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Review Article

# Multi-drug resistance in clinically relevant pathogens, *Candida* and *Staphylococcus* – an alarming situation.

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**ABSTRACT**

From the inception of civilization, human being struggles for existence, since millions of people die from various infectious diseases, thus instigating the man to endeavor for remedy from their sufferings. *Staphylococcus aureus* and *Candida tropicalis* are perhaps the pathogens of greatest concern, causing often life-threatening infections. Given the vast array of effective antimicrobial agents, virtually all infections should be treatable. However, emergence of resistance to multiple antibiotics has been particularly observed and is now the norm among such pathogens as *S. aureus* and *C. tropicalis*. Inevitably this has left fewer effective bactericidal antibiotics for treatment, since as rapidly as new antibiotics are introduced, these microorganisms have developed efficient mechanisms to tolerate them. This review focuses on such pathogenic microbes and their resistance to the commonly available drugs, thus paving a way for new drug discovery research.

**Key words:** Antimicrobial, *Candida*, Pathogens, Resistance and *Staphylococci.*

## INTRODUCTION

Although considered structurally simple, bacteria are extremely diverse from a metabolic standpoint. They are found almost everywhere on earth in vast numbers-from living in jet fuel and on the rims of volcanoes, to thriving in hydrothermal vents deep on the ocean floor. There are both beneficial and pathogenic bacteria; pathogenic bacteria can cause severe and often fatal diseases in plants, animals and humans [1]. Among such bacteria, *Staphylococci* are very widespread and among the most important etiological agents of both community and hospital acquired infections [2]. Fungi are widespread in the environment being the major pathogens of agricultural plants [3], while others are associated with animals and humans as commensals, but turn pathogenic or opportunistic after alteration of the host immune system [4]. Fungal infections, including those caused by *Candida* sp. remain a major problem for the patients with weakened or impaired immune system like in AIDS [5], cancer (leukemia) and bone-marrow transplant recipients.

The prevalence of antimicrobial resistance especially among key microbial pathogens, both bacteria and fungi is increasing at an alarming rate worldwide[6]. Resistance evolves because antimicrobial agents are rarely deployed in a way that completely eradicates the pathogen population, with survivors subjected to natural selection. Also, whenever the pathogen population, remains large enough over the course of drug treatement, the evolution of resistance is all but inevitable [7]. In recent years, however the increase in the number of multi-drug resistant bacteria has led to the prediction that we are re-entering the 'pre-antibiotic era'. *Staphylococcus aureus* subsp. *aureus* is clinically one of most important and successful pathogens[8] because of its exceptional virulence, stress tolerance and adaptibilty to antibiotic pressure i.e., capacity to induce antimicrobial resistance [9]. In adition, due to the increasing incidence of opportunistic fungal infections, therapy for serious *Candida* infections has been difficult because there are a limited number of antifungal drugs, especially compared with the number of antibacterial drugs [10]. Moreover, the appearance of such resistant strains to the commonly used antibiotics not only raises costs and reduces effectiveness of the treatments, but also poses a risk for the natural environment. This review specifically provides an insight into the multi-

antibiotic resistance of the widespread *Staphylococcus aureus* and *Candida tropicalis* infections, including its mechanism, against a class of commonly used drugs.

**PATHOGENESIS**

Pathogenesis involves the interaction of two partners with input from the environment [11]. *Staphylococcus aureus* is often the first bacterium to be cultured from the respiratory tract in infants and children with cystic fibrosis [12] and from the skin of around a third of the population. The organism is highly resistant to adverse environmental conditions and resists drying as well as high NaCl concentrations, enabling a probably temporary and even permanent colonization of skin and nasal mucosa [13]. These factors account it as one of the major resistant carrier strain in the nasal mucosa of general population with a mean carriage rate of 37.2% [14]. Apart from the skin, throat and nasal mucosa, *S. aureus* may be present in the colon and urine of a healthy person, where it can cause a range of illnesses. These range from minor skin inflammations (such as pimples, boils and cellulites), alimentary poisoning, osteomylitis, toxic shock syndrome (TSS), staphylococcal scalded skin syndrome (SSSS) and bacterial endocarditis to life threatening sepsis, pneumoniae and meningitis [15]. It is also prevelant in the environment, especially around people, in animals (on the skin and mucosae) and food. Moreover, methicillin resistant *S. aureus* (MRSA) is acknowledged to be a human commensal and pathogen having a number of "virulence factors" that enable them to result in disease [13].

Among fungi, *Candida* sp. produce a broad range of serious illnesses in immunocompromised [16] and in hospitalized hosts where it may turn into opportunistic pathogen causing local and systemic infection. *Candida* sp. are the third most common pathogens as causative agents of nosocomial bloodstream infections in premature infants [17] and the fourth commonest cause of bloodstream infections in pediatric ICU patients [18]. *Candida* sp., the most common etiologic agents in these infections, is a normal commensal of humans found in the respiratory, gastrointestinal and genitourinary tracts, skin as well as mucosal membranes. The spectrum of infection with *Candida* sp. thus range from superficial candidiasis of the skin and mucosa (oral and oesophageal) to more serious life threatening infections, deep seated, deeply invasive, systemic and hematogenously disseminated [19, 16] spreading to virtually any organ. Invasive *Candida* infection includes pneumonia, blood stream, GI, ophthalmological, CNS, renal, ocular, bone and joint infection due to the extent of adherence to tissues and gingival epithelial cells. This correlates with the pathogenecity in humans and animals, with *C. albicans* exhibiting the greatest adherence capacity, followed by *Candida tropicalis* [20] which is now emerging as the most important species responsible for invasive candidiasis [21]. Moreover, candidemia, has increased worldwide over the last 20 years [16] especially in cancer [22] and critically ill patients. *Candida* sp. associated with candidemia have shifted from *C. albicans* to non-*albicans Candida* (NAC) sp. with *C. tropicalis* as the most important species [23, 24, 16], accounting for approximately half of the reported cases [25, 26].

**DRUG RESISTANCE IN PATHOGENS**

Antimicrobial drug resistance, the ability of a microorganism to withstand the effects of antibiotics, is an important biological phenomenon that has a considerable impact on animal and human health [27]. The widespread and sometimes inappropriate use of antimicrobials sometimes as growth enhancers in animal feed accompanied by the relative ease of spread of antimicrobial-resistant bacteria cross geographic barriers contribute to the evolution of multi-antibiotic resistant bacterial species [28]. A report indicates increasing antimicrobial resistance in all health care associated pathogens [29] and the increasing prevelance of clinical drug resistance in recent decades due to the greater use and abuse of otherwise efficacious antimicrobial agents [27]. As a consequence of such antibiotic overuse and misuse, nosocomial infections caused by 'multi-drug resistant' pathogens represent a physician's nightmare through out the world [30]. The common mechanisms of microbial drug resistance (Figure 1) may involve either the overproduction of the target enzyme thus preventing the drug to inhibit the biochemical reaction completely or by alteration of drug target to avoid the binding of the drug to the target; or prevention of drug entering into the cell membrane/cell wall level or else the drug may be pumped out by an efflux pump; moreover the cell has a bypass pathway that compensates for the loss-of-function inhibition due to the drug activity. Enzymes also play a major role in drug resistance; some (mainly fungal) that convert an inactive drug to its active form are inhibited or some enzymes which degrade the drug may be secreted to the extracellular medium by the cell [31].

**Bacterial drug resistance:**

Drug resistance has emerged in a burgeoning number of bacterial genera and species accounting in more than 70% [32] throughout the world over the past 50 years [33]. Bacterial cells have multiple drug targets [34], thus exposing various sites for antibiotic resistance (Figure 2). Only a few decades after the introduction of antibiotics into clinical practice, resistance by

opportunistic bacterial pathogens, both Gram positive and Gram negative bacteria, have become a major health concern especially towards the antibiotics in common use [35]. There has been a great concern about the development of resistance especially, among Gram positive pathogens, the so-called methicillin-resistant bacteria and the particular strains which are causing problems at the moment are the methicillin resistant *S. aureus* (MRSA) [36]. In recent years, a dramatic increase in the incidence of nosocomial infections caused by *S. aureus* strains are owing to its resistance to multiple antibiotics (Table 1) because the strains that were methicilin as well as oxacillin resistant, (historically termed MRSA) have become less susceptible to, including the beta-lactam antibiotics (flucloxacillin, dicloxacillin), penicillin/beta-lactamase inhibitor combinations like, cephalosporins (cefazolin, cephalothin and cephalexin), carbapenems, non beta-lactam antibiotics like, macrolides and azalides, lincosamides, tetracyclines and aminoglycosides including clindamycin, lincomycin and erythromycin [37, 13]. Teicoplanin and vancomycin often used as an antibiotic of last resort [38], in recent times, have proved to be ineffective, with linezolid resistance worsening the situation. Probably, the last and only alternative for the treatement is using a combination of two oral antimicrobials, typically rifampicin and fusidic acid, since resistance develops rapidly if they are used as single agents. In the recent past, there has been a reemergence of antibiotic-resistant *S. aureus* in the genomics era [39].

Mechanisms of antibiotic resistance in *S. aureus* mainly include enzymatic inactivation of the antibiotic as in case of penicillinase and aminoglycoside-modification enzymes. Staphylococcal resistance to penicillin is mediated by *blaZ* gene that encodes β-lactamase, an extracellular enzyme, synthesized on exposure to β-lactam antibiotics. This enzyme hydrolyzes the β-lactam ring, affecting the activity of penicillin [40]. Resistance to aminoglycoside antibiotics occurs mainly due to genes encoding aminoglycoside-modifying-enzymes (AMEs) [41].

Antibiotic resistance in *S. aureus* may also involve alteration of the target with decreased affinity for the antibiotic, notable examples being penicillin-binding protein 2α (PBP2α) of methicillin-resistant *S. aureus* and D-Ala-D-Lac of peptidoglycan precursors of vancomycin-resistant strains; or *S. aureus* acquires complex genetic arrays like staphylococcal chromosomal cassette *mec* elements or the *vanA* operon through horizontal gene transfer [42]. Methicillin resistance requires the presence of the chromosomally localized *mecA* gene [40], responsible for synthesis of PBP2α, a 78-kDa protein. PBPs are membrane-bound enzymes that catalyze the transpeptidation reaction that is necessary for cross-linkage of peptidoglycan chains. Thus, resistance to methicillin confers resistance to all β-lactam agents, including cephalosporins. Resistance to oxacillin arise due to β-lactamase hyperproduction and one or more PBPs mutations [43]. Structural changes within the bacterial PBPs due to acquisition of metallo-beta-lactamases capable of rapidly degrading carbapenems results in resistance. Moreover resistance may be associated with changes in membrane permeability due to loss of specific outer membrane porins [44].

Acquisition of a natural resistance gene, *cfr* (chloramphenicol-florfenicol resistance) is responsible for linezolid resistant *S. aureus* [45, 46]. Resistance to macrolides has been associated with the presence of erythromycin ribosome methylase (*erm*) genes which also confers resistance to lincosamide and streptogramin B antibiotics ($MLS_B$ phenotype), with the macrolide streptogramin resistance (*msr*) drug efflux mechanism yielding an $MS_B$ phenotype [47].

Drug resistance in *S. aureus* may also involve trapping of the antibiotic for vancomycin and possibly daptomycin. Two forms of *S. aureus* resistance to vancomycin have now been identified. One form has been identified in the VISA strains. The reduced susceptibility to vancomycin appears to result from changes in peptidoglycan biosynthesis resulting in irregularly shaped, thickened cell walls, accompanied with decreased cross-linking of peptidoglycan strands reduced amounts of L-glutamine that is available for amidation of D-glutamate in the pentapeptide bridge, thus resulting in more D-Ala-D-Ala residues available to bind and trap vancomycin and preventing the molecule from getting to its bacterial target [48]. The second form of vancomycin resistance has resulted because of the acquisition by probable conjugal transfer of the *vanA* operon from an *Enterococcus* that allows synthesis of a cell wall precursor that ends in D-Ala-D-Lac dipeptide rather than D-Ala-D-Ala. Synthesis of D-Ala-D-Lac occurs only with exposure to low concentrations of vancomycin, thus allowing continued peptidoglycan assembly [49]. As a result, the additional biosynthetic demands are limited and the VRSA strain is ecologically fit [50]. This ecological fitness, and the resistance of these strains to both β-lactams and glycopeptides all increase the likelihood that VRSA strains will rapidly become more prevalent [51]. Daptomycin resistance (DAP-R) in *S. aureus* often exhibit progressive accumulation of single nucleotide polymorphisms in the multipeptide resistance factor gene (mprF) and the yycFG components, involved in key cell membrane (CM) events, with mprF being responsible for the synthesis

and outer CM translocation of the positively charged phospholipid, lysyl-phosphotidylglycerol, while the yyc operon is involved in the generalized response to stress agentsf such as antimicrobials. Extremes in CM order, resistance to CM depolarization and permeabilization, and reduced surface binding of DAP along with modifications of the cell wall (CW) leading to enhanced expression of the dlt operon (involved in d-alanylation of CW teichoic acids) and progressive CW thickening are the major causes for appearance of DAP-R strains [52].

Spontaneous mutations and positive selection are the main cause of resistance to other antibiotics, including some of the most recent ones like, including the fluoroquinolones, linezolid and daptomycin [42]. The mechanism of resistance in *S. aureus* to quinolones results from the stepwise acquisition of chromosomal mutations. Mutations generally occur due to the limited quinolone concentrations at staphylococcal infection sites accompanied with high bacterial population and presence of resistant bacteria [53]. Since quinolones act on DNA gyrase and topoisomerase IV, responsible for relieving DNA supercoiling and separation of the concatenated DNA strands, any change in amino acids present in critical regions of the enzyme-DNA complex (quinolone resistance–determining region) results in a reduction of quinolone affinity for both of these enzymes. Efflux pumps may also be involved in exhibiting resistance mechanism against fluoroquinolones and tetracycline by *S. aureus.* Resistance occurs due to induction of the NorA multidrug resistance efflux pump in *S. aureus*; increased expression of this pump can result in low-level quinolone resistance [54]. Two mechanisms of tetracycline resistance have been identified in *Staphylococcus* species: (i) active efflux resulting from the acquisition of the *tetK* and *tetL* genes located on a plasmid; and (ii) ribosomal protection mediated by *tetM* or *tetO* determinants located on either a transposon or the chromosome [55].

Rifampicin resistance in *S. aureus* was closely associated with mutations in the *rpoB* gene [56]. Resistance occurs even with isolates from individuals who have never been exposed to fusidic acid. *S. aureus* produces spontaneous single step chromosomal mutations in the gene coding for elongation factor G i.e., EF-G; resistance to fusidic acid may also arise from plasmid mediated decreased cell wall or membrane permeability [57, 58].

**Fungal drug resistance:**

With limited availability and increased use of antifungal agents, emergence of antifungal drug resistance among a number and variety of fungal species is inevitable [59]. Antifungal drug resistance has been studied most extensively with the yeast *Candida albicans* owing to its importance as an opportunistic pathogen [7]. It has also been studied in non-*albicans Candida* (NAC) (including *C. tropicalis*) species, where the extensive prophylactic use of antifungals has lead to increasing colonization i.e., the capacity of yeasts to attach to a wide range of inanimate surfaces and hence protect them from immune response and antifungal agents [60], thus requiring a greater dosage [22]. Drug targets that distinguish pathogen from host are more difficult to identify in fungi (Figure 3) than in bacteria, at least in part because fungi and animals are relatively closely related as crown eukaryotes, whereas bacteria are much more distantly related to their human hosts [61]. Also, antifungal drugs have similar targets in the host, it becomes difficult to ascertain the safety of these agents [59].

Initially sensitive fungal pathogens have become resistant to the currently available, clinically important antifungal drugs (Table 2). Mechanisms of resistance against various commonly available antifungal agents have been quite extensively studied in yeast *Candida tropicalis*. With the introduction of azole antifungal agents i.e., imidazoles and the triazoles, the approach to the treatement of serious *Candida* infections began to change [62], and fluconazole, a water soluble triazole is used as therapy for oropharyngeal candidiasis in patients with advanced HIV infections and AIDS. There are various mechanisms that lead to antifungal resistance, including mutations in drug target and overexpression of the enzyme changes that alter the drug target [63, 64], and there are reports on acquisition of fluconazole resistance in *C. tropicalis* [65]. The up-regulation, overexpression and mutations of ERG11, the gene coding for lanosterol 14α-demethylase mediate azole resistance of *C. tropicalis* [66, 67].

In the wake of increasing resistance to azoles, amphotericin B, the main systemic antifungal polyene in clinical use as a sole drug for nearly 30 years [31], remains the initial drug of first choice in hemodynamically unstable critically ill children at risk of NAC candidemia [16, 22]. Evidence based guidelines for the treatment of candidiasis, published by the Infectious Disease Society of America (IDSA) in 2004 [68] indicates that the first line therapy includes amphotericin B and fluconazole, approved for use in pediatrics [16]. Candidiasis like candidaemia, acute and chronic disseminated are treated using amphotericin B/fluconazole, amphotericin B/fluconazole and fluconazole respectively [69].

**Table 1**
***S. aureus* resistance to commonly used antibacterial agents**

| S. No. | Antibacterial agents | Resistance mechanism | References |
|---|---|---|---|
| 1. | Penicillin | Enzymatic inactivation by *blaZ*, the gene encoding β-lactamase | 39 |
| 2. | Methicilin | Alteration of the target with chromosomally localized *mecA* gene, responsible for synthesis of penicillin-binding protein 2α that catalyze the transpeptidation reaction necessary for cross-linkage of peptidoglycan chains | 36 |
| 3. | Oxacillin | β-lactamase hyperproduction and one or more PBP (penicillin binding protein) mutations | 42 |
| 4. | Beta-lactam antibiotics (flucloxacillin, dicloxacillin) | Mediated by *blaZ*, the gene that encodes β-lactamase | 39 |
| 5. | Fluoroquinolones | Induction of the NorA multidrug resistance efflux pump & amino acid changes in critical regions of the enzyme-DNA complex thus, reducing quinolone affinity for both of its targets i.e., DNA gyrase and topoisomerase IV. | 52, 53 |
| 6. | Lipopeptide Daptomycin | Daptomycin resistance (DAP-R) by progressive accumulation of single nucleotide polymorphisms in the multipeptide resistance factor gene (mprF) and the yycFG components, involved in key cell membrane (CM) events and cell wall modifications | 51 |
| 7. | Cephalosporins (cefazolin, cephalothin and cephalexin) | Mutations in *fusA* and *fusB* confer resistance | 50 |
| 8. | Carbapenems | Resistance to carbapenems develops when bacteria acquire or develop structural changes within their PBPs, when they acquire metallo-beta-lactamases that are capable of rapidly degrading carbapenems, or when changes in membrane permeability arise as a result of loss of specific outer membrane porins. | 43 |
| 9. | Non beta-lactam antibiotics (macrolides, azalides eg. azithromycin, erythromycin, clindamycin) | Drug efflux mechanism and erythromycin ribosome methylase (*erm*) confers resistance to macrolides | 46 |
| 10. | Lincosamides-Streptogramin B | Drug efflux mechanism and erythromycin ribosome methylase (*erm*) | 46 |
| 11. | Tetracyclines | Active efflux resulting from the acquisition of the *tetK* and *tetL* genes located on a plasmid; and (ii) ribosomal protection mediated by *tetM* or *tetO* determinants located on either a transposon or the chromosome | 54 |
| 12. | Aminoglycosides (neomycin, kanamycin and erythromycin) | Enzymatic inactivation by genes encoding aminoglycoside-modifying-enzymes (AMEs) | 40 |
| 13. | Teicoplanin | Mutation involving the regulation of expression of both polypeptides of PBP2 and a 35-kDa membrane protein. | 38 |
| 14. | Glycopeptide (Vancomycin) | More D-Ala-D-Ala residues to bind and trap vancomycin & conjugal transfer of the *vanA* operon that allows synthesis of a cell wall precursor that ends in D-Ala-D-Lac dipeptide. | 50 |
| 15. | Linezolid | Acquisition of a natural resistance gene, *cfr* (chloramphenicol-florfenicol resistance) | 44, 45 |
| 16. | Fusidic acid | Spontaneous single step chromosomal mutations in *fusA* the gene coding for elongation factor G (EF-G) which is the target of fusidic acid action; resistance may also arise from plasmid mediated decreased cell wall or membrane permeability | 54, 57 |
| 17. | Rifampicin | Mutations in the *rpoB* gene | 55 |

**Table 2**
***C. tropicalis* resistance to commonly used antifungal agents**

| S. No. | Antifungal agents | Resistance mechanism | References |
|---|---|---|---|
| 1. | Azole antifungal agents i.e., imidazoles and fluconazole | The up-regulation, overexpression and mutations of ERG11, the gene coding for lanosterol 14α-demethylase | 69, 70 |
| 2. | Polyene antibiotic i.e., Amphotericin B | Ratio changes of sterol to phospholipids, sterols in polyene bond replacement by weaker bond, such as replacement of ergosterol by 3'-hydroxy or 3-oxosterol, and masking ergosterol which has been formed thus causing a decrease in ergosterol | 63, 68, 69 |
| 5. | Flouropyrimidine i.e., Flucytosine (5 FC) | Increased transcription of all the genes involved in the de novo pyrimidine biosynthetic pathway (including *URA3* i.e., orotidine 5′-phosphate decarboxylase, ODCase) to overproduce UMP (uridyl-monophosphate) thus affecting nucleic acid synthesis | 74 |
| 6. | Echinocandins i.e., Capsofungin and its analogues i.e., Pneumocandins | Altered glucan synthesis enzyme complex by mutations in 1,3-β<br>-glucan synthase | 63, 73 |

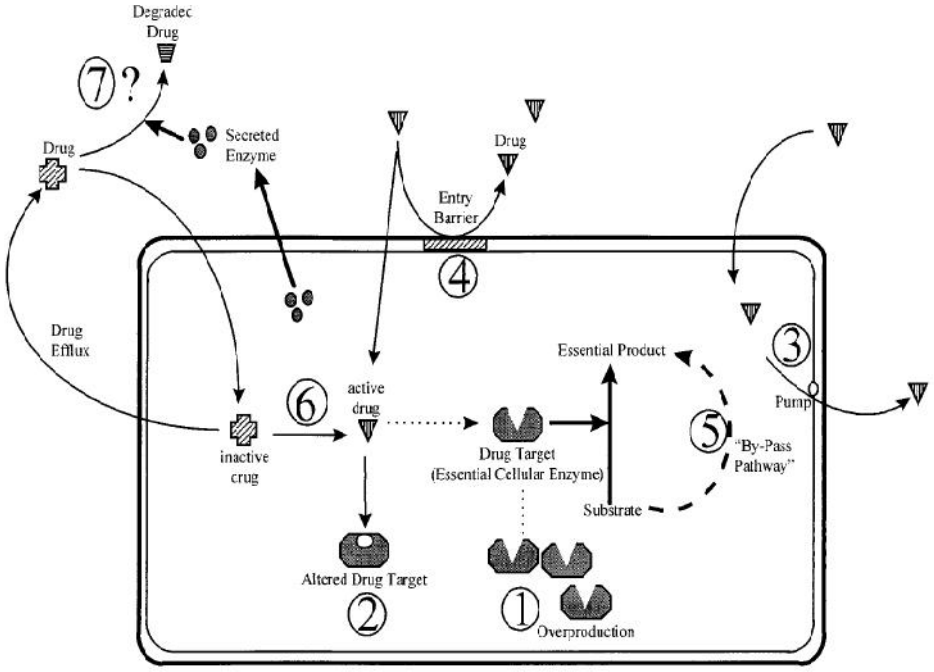


**Figure 1**
**Mechanisms by which microbial cells might develop resistance (Ghannoum and Rice, 1999)[31]**

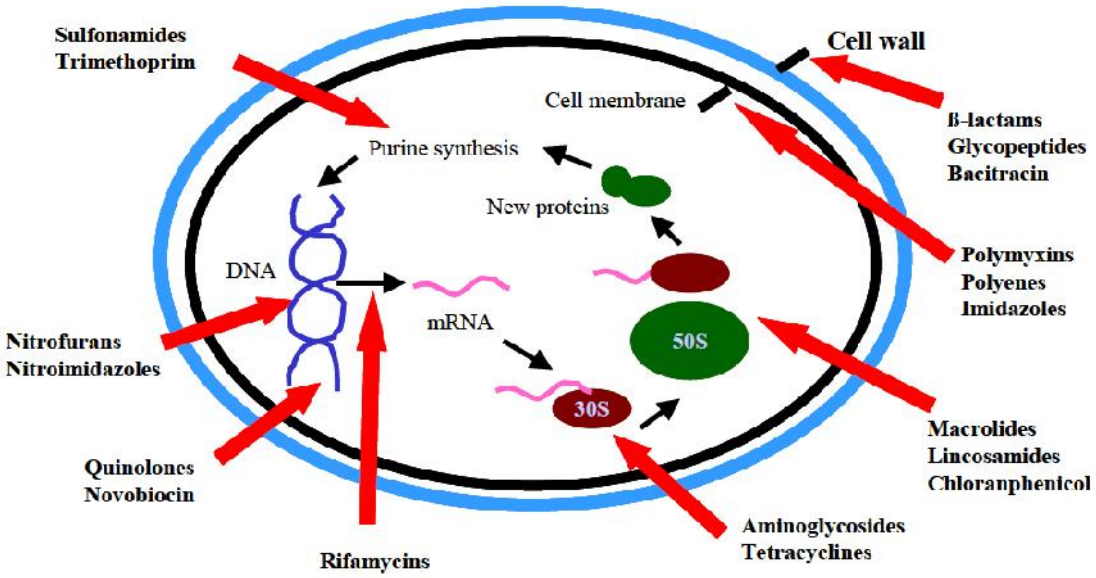


**Figure 2**
**Target of various antibacterial drugs**

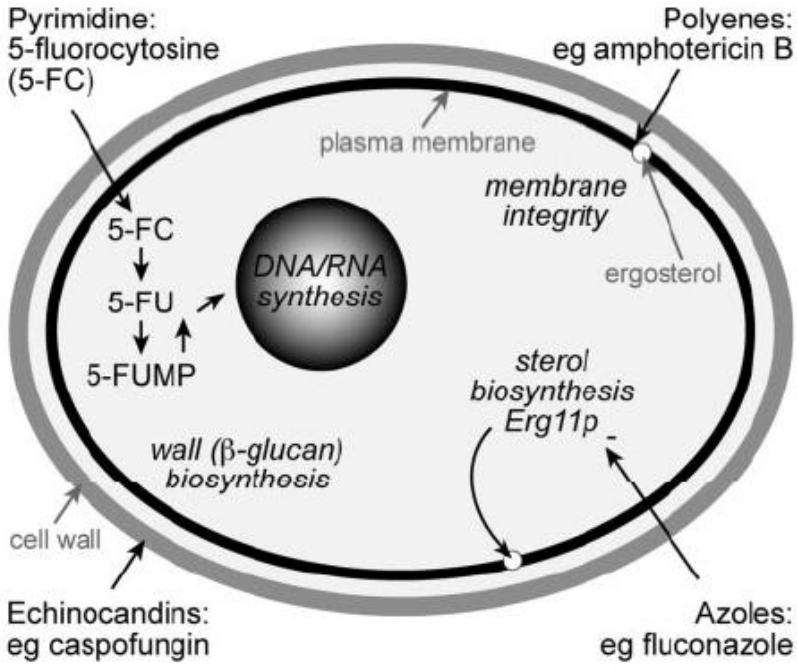


**Figure 3**
**Target of various antifungal drugs**

However, *C. tropicalis* with mutations of the azole target (Erg11p) with or without alterations of the ergosterol biosynthesis pathway involving defective activity of sterol 14α-demethylase and sterol Δ(5,6)-desaturase lead to azole-polyene cross-resistance between fluconazole, voriconazole and amphotericin B [70, 71]. According to a recent study, resistance rates for the azole group of antifungal drugs, particularly fluconazole have been found to be more as compared to amphotericin B [72].

Capsofungin, belonging to the newest cyclic lipopeptides [69] also called echinocandins [7] exhibit activity both *in vivo* and *in vitro* against clinical pathogens like *Candida* sp. IDSA indicated capsofungin as first line treatment of candidiasis in adults [16]. The pneumocandins, echinocandin analogues are cyclic hexapeptides and possess activity against *Candida* sp. among others [73]. The other mechanisms of resistance to antifungal drugs involve the reduction of drug accumulation, prevention of drug entering the cell and activation of the dispensing of cells [64]. Resistance mechanism to echinocandins (Caspofungin, anidulafungin, and micafungin) that has been characterized in *C. tropicalis*, is one of an altered glucan synthesis enzyme complex that shows a decreased sensitivity to inhibition by agents within the class [74].

Flucytosine, the main flouropyrimidine antifungal agent in clinical use offers a limited activity spectrum [10] against some yeast, including *Candida* [31]. One of the resistance mechanisms of *C. tropicalis* against 5-flucytosine (5 FC) consists of increasing the transcription of all the genes involved in the de novo pyrimidine biosynthetic pathway (including *URA3* i.e., orotidine 5′-phosphate decarboxylase, ODCase) to overproduce UMP (uridyl-monophosphate) thus affecting nucleic acid synthesis [75].

The allylamine and thiocarbamate antifungal agent as well as cationic peptides possess antifungal activity against *Candida* sp. [76, 77, 78]. Limited activity of allylamine terbinafine against *Candida* sp. has been reported [79]. Unusual resistance of *Candida* sp. has also been seen towards cationic antifungal proteins [80].

## CONCLUSION

The past decade has witnessed a significant increase in the prevalence of resistance to antibacterial as well as antifungal agents. Driven by the increasing importance of opportunistic pathogens and the pharmacological limitations of antibiotic potencies, with resistance compromising the effectiveness of all but the newest, management of microbial infections has become a challenging problem. Thus there is an ever-pressing and critical need to develop innovative therapeutic agents or alternative chemotherapeutic strategies, to combat these evolving pathogens and to replace the invalidated antibiotics, to which bacteria are less likely to become resistant. Therefore the search for new antimicrobial agents through previously unexplored targets with distinctly different chemical structures and mechanism of action from the known antibiotics is imperative.

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