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Research Article

# Detection and Antibiotic Susceptibility Patterns of *Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus* spp. Isolated from Sputum of Patients with Respiratory Tract Infections

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## ABSTRACT

*Objectives:* Respiratory tract infections which are transmitted from patients to other patients or non-patients, and affect all ages, range from lower tract to upper tract infections. Sputum production represents one of the symptoms of these infections in some cases. These infections are associated with pathogenic gram-positive bacteria such as *Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus* spp.

*Materials and Methods:* Sputum specimens were collected at Imam Ali Hospital in Kotha District, Babylon Province for the detection of *S. aureus, S. pyogenes* and *Streptococcus* spp. using the bio-chemical and VITEK 2 system tests, as well as for identifying the antibiotic sensitivity patterns against these bacterial species using the standard disk diffusion procedure on the Mueller–Hinton agar.

*Results:* The percentage of positive growth of the pathogenic gram-positive bacteria was 46.0%, and for *S. aureus, S. pyogenes* and *Streptococcus spp.,* the values were 29.166%, 37.500% and 33.334%, respectively. Males were found to be more susceptible to the infection than females (87.5% and 12.5%, respectively). The antibiotic sensitivity patterns showed that ceftriaxone, azithromycin and amoxiclav were effective against *S. aureus*, whereas ciprofloxacin, azithromycin, levofloxacin and amoxicillin were effective against *S. pyogenes.* Ciprofloxacin, levofloxacin, meropenem and azithromycin were found to be effective against *Streptococcus* spp.

*Conclusions:* The growth percentages for *S. aureus, S. pyogenes* and *Streptococcus* spp. were 29.166%, 37.5% and 33.334%, respectively. Males were more susceptible to infection than females and among all the antibiotics used in this study, only azithromycin was effective against all *S. aureus, S. pyogenes* and *Streptococcus* spp. isolates.

**Keywords:** Communicable Respiratory Tract Disease, Sputum, *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus* spp., Antibiotics

## Introduction

Sputum production represents one of the symptoms associated with respiratory tract infections in some cases. These infections range from lower tract to upper tract infections and affect all ages. Pathogenic gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes* and Streptococcus spp. constitute the main cause of respiratory tract infections and play an important role in the increase of antibiotic use and resistance. These infections are one of the leading diseases globally[1] and incur a heavy public health burden.[2]

Respiratory infections are divided into upper and lower respiratory tract infections and are the main cause of morbidity and mortality, mostly in developing countries.[3] Infections of the upper respiratory tract involve laryngitis, pharyngitis, tonsillitis, otitis media, sinusitis, and common cold.[4] Infections of the lower respiratory tract are more prevalent among humans.[5] These include acute trachea bronchitis, acute bronchitis, chronic bronchitis, and pneumonia. About 4.4% of the patients suffering from these diseases may need hospital admissions, with excessive health costs and high morbidity and mortality rates.[6–8] The most common gram-positive bacteria causing respiratory infections are *Staphylococcus* and *Streptococcus*.[9] *S. aureus* and *S. pyogenes* have been isolated from upper respiratory tract infections,[10] and *S. aureus*, *Streptococcus pneumoniae* etc. have been isolated from lower respiratory tract infections[11,12]. The majority of patients with symptomatic respiratory tract infections are mostly treated.[13] Antimicrobial resistance has led to the failure of the therapeutic process.[14] Bacteria may develop antibiotic resistance through the following mechanisms: decreased cell membrane permeability, active efflux, antibiotic inactivation, and modification of the antibiotic target.[15] Factors attributed to the emergence of bacterial resistance include poor use of antibiotics and the transmission of resistant bacteria between patients or from patients to healthcare workers or from healthcare workers to patients, in addition to poor guidelines regarding the administration of antibiotics.[16] Various types of research have been conducted which show that a better understanding of the mechanisms of resistance in respiratory pathogenic bacteria and a correct identification of causal respiratory inflammatory factors can lead to an improvement in the patient's health and reduce morbidity and mortality rates along with a reduction in antibiotic resistance.[17]

## Material and Methods

### Specimens

Fifty-two sputum specimens were collected from patients with respiratory tract infections in the microbiological laboratory at Imam Ali Hospital in Kotha District, Babylon Province using a sterile container through the period from Jan 2021 to Dec 2021 after obtaining the acceptance of the ethics committee. These specimens were used for the identification of pathogenic gram-positive bacteria, especially the *S. aureus, S. pyogenes* and Streptococcus spp. and samples were analysed using the Excel and SPSS programmes.

### Detection of Pathogenic Gram-Positive Bacteria

The pathogenic gram-positive bacteria isolated from the sputum specimens included *S. aureus, S. pyogenes* and *Streptococcus* spp. These bacterial species were detected using the bio-chemical and VITEK 2 system tests, as illustrated in Table 1.

**Table 1.Detection of Pathogenic Gram-Positive Bacteria in Sputum Samples**

| S. No. | Pathogenic Bacteria | Tests |
|---|---|---|
| 1.<br>2.<br>3. | *Staphylococcus aureus*<br>*Streptococcus pyogenes*<br>*Streptococcus spp.* | VITEK 2 system<br>and<br>bio-chemicals |

### Detection of Antibiotic Susceptibility

The identification of the antibiotic susceptibility pattern with respect to *S. aureus, S. pyogenes* and *Streptococcus* spp. was done in this study using the method of standard disk diffusion. This method involved the incubation of pathogenic gram-positive bacteria with the used antibiotics on the Mueller–Hinton medium for 24 hours and the measurement of clear (inhibition) zones in bacterial culture by a special millimetre ruler.

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## Results

The sputum specimens were collected for the isolation and detection of *S. aureus, S. pyogenes* and *Streptococcus* spp. and the identification of the antibiotic pattern of sensitivity with respect to these bacterial species. Figure 1 shows that among all sputum specimens, 24 (46.0%) showed bacterial growth. Table 2 shows that males were more susceptible to the infection than females (87.5% and 12.5%, respectively). Individuals belonging to the age category of 30–39 years were found to be more susceptible to the infection with

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a positive growth percentage of 41.666%. Table 3 reveals that the growth percentages of *S. aureus, S. pyogenes* and *Streptococcus* spp. were 29.166%, 37.500%, and 33.334%, respectively. As per Figure 2, the growth rate was lower. Figure 3 shows that the age category of 30–39 years had a higher growth rate, while for Figure 4 and other age categories, the growth rate was lower. Table 4 reveals the antibiotic susceptibility patterns, showing that ceftriaxone, azithromycin and amoxiclav were effective against *S. aureus,* while ciprofloxacin, levofloxacin, azithromycin and amoxicillin were effective against *S. pyogenes*, and ciprofloxacin, levofloxacin, meropenem and azithromycin were effective against *Streptococcus* spp.

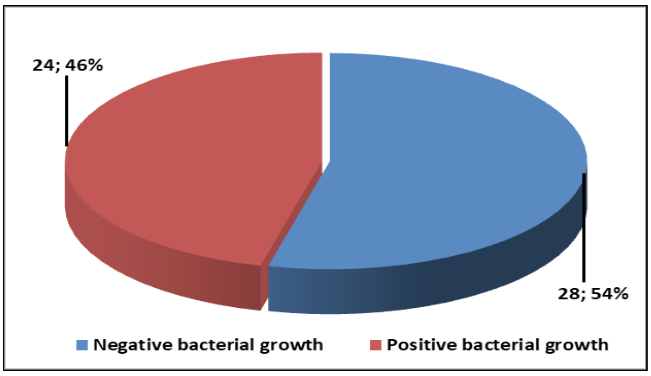


**Figure 1.Specimens with Positive and Negative Bacterial Growth**

**Table 2.Distribution of Pathogenic Gram-Positive Bacteria According to the Gender and Age Categories**

| S. No. | Gender | Positive Cases n (%) | Negative Cases n (%) |
|---|---|---|---|
| 1. | Male | 21 (87.500) | 20 (71.428) |
| 2. | Female | 3 (12.500) | 8 (28.572) |
| **S. No.** | **Age categories (years)** | **Positive Cases n (%)** | **Negative Cases n (%)** |
| 1. | 10–19 | 4 (16.667) | 3 (10.715) |
| 2. | 20–29 | 3 (12.500) | 14 (50.000) |
| 3. | 30–39 | 10 (41.666) | 4 (14.285) |
| 4. | 40–49 | 4 (16.667) | 3 ( 10.715) |
| 5. | 50–59 | 3 (12.500) | 4 (14.285) |
| Total | | 24 (100.000) | 28 (100.000) |

**Table 3.Percentage of Pathogenic Gram-Positive Bacteria in the Sputum Specimens**

| S. No. | Bacterial Species | Positive Cases (%) |
|---|---|---|
| 1. | *Staphylococcus aureus* | 29.166 |
| 2. | *Streptococcus pyogenes* | 37.500 |
| 3. | *Streptococcus spp.* | 33.334 |
| 4. | Total | 100.000 |

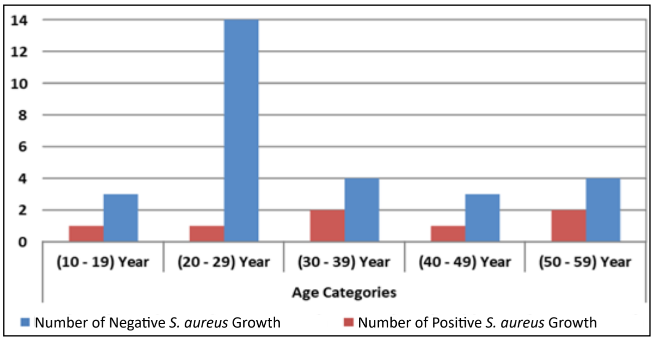


**Figure 2.Comparison Between the Numbers of *S. aureus* in Specimens with Positive and Negative Bacterial Growths**

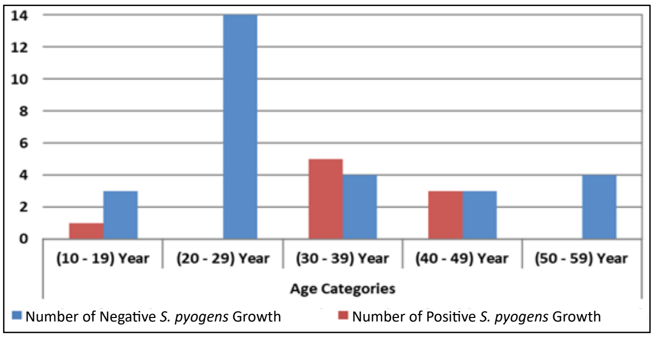


**Figure 3.Comparison between the Numbers of *S. pyogenes* in Specimens with Positive and Negative Bacterial Growth**

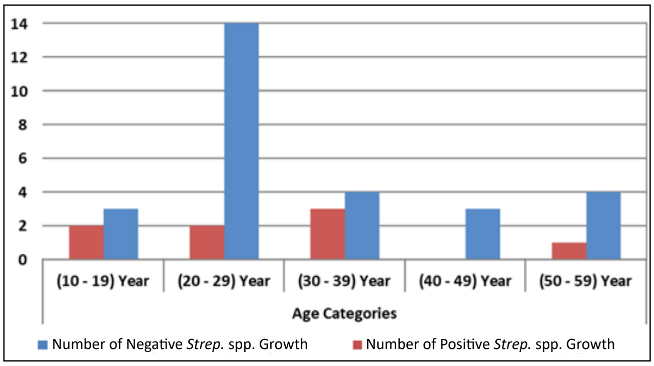


**Figure 4.Comparison between the Numbers of *Streptococcus* spp. in Specimens with Positive and Negative Bacterial Growths**

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**Table 4.Antibiotic Sensitivity of Pathogenic Gram-Positive Bacteria in Sputum Specimens**

| S. No. | Bacterial Type | Antibiotic-sensitive Type | Antibiotic-resistant Type |
|---|---|---|---|
| | *S. aureus* | Ciprofloxacin, ceftriaxone | Nalidixic acid, norfloxacin |
| | *S. aureus* | Azithromycin, norfloxacin, amoxiclav, ceftriaxone | Nalidixic acid, ciprofloxacin, cefixime |
| | *S. aureus* | Ceftriaxone, ciprofloxacin, cefixime | Nalidixic acid, norfloxacin |
| | *S. aureus* | Ceftriaxone, ciprofloxacin, cefotaxime | Nalidixic acid, norfloxacin |
| | *S. aureus* | Ceftriaxone, ciprofloxacin | Nalidixic acid, norfloxacin |
| | *S. pyogenes* | Ciprofloxacin, levofloxacin, azithromycin | Nalidixic acid, gentamycin, nitrophoridntid |
| | *S. pyogenes* | Levofloxacin, ciprofloxacin, amoxicillin | Trimethoprim, ceftriaxone |
| | *S. pyogenes* | Levofloxacin, amoxicillin, ciprofloxacin | Trimethoprim, cefepime |
| | *S. pyogenes* | Azithromycin, amoxicillin, ciprofloxacin | Amoxiclav, trimethoprim, ampicillin |
| | *S. pyogenes* | Levofloxacin, norfloxacin, tetracycline, clindamycin | Erythromycin, ceftriaxone |
| | *Streptococcus spp.* | Ciprofloxacin, levofloxacin | - |
| | *Streptococcus spp.* | Gentamycin, meropenem, azithromycin | Trimethoprim, norfloxacin, nalidixic acid |
| Antibiotics Dose/mg | | | |

Ciprofloxacin: 500 mg; Ceftriaxone: 1000 mg; Meropenem: 250 mg; Naladxic acid: 500 mg; Norfloxacin: 400 mg; Cefepime: 1000 mg; Azithromycin: 500 mg; Amoxiclave: 625 mg; Clindamycin: 300 mg; Cefexem: 400 mg; Cefotaxim: 1000 mg; Trimethoprim: 200 mg; Nitrofurantoin: 100 mg; Gentamycin: 80 mg; Tetracycline: 250 mg; Levofloxacin: 500 mg; Amoxicillin: 500 mg; Erythromycin: 500 mg

**Table 5.Antibiotic activity percentage against Pathogenic Gram-Positive Bacteria**

| Pathogenic Gram-Positive Bacteria in Sputum Specimens | | | |
|---|---|---|---|
| Pathogenic Bacteria | Antibiotic Type | Sensitivity Percentage | Resistance Percentage |
| *Staphylococcus aureus* | Ciprofloxacin | 90 | 10 |
| | Ceftriaxone | 80 | 20 |
| | Azithromycin | 90 | 10 |
| | Norfloxacin | 10 | 90 |
| | Amoxiclav | 80 | 20 |
| | Cefotaxime | 80 | 20 |
| | Cefixime | 50 | 50 |
| | Nalidixic acid | 30 | 70 |
| *Streptococcus pyogenes* | Ciprofloxacin | 80 | 20 |
| | Levofloxacin | 80 | 20 |
| | Azithromycin | 90 | 10 |
| | Amoxicillin | 80 | 20 |
| | Tetracycline | 80 | 20 |
| | Clindamycin | 80 | 20 |
| | Nalidixic acid | 30 | 70 |
| | Gentamycin | 30 | 70 |
| | Trimethoprim | 30 | 70 |
| | Erythromycin | 50 | 50 |
| | Norfloxacin | 30 | 70 |
| | Tetracycline | 30 | 70 |
| | Clindamycin | 30 | 70 |
| *Streptococcus spp.* | Ciprofloxacin | 80 | 20 |
| | Levofloxacin | 80 | 20 |
| | Gentamycin | 80 | 20 |
| | Meropenem | 80 | 20 |
| | Azithromycin | 90 | 10 |
| | Trimethoprim | 30 | 70 |
| | Norfloxacin | 10 | 90 |
| | Nalidixic acid | 10 | 90 |
| Antibiotics Dose/mg | | | |

Ciprofloxacin: 500 mg; Ceftriaxone: 1000 mg; Meropenem: 250 mg; Naladxic acid: 500 mg; Norfloxacin: 400 mg; Cefepime: 1000 mg; Azithromycin: 500 mg; Amoxiclave: 625 mg; Clindamycin: 300 mg; Cefexem: 400 mg; Cefotaxim: 1000 mg; Trimethoprim: 200 mg; Nitrofurantoin: 100 mg; Gentamycin: 80 mg; Tetracycline: 250 mg; Levofloxacin: 500 mg; Amoxicillin: 500 mg; Erythromycin: 500 mg

## Discussion

The present study shows that among all sputum specimens, 46.0% showed bacterial growth (Figure 1). It was also seen in this study that males were more susceptible to the infection than females with infection percentages of 87.5% and 12.5%, respectively (Table 2). The study also revealed the growth percentages of *S. aureus, S. pyogenes* and *Streptococcus* spp. to be 29.166%, 37.500%, and 33.334%, respectively (Table 3). In a study conducted by Miriti et al., the percentage of pathogenic bacteria was found to be 45.6% of all samples. The isolation percentages for *S. aureus, S. pyogenes,* and *Streptococcus pneumoniae* were equal to 16.6%, 13.7% and 10.3%, respectively. Males were found to be more susceptible to the infection in this study too (61.3% vs 27.8%, respectively).[3] However, in a study by Atia et al., the percentage of positive culture was found to be equal to 83.7% of sputum specimens, and the isolation percentages for *S. aureus and S. pneumoniae* were 13.0% and 48.0%, respectively; however, males were found to be more susceptible than females in this study (41.0% vs 59.0%, respectively).[18] A study conducted by Watanabe et al. showed that the percentage of pathogenic gram-positive bacteria was 52.8% of all samples, and for *S. aureus, S. pyogenes, S. pneumoniae,* and *S. agalactiae,* the isolation percentages were 25.5%, 6.2%, 17.4% and 2.0%, respectively.[19]

It has been seen in the present study that the individuals belonging to the age category of 30–39 years were more susceptible to infection with a positive growth percentage of 41.666% (Table 2). In a study by Miriti et al., people belonging to the age category of 25–34 years were found to be more susceptible to infection (30.3%).[3] A study carried out in Kenya revealed a higher incidence of acute bacterial respiratory infections in the age group of 17–50 years.[20] Likewise, another study in Nigeria recorded more cases of infection of the lower respiratory tract in patients aged between 21 and 40 years.[21] The disparities in these studies could be due to the fact that most of the patients in these age groups are working and therefore have higher mobility and are more able to socialise, which makes them exposed to risk factors such as external contaminants like pathogenic microorganisms, especially in crowded places. The differences in the study period, geographical location, and socioeconomic status of the participants in these studies could also be a cause of the variation.

The current study showed the antibiotic sensitivity patterns with respect to *S. aureus, S. pyogenes,* and *Streptococcus* spp. Ceftriaxone, azithromycin and amoxiclav were found to be effective against *S. aureus,* while ciprofloxacin, levofloxacin, azithromycin and amoxicillin were effective against *S. pyogenes.* Ciprofloxacin, levofloxacin, meropenem, and azithromycin were found to be effective against *Streptococcus* spp. (Tables 4 and 5). Miriti et al. conducted a study and found the resistance percentages of *S. aureus* against amoxicillin, ampicillin, ciprofloxacin, ceftazidime, piperacillin/ tazobactam, gentamicin, amikacin, and cefuroxime to be 100.0%, 100.0%, 92.0%, 89.0%, 67.0%, 0.0%. 0.0% and 0.0%, respectively, whereas the resistance percentages of *S. pyogenes* against amoxicillin, ampicillin, ciprofloxacin, ceftazidime, cephalexin, gentamicin, amikacin, and cefuroxime were 97.6%, 100.0%, 100.0%, 91.7%, 83.3%, 0.0%, 0.0% and 0.0%, respectively, and the resistant percentages of *S. pneumonia* against amoxicillin, ampicillin, piperacillin-tazobactam, cephalexin, gentamicin, amikacin, cefuroxime, and ceftazidime were 100.0%, 100.0%, 100.0%, 80.0%, 70.0%, 0.0%, 6.0% and 10.0%, respectively.[3] In a study by Watanabe et al., the activity percentages of ampicillin, ciprofloxacin, cefaclor, cefteram, cefixime, and ofloxacin against *S. aureus* were found to be 6.25%, 0.78%, 3.13%, 6.25%, 25.00% and 0.78%, respectively, whereas, against *S. pyogenes*, the values were 0.050%, 3.130%, 0.780%, 0.025%, 0.390% and 0.200%, respectively. Against *S. pneumonia*, the values were 0.78%, 1.56%, 6.25%, 0.39%, 3.13%, and 3.13%, respectively.[19] The variations which appear in the susceptibility of antibiotics against pathogenic bacteria in the studies may be attributed to the type and structure of antibiotics, dosages used, industrial company origin, differences in geographical regions, use of antibiotics without a proper prescription by a specialist, use of antibiotics without laboratory guidance, misuse of the drug through improper concentrations and/ or incorrect dosing schedule, and differences in study areas and bacterial types under study.[22–25]

## Conclusion

The percentage of positive bacterial growth for all sputum specimens in this study was 46.0%. The growth percentage values were 29.166%, 37.500%, and 33.334% for *S. aureus, S. pyogenes* and *Streptococcus* spp., respectively. Males were more susceptible to infection than females with infection percentages of 87.5% and 12.5%, respectively. Among all antibiotic types which were used in this study, only azithromycin was found to be effective against all *S. aureus, S. pyogenes* and *Streptococcus* spp. isolates.

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## Conflict of Interest

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