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**Original research article**

# To study *Staphylococcus aureus* for quinolone resistance detection in a tertiary care hospital

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**Abstract**

**Introduction and Background:** *Staphylococcus aureus* is a gram-positive spherical bacterium commonly present in the nasal passages, respiratory system, and skin. The aim and objectives of this investigation were to identify phenotypic and genotypic resistance in *Staphylococcus aureus* and to isolate it.

**Materials and Methods:** This study was cross-sectional and prospective. Isolates were collected from different clinical samples for this study conducted at the Department of Microbiology, Madha Medical College and Research Institute in Chennai, Tamil Nadu, India. The study was conducted from September 2017 to August 2018.

**Results:** *Staphylococcus aureus* is a significant pathogen that colonises around 30% of asymptomatic individuals. Drug resistance has developed as a result of the growing unintentional utilisation of potent antibiotics, leading to the rapid emergence of multidrug-resistant microorganisms. Real-time PCR offers great sensitivity and a significantly shorter turnaround time compared to previous procedures. Laboratories equipped with molecular testing facilities for Real-time PCR can utilise it as a standard diagnostic technique.

**Conclusion:** Improper antibiotic use has led to extensive resistance. Novel chemicals have been introduced, leading to the inadvertent development of resistance pathogens by doctors.

**Keywords:** *Staphylococcus aureus*, quinolone resistance, tertiary care hospital

## Introduction

The gram-positive bacterium *Staphylococcus aureus* is common in the skin, respiratory system, and nose. Skin diseases like abscesses, respiratory infections like sinusitis, and food poisoning can all be caused by *Staphylococcus aureus*, although this does not mean it is always hazardous. The infectious agents that cause infections are highly pathogenic microorganisms that secrete powerful protein toxins and have surface proteins on their cells that bind and render antibodies ineffective [1, 2]. *Staphylococcus aureus* is responsible for a wide variety of infections, from fairly harmless skin conditions like boils, pimples, impetigo, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses to potentially fatal respiratory tract, central nervous system, and bone diseases like toxic shock syndrome, bacteremia, and sepsis. It frequently causes infections in patients after surgery and is still among the top five causes of healthcare-associated illnesses [1, 3].

When it comes to bloodstream infections in the intensive care unit (ICU), *Staphylococcus aureus* is by far the most common culprit, and staphylococci are the most common species seen in positive blood cultures. Given its inherent aggressiveness, adaptability to various environmental conditions, and potential to produce a varied array of life-threatening infections, *Staphylococcus aureus* may be the most worrisome organism. There are effective antibiotics available, however the mortality rate of *Staphylococcus aureus* bacteremia is still around 20-40%. With an increasing number of patients receiving treatment outside of hospitals, *Staphylococcus aureus* has emerged as the top source of nosocomial infections. This is a growing issue in the community [4, 6].

Resistant to methicillin one bacterium that causes numerous intractable human illnesses is Methicillin-resistant *Staphylococcus aureus*, more commonly known as MRSA. Multi-drug-resistant *Staphylococcus aureus* (MRSA) can be defined as any strain of *Staphylococcus aureus* that has acquired resistance to multiple quinolone and beta-lactam antibiotics by horizontal gene transfer and natural selection. A major issue in clinical medicine around the world is the rise of *Staphylococcus aureus* strains that are resistant to antibiotics, such as Methicillin-resistant *Staphylococcus aureus* [7, 9].

Nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) typically affect individuals with compromised immune systems, open wounds, or invasive medical devices. This is more

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typical in institutional settings like nursing homes, jails, and hospitals than in the wider community. Because of this differentiation, MRSA that is linked with healthcare facilities is referred to as HA-MRSA, but MRSA that is connected with the population is known as CA-MRSA or LA-MRSA [10, 11]. Superstition against methicillin both the general public and healthcare facilities deal with the constant threat of *Staphylococcus aureus*. Rapid development of quinolone resistance in *Staphylococcus aureus* occurred, with methicillin-resistant strains showing the most rapid emergence. The effectiveness of fluoroquinolones as anti-staphylococcal drugs was significantly diminished, and they are not useful when administered in this manner. It is unclear why quinolone resistance rates among MSSA and MRSA are so different. The selection and spread of MRSA strains that are resistant to antibiotics is likely due, in part, to antibiotic selective pressure, which is particularly prevalent in healthcare settings. This population of resistant strains serves as a breeding ground for new diseases [9, 11].

In both healthcare and community settings, the concerning increase in antibiotic resistance among harmful bacteria remains a recurring problem with antibiotic treatment. Patients in both hospitals and the community seem to be warming up to the fluoroquinolone class of antimicrobials, and their use is on the rise. Despite this, the use of several drugs in this class - including trovafloxacin, gatifloxacin, temafloxacin, and grepafloxacin - has been limited or discontinued due to side effects. The most prevalent kind of infection is one that affects the respiratory system, and new fluoroquinolone medications are being researched and authorised for use in treating this condition. The convenience of taking fluoroquinolones orally once or twice day has led to their increased usage. Already, in certain therapeutic contexts and among certain bacterial species, resistance to fluoroquinolones has developed as we near the midpoint of the second decade of their use [10, 12].

The goals of this research are to (1) identify potential epidemiologic variables that have led to the epidemic of antibiotic resistance in clinical settings and (2) investigate the processes by which fluoroquinolone resistance has developed. *Staphylococcus aureus* phenotypic and genotypic resistance detection, as well as clinical isolate isolation and characterization, were the aims and objectives of this investigation.

**Materials and Methods**

The prospective cross-sectional study was this. This study was conducted in the Department of Microbiology, Madha Medical College and Research Institute, Chennai, Tamil Nadu, India, using isolates taken from a variety of clinical samples. From September 2017 through August 2018, researchers conducted this investigation.

**Sample Collection**

The investigation encompassed all clinical samples that were sent to the diagnostic microbiology laboratory. We processed about 100 clinical isolates of *Staphylococcus aureus* that developed beta haemolytic golden yellow colonies on 5% sheep blood agar. These isolates came from a variety of clinical materials including blood, wound swabs, pus, sputum, broncho-alveolar lavage, and tracheal aspirate.

**Results**

A **t**otal of 1200 samples of pus, blood, wound swab, tracheal aspirate and urine were processed in microbiology laboratory between September 2017 to August 2018. The incidence of *Staphylococcus aureus* from the samples were 3.5%.

**Table 1:** Antibiotic susceptibility pattern of *Staphylococcus aureus* isolates

| Sr. No. | Antibiotic (µg) | Sensitive (%) | Resistance (%) |
|---|---|---|---|
| 1. | Vancomycin (30 µg) | 100 | 0 |
| 2. | Linezolid (30 µg) | 100 | 0 |
| 3. | Amoxyclav (30 µg) | 80 | 25 |
| 4. | Clindamycin (2 µg) | 89 | 15 |
| 5. | Erythromycin (15 µg) | 70 | 30 |
| 6. | Penicillin (6 µg) | 15 | 80 |
| 7. | Ciprofloxacin (5 µg) | 0 | 100 |
| 8. | Levofloxacin (5 µg) | 0 | 100 |
| 9. | Ofloxacin (5 µg) | 0 | 100 |
| 10. | Cefoxitin (10 µg) | 40 | 70 |

Table 1 illustrates the susceptibility pattern of *Staphylococcus aureus* isolates to different drugs.

**Table 2:** MIC for *Staphylococcus aureus* resistant to Fluoroquinolones

| Sr. No. | Antibiotic | MIC 8-16 (µg) | MIC 16-32 (µg) | MIC 32-64 (µg) |
|---|---|---|---|---|
| 1. | Ciprofloxacin | 50 | 30 | 32 |

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| | | | | |
|---|---|---|---|---|
| 2. | Levofloxacin | 20 | 40 | 40 |
| 3. | Ofloxacin | 10 | 38 | 45 |

Table 2 shows the Minimum Inhibitory Concentration (MIC) for *Staphylococcus aureus* that is resistant to fluoroquinolones. The molecular characteristics of gyrA, gyrB, grlA, and grlB were determined using Real-time PCR and amplified using gel electrophoresis.

**Discussion**

A major pathogen, *Staphylococcus aureus*, is colonised by about 30% of the asymptomatic population. The increased accidental use of powerful antibiotics has led to the evolution of drug resistance, and the emergence of bacteria that are resistant to more than one treatment is happening at a rapid pace. In our investigation, we discovered that 74% of cases involved MRSA. Other research in and throughout the world have found a strong correlation with this. The percentage of MRSA isolates reported in different regions of the world varies from 28% to 87% [13, 15].

According to literature reviews, a disproportionate number of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are resistant to fluoroquinolones. We found that 74% of MRSA were resistant to fluoroquinolones. Ofloxacin, levofloxacin, and ciprofloxacin were the most resistant, with 43.3% being the most resistant. The impact of fluoroquinolones and cotrimoxazole on MRSA colonisation and infection in patients is related. An analysis of MRSA isolates from patients revealed that 74% were ciprofloxacin resistant and 68% were cotrimoxazole resistant. Of the MSSA clinical isolates tested, 26% showed resistance to ciprofloxacin and 32% to cotrimoxazole [16, 18].

Pyogenic infections were the most common source of MRSA, followed by infections in the bloodstream. The study found that blood culture specimens from patients in the intensive care unit (ICU) had a prevalence of 43% and ward samples of 35% MRSA, respectively [19]. Results from tests for antimicrobial susceptibility to *Staphylococcus aureus* isolates demonstrated that linezolid, clindamycin, and amoxyclav were the most effective. Among the antibiotics tested, ciprofloxacin and ofloxacin showed the strongest resistance. Patients who are unable to take anti-staphylococcal penicillins typically rely on first-generation cephalosporins as their primary treatment for methicillin-sensitive Staphylococcal infections. It is recommended to de-escalate the patient to beta lactams if the patient has been empirically started on vancomycin and the culture report shows MSSA. Glycopeptides, such as teicoplanin and vancomycin, are reserved for extreme cases [20, 22].

The agar dilution method was used to investigate the fluroquinolone susceptibility profile to minimal inhibitory concentrations. The minimum inhibitory concentration (MIC) values for ciprofloxacin, ofloxacin, and levofloxacin were 32 to 64 µg, 16 to 32 µg, and 8 to 16 µg, respectively, out of 100 *Staphylococcus aureus* isolates. Fluoroquinolones had MIC values ranging from 32 to 64 µg for MRSA, whereas MSSA had values ranging from 8 to 16µg. In contrast to Levofloxacin's 25.5% resistance rate, studies on ocular infections have shown ciprofloxacin resistance rates of 3–11%. Anxieties in infection management are heightened by the fact that ciprofloxacin and levofloxacin are becoming less effective against certain strains of *Staphylococcus aureus*. While previous studies have shown fluoroquinolone resistance in *Staphylococcus aureus* ranging from 13% to20.7%, a recent study indicated a significantly higher prevalence of 41.8% [23, 25].

There has been a lot of research on how *Staphylococcus aureus* develops genotypic resistance to fluoroquinolones. Single mutations in DNA gyrase and DNA topoisomerase IV have been detected in most isolates. *Staphylococcus aureus* mutations in grlA are the initial step towards fluoroquinolones, which target DNA topoisomerase IV. Our investigation involved using a real-time PCR technique to detect topoisomerase iv and gyrase in fifty isolates of resistant *Staphylococcus aureus* that were obtained using disc diffusion and agar dilution. Our investigation found that grlA and gyrA were expressed by the majority of *Staphylococcus aureus* isolates. There is a good agreement between this and other studies that have found the same thing. Pulsed field gel electrophoresis investigations have suggested that ciprofloxacin resistance in *Staphylococcus aureus* isolates is primarily caused by one or two point mutations in the QRDR domains of the grlA and gyrA genes [26, 28].

Of the isolates analysed, 43.3% showed high levels of ciprofloxacin resistance. Commonly linked to mutations in grlA and gyrA is this extreme resistance. Other research also found a good match with this result. Restricted fragment length polymorphism, high performance liquid chromatography, and gene sequencing are some other molecular approaches for fluroquinolone identification; nevertheless, these procedures are labor-intensive and inconvenient. Compared to previous approaches, real-time polymerase chain reaction has a significantly shorter turnaround time and very high sensitivity. Regular use of real-time polymerase chain reaction as a diagnostic tool is possible in labs that have molecular testing capabilities [29, 32].

**Conclusion**

Resistance to antibiotics has emerged due to the overuse of these drugs. Although there are newer chemicals available, we now face the challenge of eliminating bacteria that have developed resistance

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due to clinicians' accidental use of these medications. Only by enforcing antibiotic policies that are specific to each hospital's needs and conducting frequent audits to ensure compliance can we protect populations from emerging diseases and bacteria that are resistant to multiple drugs.

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**Conflict of Interest**
None.

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