Microbiology, 001318.
4. Farooqi, J. Q., Soomro, A. S., Baig, M. A., Sajjad, S. F., Hamid, K., Jabeen, K., ... & Asghar, R. J. (2020). Outbreak investigation of Candida auris at a tertiary care hospital in Karachi, Pakistan. *Journal of Infection Prevention*, *21*(5), 189-195.
5. Chybowska, A. D., Childers, D. S., & Farrer, R. A. (2020). Nine things genomics can tell us about candida auris. *Frontiers in genetics*, *11*, 351.
6. Sardi, J. D. C. O., Silva, D. R., Mendes-Giannini, M. J. S., & Rosalen, P. L. (2018). Candida auris: epidemiology, risk factors, virulence, resistance, and therapeutic options. *Microbial pathogenesis*, *125*, 116-121.
7. Sarma, S., & Upadhyay, S. (2017). Current perspective on emergence, diagnosis and drug resistance in Candida auris. *Infection and drug resistance*, *10*, 155.
8. Saris, K., Meis, J. F., & Voss, A. (2018). Candida auris. Current opinion in infectious diseases, 31(4), 334-340
9. Sardi, J. D. C. O., Silva, D. R., Mendes-Giannini, M. J. S., & Rosalen, P. L. (2018). Candida auris: epidemiology, risk factors, virulence, resistance, and therapeutic options. *Microbial pathogenesis*, *125*, 116-121.
10. Spivak, E. S., & Hanson, K. E. (2018). Candida auris: an emerging fungal pathogen. *Journal of clinical microbiology*, *56*(2).
11. Osei Sekyere, J. (2018). Candida auris: A systematic review and meta-analysis of current updates on an emerging multidrug-resistant pathogen. *Microbiologyopen*, *7*(4), e00578.
12. Chaabane F, Graf A, Jequier L and Coste AT (2019) Review on Antifungal Resistance Mechanisms in the Emerging Pathogen Candida auris. Front. Microbiol. 10:2788.doi: 10.3389/fmicb.2019.02788.
13. Lockhart, S. R., Etienne, K. A., Vallabhaneni, S., Farooqi, J., Chowdhary, A., Govender, N. P., et al. (2017). Simultaneous emergence of multidrug-resistant Candida auris on 3 continents confifirmed by whole-genome sequencing and epidemiological analyses. Clin. Infect. Dis. 64, 134–140. doi: 10.1093/cid/ciw691
14. Ben-Ami, R., Berman, J., Novikov, A., Bash, E., Shachor-Meyouhas, Y., Zakin, S., et al. (2017). Multidrug-resistant *Candida haemulonii* and *C. auris*, Tel Aviv, Israel. *Emerg. Infect. Dis.* 23, 195–203. doi: 10.3201/eid2302.161486.
15. Osei Sekyere, J. (2018). *Candida auris*: a systematic review and meta analysis of current updates on an emerging multidrug-resistant pathogen. *MicrobiologyOpen* 7:e00578. doi: 10.1002/mbo3.578.
16. Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H. Candida auris sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. Microbiol Immunol.212009;53(1):41–4.
17. Cortegiani A, Misseri G, Chowdhary A. What's new on emerging resistant Candida species. Intensive Care Med. 2018 Online First doi: https://doi.org/10.1007/s00134-018-5363-x.

18. Rudramurthy SM, Chakrabarti A, Paul RA, Sood P, Kaur H, Capoor MR, et al. Candida auris candidaemia in Indian ICUs: analysis of risk factors. J Antimicrob Chemother. 2017;72:1794–801.
19. Chowdhary A, Anil Kumar V, Sharma C, Prakash A, Agarwal K, Babu R, et al. Multidrug-resistant endemic clonal strain of Candida auris in India. Eur J Clin Microbiol Infect Dis. 2014;33:919–26.
20. Sharma C, Kumar N, Pandey R, Meis JF, Chowdhary A. Whole genome sequencing of emerging multidrug resistant Candida auris isolates in India demonstrates low genetic variation. New Microbes New Infect. 2016;13:77–82.
21. Girard V, Mailler S, Chetry M, Vidal C, Durand G, van Belkum A, et al. Identification and typing of the emerging pathogen Candida auris by matrix-assisted laser desorption ionisation time of flight mass spectrometry. Mycoses. 2016;59:535–8.
22. Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, et al. Simultaneous emergence of multidrug-resistant Candida auris on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clin Infect Dis. 2017;64:134–40.
23. Larkin E, Hager C, Chandra J, Mukherjee PK, Retuerto M, Salem I, et al. The emerging pathogen Candida auris: growth phenotype, virulence factors, activity of antifungals, and effect of SCY-078, a novel glucan synthesis inhibitor, on growth morphology and biofilm formation. Antimicrob Agents Chemother. 2017; 61:e02396–16.
24. Wattal C, Oberoi JK, Goel N, Raveendran R, Khanna S. 2017. Matrix assisted laser desorption ionization time of flflight mass spectrometry (MALDI-TOF MS) for rapid identifification of micro-organisms in the routine clinical microbiology laboratory. Eur J Clin Microbiol Infect Dis 36:807– 812. https://doi.org/10.1007/s10096-016-2864-9.
25. Oh BJ, Shin JH, Kim MN, Sung H, Lee K, Joo MY, Shin MG, Suh SP, Ryang DW. 2011. Biofifilm formation and genotyping of Candida haemulonii, Candida pseudohaemulonii, and a proposed new species (Candida auris) isolates from Korea. Med Mycol 49:98 –102. https://doi.org/10.3109/ 13693786.2010.493563.
26. European Committee on Antimicrobial Susceptibility Testing. 2015. Clinical breakpoints—fungi (v 8.0). 48.
27. Clinical and Laboratory Standards Institute. 2008. M27-A3 reference method for broth dilution antifungal susceptibility testing of yeasts, 3rd ed. Clinical and Laboratory Standards Institute, Wayne, PA.
28. Magobo, R. E., Corcoran, C., Seetharam, S., Govender, N., & Naicker, S. (2014). Candida auris: an emerging, azole-resistant pathogen causing candidemia in South Africa. *International Journal of Infectious Diseases*, *21*, 215.
29. Larkin, E., Hager, C., Chandra, J., Mukherjee, P. K., Retuerto, M., Salem, I., ... & Ghannoum, M. (2017). The emerging pathogen Candida auris: growth phenotype, virulence factors, activity of antifungals, and effect of SCY-078, a novel glucan synthesis inhibitor, on growth morphology and biofilm formation. *Antimicrobial agents and chemotherapy*, *61*(5), e02396-16.
30. Sherry, L., Ramage, G., Kean, R., Borman, A., Johnson, E. M., Richardson, M. D., & Rautemaa-Richardson, R. (2017). Biofilm-forming capability of highly virulent, multidrug-resistant Candida auris. Emerging infectious diseases, 23(2), 328.
31. Fasciana, T., Cortegiani, A., Ippolito, M., Giarratano, A., Di Quattro, O., Lipari, D., & Giammanco, A. (2020). Candida auris: An Overview of How to Screen, Detect, Test and Control This Emerging Pathogen. Antibiotics, 9(11), 778.

32. Sabino, R., Veríssimo, C., Pereira, Á. A., & Antunes, F. (2020). Candida auris, an agent of hospital-associated outbreaks: which challenging issues do we need to have in mind? Microorganisms, 8(2), 181.
33. Du, H., Bing, J., Hu, T., Ennis, C. L., Nobile, C. J., & Huang, G. (2020). Candida auris: Epidemiology, biology, antifungal resistance, and virulence. *PLoS pathogens*, *16*(10), e1008921.
34. Satoh, K.; Makimura, K.; Hasumi, Y.; Nishiyama, Y.; Uchida, K.; Yamaguchi, H. Candida aurissp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. Microbiol. Immunol. 2009, 53, 41–44.
35. Lee, W.G.; Shin, J.H.; Uh, Y.; Kang, M.G.; Kim, S.H.; Park, K.H.; Jang, H.-C. First Three Reported Cases of Nosocomial Fungemia Caused by Candida auris. J. Clin. Microbiol. 2011, 49, 3139–3142.
36. Magobo, R.E.; Corcoran, C.; Seetharam, S.; Govender, N.P. Candida auris–Associated Candidemia, South Africa. Emerg. Infect. Dis. 2014, 20, 1250–1251.
37. Calvo, B.; Melo, A.S.; Perozo-Mena, A.; Hernandez, M.; Francisco, E.C.; Hagen, F.; Meis, J.F.; Colombo, A.L. First report of Candida auris in America: Clinical and microbiological aspects of 18 episodes of candidemia. J. Infect. 2016, 73, 369–374.
38. Ruiz-Gaitán, A.; Moret, A.M.; Tasias-Pitarch, M.; Aleixandre-Lopez, A.I.; Martínez-Morel, H.; Calabuig, E.; Salavert-Lletí, M.; Ramirez, P.; Hontangas, J.L.L.; Hagen, F.; et al. An outbreak due to Candida auris with prolonged colonisation and candidaemia in a tertiary care European hospital. Mycoses 2018, 61, 498–505.
39. Kordalewska, M.; Perlin, D.S. Identification of Drug Resistant Candida auris. Front. Microbiol. 2019, 10, 1918.
40. Sexton, D.J.; Kordalewska, M.; Bentz, M.L.; Welsh, R.M.; Perlin, D.S.; Litvintseva, A.P. Direct detection of emergent fungal pathogen Candida auris in clinical skin swabs by SYBR Green-Based Quantitative PCR Assay. J. Clin. Microbiol. 2018, 56, e01337-18.
41. Teresa Fasciana, Andrea Cortegiani, Mariachiara Ippolito, Antonino Giarratano, Orazia Di Quattro, Dario Lipari, Domenico Graceffa and Anna Giammanco: Candida auris: An Overview of How to Screen, Detect, Test and Control This Emerging Pathogen, Antibiotics 2020, 9, 778; doi:10.3390/antibiotics9110778.
42. Leach, L.; Zhu, Y.; Chaturvedi, S. Development and validation of a Real-Time PCR Assay for rapid detection of Candida auris from surveillance samples. J. Clin. Microbiol. 2017, 56.
43. Khan, Z.; Ahmad, S.; Al-Sweih, N.; Joseph, L.; Alfouzan, W.; Asadzadeh, M. Increasing prevalence, molecular characterization and antifungal drug susceptibility of serial Candida auris isolates in Kuwait. PLoS ONE 2018, 13, e0195743.
44. Ruiz-Gaitán, A.C.; Fernández-Pereira, J.; Valentin, E.; Tormo-Mas, M.A.; Eraso, E.; Pemán, J.; De Groot, P.W. Molecular identification of Candida auris by PCR amplification of species-specific GPI protein-encoding genes. Int. J. Med Microbiol. 2018, 308, 812–818.
45. Arastehfar, A.; Fang, W.; Badali, H.; Vaezi, A.; Jiang, W.; Liao, W.; Pan, W.; Hagen, F.; Boekhout, T. Low-Cost Tetraplex PCR for the global spreading multi-drug resistant fungus, Candida auris and its phylogenetic relatives. Front. Microbiol. 2018, 9, 1119.
46. Ahmad, A.; Spencer, J.E.; Lockhart, S.R.; Singleton, S.; Petway, D.J.; Bagarozzi, D.A., Jr.; Herzegh, O.T. A high-throughput and rapid method for accurate

identification of emerging multidrug-resistant Candida auris. Mycoses 2019, 62, 513–518. [CrossRef] [PubMed] Antibiotics 2020, 9, 778 12 of 14

47. Theill, L.; Dudiuk, C.; Morales-López, S.E.; Berrio, I.; Rodriguez, J.Y.; Marín, A.; Gamarra, S.; Garcia-Effron, G. Single-tube classical PCR for Candida auris and Candida haemulonii identification. Revista Iberoamericana de Micología 2018, 35, 110–112.
48. Arastehfar, A.; Fang, W.; Pan, W.; Lackner, M.; Liao, W.; Badiee, P.; Zomorodian, K.; Badali, H.; Hagen, F.; Lass-Flörl, C.; et al. YEAST PANEL multiplex PCR for identification of clinically important yeast species: Stepwise diagnostic strategy, useful for developing countries. Diagn. Microbiol. Infect. Dis. 2019, 93, 112–119.
49. Martínez-Murcia, A.J.; Navarro, A.; Bru, G.; Chowdhary, A.; Hagen, F.; Meis, J.F. Internal validation of GPS™ MONODOSE CanAur dtec-qPCR kit following the UNE/EN ISO/IEC 17025:2005 for detection of the emerging yeast Candida auris. Mycoses 2018, 61, 877–884.
50. Sexton, D.J.; Bentz, M.L.; Welsh, R.M.; Litvintseva, A.P. Evaluation of a new T2 Magnetic Resonance assay for rapid detection of emergent fungal pathogen Candida auris on clinical skin swab samples. Mycoses 2018, 61, 786–790.
51. Yamamoto, M.; Alshahni, M.M.; Tamura, T.; Satoh, K.; Iguchi, S.; Kikuchi, K.; Mimaki, M.; Makimura, K. Rapid Detection of Candida auris based on Loop-Mediated Isothermal Amplification (LAMP). J. Clin. Microbiol. 2018, 56, e00591-18.
52.