

Isolation and Identification of *Staphylococcus Aureus* and *Candida* SPP from Throat Infections

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ABSTRACT: This research aimed to isolate and identify *Staphylococcus aureus* and *Candida* species from throat infections, as well as assess their antibiotic resistance. Samples were collected from both infected patients and healthy individuals, encompassing various ages and genders, in hospitals of Najaf province between December 2022 and March 2023. The findings revealed that *S. aureus* constituted 36.10% of isolates, followed by *S. warneri* at 22.81%, and *S. lugdunensis* at 15.29%. Notably, *S. aureus* isolates displayed 100% resistance to Penicillin (P), Aztreonam (ATM), and Oxacillin (OX), Amoxicillin (AMC) and Tetracycline (TE) exhibiting resistance rates of 81.95% and 78.19%, respectively. The study concluded that *S. aureus* was the predominant strain, followed by *S. warneri*. Antibiotic susceptibility testing on all *S. aureus* isolates (n=21) was conducted using seven selected antibiotics. *Candida* species identified included *Candida albicans*, *Candida parapsilosis*, and *Candida glabrata*, with maximum resistance observed against fluconazole (FLU) and polymyxin B (PB), followed by miconazole (MCL).

KEYWORDS: *Staphylococcus aureus*, *Candida*, throat infections

INTRODUCTION

The human throat offers a variety of niches for microbial colonization. Some of these microorganisms adapted to be as normal flora in throat. It was found that about 1:4 of *Staphylococcus aureus* carriers have been in throat carriage exclusively (Kumpitsch, et. al.2019). Bacterial communities in throat and pharynx characterized with gram positive and negative species. This distinct environment has the potential to contain a distinct microbiota and play an important role in disease (Bassis , et. al. , 2014). Few studied have focused on the infections of the human throat as a microbial carriage site. However, different isolation rates have been investigated in the previous studies (Nilsson, P., & Ripa, 2006). Many coagulase-positive and negative staphylococci are normal microbiota of the throat. In contrast, some of *Staphylococcus* species pose public health problems due to their capacity to produce staphylococcal infections and diseases in humans (Zondervan , et. al. , 2021). The main reservoir site for *Staphylococcus aureus* is the anterior nares, vestibules and throat (Sakr , et. al. , 2018). *S. aureus* is an important cause of throat infections due to persistent carriage of different *S. aureus* strains (Bassis et al. , 2014 ; Steed et al. , 2014). *S. aureus* can disseminate to other deep organs in human to cause a big health problem in patients, especially when these strains characterized with ability of antibiotic resistance (Mohajeri , el. al. , 2013). Methicillin-resistant *S. aureus* (MRSA) strains are found worldwide, and it is resistant to all beta-lactam antibiotics including penicillin, cephalosporins, and cephamycins except ceftaroline (Iyer , et. al. , 2014; Zainab et al., 2020). Presence of *S. aureus* throat colonization can provide an indication of a higher risk for subsequent infection, including with MRSA (Dogramachy , 2018) .

On the other hand, *Candida* species is the most frequently encountered pathogenic human fungal species and commonly colonizes host mucosal and moist skin surfaces. However, under conditions of immune dysfunction, this opportunistic microbe can rapidly transition from commensal to pathogen, causing an array of infections ranging from localized mucosal to severe to vaginal to systemic infections with high morbidity and mortality rates (Bartie , et. al. , 2004; Teeba et al. 2021).

This research aimed to isolate and identify *Staphylococcus aureus* and *Candida* species from throat infections, as well as assess their antibiotic resistance.

MATERIALS AND METHODS

Patient's Specimens

Specimens were collected from patients with (throat infections) and healthy. The samples were collected from patients of both genders of different ages from Hospitals of Najaf province between December 2022 and March 2023. Specimens were taken from patients by sterile swabs .The swabs were placed in sterile tubes with transport media, and then were transported to Public Health

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Laboratory within 1 hour to be inoculated on blood agar, MacConkey agar, mannitol agar and SAD incubated at 37 °C for 24 to 48 hours (Forbes et al. , 2007).

Samples Inoculation

The collected specimens were inoculated on culture media which included blood agar, mannitol salt agar, MacConkey agar which considered as predominant enrich media, selective and differential media for the isolation, purification and identification of many types of bacteria. The plates were incubated at 37°C for 24 hours then a single pure isolated colony was transferred to brain heart infusion agar slant for the preservation and to carry out other biochemical tests that confirmed the identification of isolates. The specimens also inoculated on Sabouraud's Dextrose Agar (SDA), then incubated at 30°C for 48-72 hours, and covered the plates using the parafilm to prevent contamination and preserve the culture when saved it in the refrigerator (Bhavan et al. , 2010). Sabouraud dextrose agar (SDA) is standard medium for isolation *Candida* spp. This medium has special properties one of them the pH of medium is low so many bacteria could not grow on it (Budzyńska et al. , 2014).

Identification of Staphylococci

Staphylococci was identified depending on the morphological features (colony size, shape, color, hemolysis, translucency, edge, elevation, and texture) on culture media and biochemical tests. All samples were examined microscopically to determine the presence of staphylococcal cells. (MacFaddin, 2000).

Identification of Fungi

Culture was examined for creamy, pasty, and smooth white colonies. Colony morphology was tested and rehabilitated after cultivation and incubation on SDA (Hameed et al., 2017). The *Candida* colonies were initially identified by colonial color when compared with standard color photographs supplied by the manufacturer and also presented (Williams & Lewis, 2000).

Kinds of Antibiotic and Concentration

The antibiotic discs that were used in the present study are listed in table (1) and global values of antibiotics are based on this study according to CLSI, 2019.

Table 1.-Antibiotic discs used in this study.

NO.	Antibiotic	Symbol	Concentration (µg)	Company
1	Penicillin	PG	12	Merseyside (U.K)
2	Tetracycline	TE	11	Bioanalyse (Turkey)
3	Amoxicillin	AMC	31	Liofilchem (Italy)
4	Azithromycin	AZM	16	Liofilchem (Italy)
5	Azteronam	ATM	31	Liofilchem (Italy)
6	Oxaxillin	OX	11	Liofilchem (Italy)
7	Erythromycin	E	14	Liofilchem (Italy)
8	Polymyxin B	PB	11	Liofilchem (Italy)
9	Miconazole	MCL	11	Liofilchem (Italy)
10	Fluconazole	FLC	24	Liofilchem (Italy)

STATISTICAL ANALYSIS

Descriptive statistics have used, percentage and were used in tables and figures. Level of significance of ≤ 0.05 was considered as significant difference with equal and unequal observations (Al-fahham, 2018).

RESULTS

Identification by VITEK compact

The VITEK technique was used to identify the *Staphylococcus* species. The results indicated that *S. aureus* was the most prevalent with 8 isolate (36.10%), followed by *S. warneri* with 5 isolate (22.81%), followed by *S. lugdunensis* with 3 isolate (15.29%). (Figure 1).

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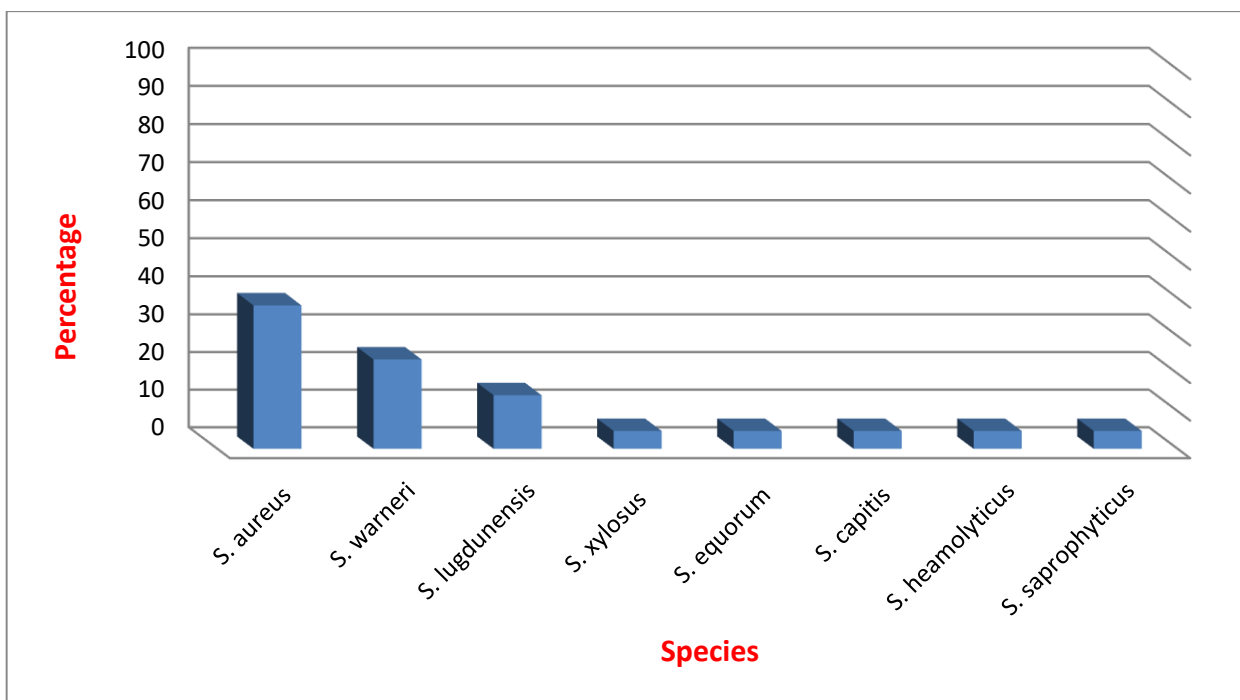


Figure 1. Frequency percent of Staphylococcus species from total positive samples. Staphylococcus identification by Vitek system. S. = Staphylococcus

Antibiotic susceptibility of *S. aureus*

Notably, *S. aureus* isolates displayed 100% resistance to Penicillin (P), Aztreonam (ATM), and Oxacillin (OX), Amoxicillin (AMC) and Tetracycline (TE) exhibiting resistance rates of 81.95% and 78.19%, respectively

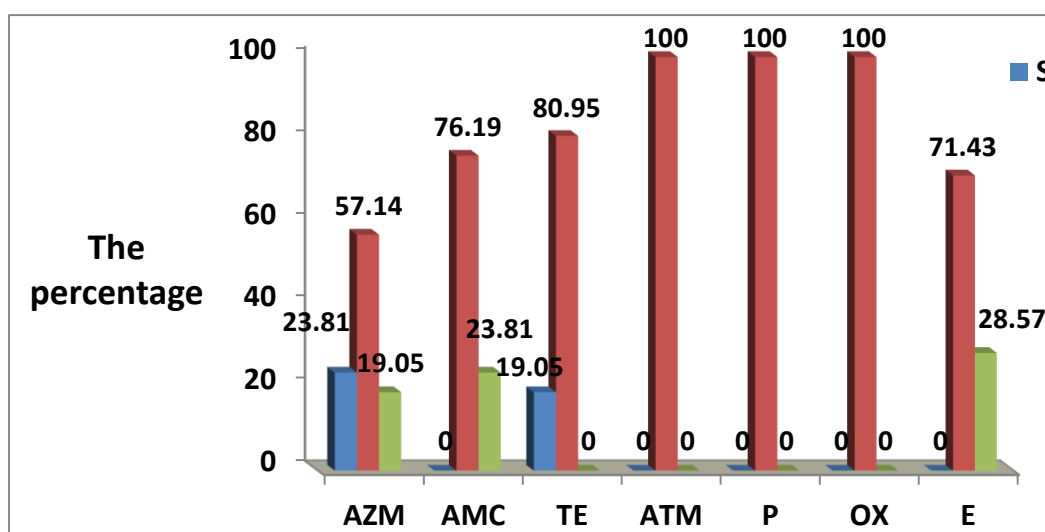


Figure 2. Antibiotic susceptibility of isolated *S. aureus*

Identification of *Candida*

Twenty samples (22.73%) of total 88 samples cultured on Sabouraud Dextrose Agar (SDA) were positive to at least one yeast species. The green color of the colonies indicates to *Candida albicans*, after incubation for 48 hours. Creamy color indicates for *Candida parapsilosis* the yeast colony in purple color had seen in isolates of *Candida glabrata*.

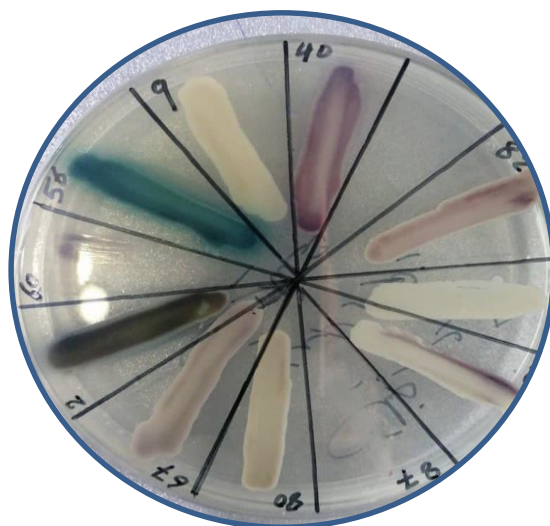


Figure 3. Growth of *Candida* species on CHROM Agar. (Green = *C. albicans* , Creamy= *C. parapsilosis* , blue = *C. tropicalis* , purple = *C. glabrata*)

Antifungal drugs susceptibility of *Candida*

All yeast isolates (n=20) were examined for Antifungal drugs susceptibility against 3 selected antibiotics (figure 4). The results revealed that there were differences in resistance rates exhibited by the targeted yeast. The maximum ratio of resistance was (35%) of *Candida* isolates against fluconazole (FLU) and polymyxin B (PB) followed by miconazole (MCL) (25%).

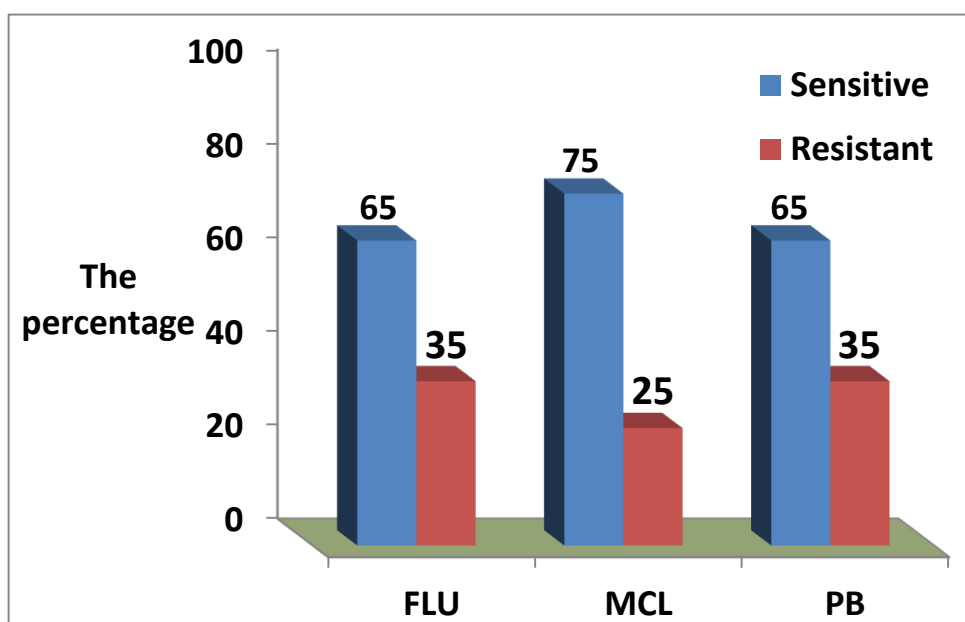


Figure 4. Antibiotic susceptibility of yeast species. FLU=Fluconazole , PB=Polymyxin B , MCL=Miconazole.

DISCUSSION

Although *Staphylococcus* spp. may be considered as normal human microbiota, *Staphylococcus* can as well act as opportunistic microorganism and produce super infection. *Staphylococcus aureus* is considered the most important throat staphylococci. In this study , *S. aureus* were isolated with 23.86%, which was relatively close to previous study *S. aureus* (Abdullahi et al. , 2021). The result of current study was near of the global reports. For example , a study in Nigeria showed that the isolation of *S. aureus* was 46.51% (Edomwande et. al. , 2014). *Staphylococcus aureus* can adapt to different environments and hosts efficiently by the help of resistance genes that play the major role in pathogenicity and various virulence factors (Rasheed & Hussein , 2020).

The antibiotic resistance of *S. aureus* has increased controlling infection since the first case was reported in the United Kingdom in 1960 (Abdullahi et al. , 2021). Therefore, in the current study, the antibiotics resistance was achieved to 100% against some antibiotics. These results were in agreement with several studies in Iraq such as in Basra (Jamalludeen et al , 2021) and in Erbil (Dogramachy , 2018) revealed high resistance antibiotics by *Staphylococcus aureus* against chloramphenicol , ciprofloxacin , clindamycin , erythromycin , gentamicin penicillin , rifampicin , trimethoprim sulfamethoxazole , and tetracycline.

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Iraq is one of six countries that characterized with very high antibiotics resistance prevalence of *S. aureus*, which has also been differentiated through the ownership of unique patterns of antimicrobial resistance and molecular characteristics (Abdullahi et al., 2021). This fact gives reasons behind the high ratio of resistance; since antibiotic resistance of *S. aureus* prevalent in Iraq has an especial pattern in resistance in general to antimicrobials.

Candida albicans is ubiquitously present, and the colonization in the pharyngeal and nasal site is common (Ickrath et al., 2021). *C. albicans* is considered the main etiologic agent of opportunistic infection, candidiasis, related with local and systemic predisposing factors (Martins et al., 2002). All isolated yeast species were subcultured on CHROMagar petridishes and the identification was done depending on colonies color, which indicates that all yeast was identified under *Candida* genus. In addition, the result of germ tube test indicated that there was only two isolates of *C. albicans*. These results were well interpreted in genetic section of this chapter.

The total percentage of yeast isolates in this study was (22.73%), which was more than the percent of *Candida* species (13.8%) isolated from immunocompromised patients with throat infections in Baghdad (Alwatar et al., 2021).

Antifungal drugs resistance was determined in isolated yeast species using fluconazole, polymyxin and miconazole, which were locally used to treat yeast infections. The highest resistance (35%) was observed against fluconazole and miconazole followed by (25%) polymyxin. This indicates that yeast species, especially *Candida* develop their abilities (for example; overexpression of plasma membrane efflux pumps) to resist these antifungals to become fully resist (Cowen et al., 2015). Another study confirmed that the consequence of inhibition is a reduction in the intracellular levels of ergosterol, the main sterol of fungi, and an increase in substrate and other abnormal sterols produced by further metabolism of the substrate. This change in sterol composition results in growth arrest. Many mechanisms of resistance include increased drug efflux, alteration or increase in the drug target, and development of compensatory pathways for producing the target sterol, ergosterol were reported in yeast species against fluconazole (Berkow & Lockhart, 2017).

CONCLUSIONS

The study determined that *S. aureus* had the highest prevalence, with *S. warneri* following closely behind. Antibiotic susceptibility testing was conducted on all 21 *S. aureus* isolates using seven selected antibiotics. The identified *Candida* species included *Candida albicans*, *Candida parapsilosis*, and *Candida glabrata*. Among the *Candida* isolates, the highest resistance ratio was 35%, observed against fluconazole (FLU) and polymyxin B (PB), followed by miconazole (MCL) with a resistance rate of 25%.

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