Prevalence of Multidrug Resistant *Staphylococcus aureus* in Clinically Suspected Atypical Pneumonia Patients

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ABSTRACT

BACKGROUND

Staphylococcus aureus (*S. aureus*) is one of the common pathogens frequently causing pneumonia. *S. aureus* is a gram-positive organism, which belongs to the family *Micrococcaceae*. Generally atypical pneumonia is caused by *Mycoplasma pneumoniae*, *Legionella pneumophila* and *Chlamydophila pneumoniae*. Typical pathogen *S. aureus* is the most frequent and common nosocomial acquired infection. Now-a-days, antibiotic resistant bacterial strains like *S. aureus* have increased in hospital settings. Developing antibiotic resistance is a major problem with *S. aureus*. We wanted to estimate the non atypical pathogens particularly *S. aureus* in clinically suspected atypical pneumonia cases.

METHODS

250 clinical samples of sputum, broncho alveolar lavage (BAL) and pleural fluid were collected and investigated with Gram staining, culture and antibiotic sensitivity tests.

RESULTS

Out of a total of 250 cases, *S. aureus* was isolated in 19 cases. *S. aureus* showed highest resistance to Ampicillin (15) (6%), and Penicillin (15) (6%). *S. aureus* showed highest sensitivity to Vancomycin 19 (7.6%) and Tetracycline 19 (7.6%).

CONCLUSIONS

Based on clinical symptoms alone, we cannot identify the organism causing the infection. It is important to determine as to whether it is caused by atypical pathogen or typical pathogen. Sometimes co-infections might be the reason. That's the reason why screening is essential for both atypical and typical pathogens. Clinicians must follow the authentication-based guidelines and proper patient management which will help in reducing the severity of the disease. Mainly screening will help clinicians to give proper treatment as well as decrease the drug resistance.

KEY WORDS

Pneumonia, Nosocomial Acquired Infection, Sputum, Penicillin G, Drug Resistance

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BACKGROUND

The word "pneumonia" is derived from the prehistoric Greek word "pneumon" which means "lung," hence the word "pneumonia" becomes "lung disease. Inflammation of lung's parenchyma may be in one or both lungs". Pneumonia is caused by various pathogens like bacteria, viruses, fungi and parasites. Usually Bacterial Pneumonia caused by both typical and atypical organisms. Atypical pneumonia caused by *Mycoplasma pneumoniae*, *Legionella pneumophila* and *Chlamydophila pneumoniae*. Typical pneumonia caused by *Klebsiella pneumoniae*, *Pseudomonas*, *E. coli*, *Acinetobacter*, *Streptococcus pneumoniae*, *Staphylococcus aureus* etc.¹ *S. aureus* is a common typical pathogen frequently causing pneumonia. *S. aureus* is a gram-positive organism belongs to the family *Micrococcaceae*, aerobic, catalase and coagulase positive bacteria. Generally, *Staphylococcus* species are normal inhabitants in human beings. 50% of *S. aureus* causing pneumonia isolates was turned in to methicillin-resistant *S. aureus* (MRSA), because of lack of proper clinical management.² Pneumonia caused by *S. aureus*, is one of the severe infections and often associated with clinical difficulties and a high death rate. High mortality rate due to *S. aureus* pneumonia in the pre-antibiotic period, diverse is between 50% and 90%. In spite of proper antibiotic therapy, the mortality rate is high approximately 30-50%.³

Various virulence factors play an important role in staphylococcal infections and distinctively concerned in the development of pleuropulmonary infections. Several appear to be distinctively, especially in the most severe manifestation of *S. aureus* pneumonia, the haemorrhagic necrotizing phenotype. Enormous polymorphonuclear leukocyte invasion into the lung parenchyma and the development of micro abscesses are distinctive findings of pneumonia caused by *S. aureus*.⁴

WHO identifies antibiotic resistance as a worldwide public health problem as a consequence of its effect on system of health care together with prolonged hospitalisation, higher costs and increased death rate. In USA, approximately 2 million people acquire severe infections caused by bacteria resistant to as a minimum one suggested antibiotic. *Staphylococcus aureus* pneumonia is a major public health problem by increasing rates of antibiotic resistance due to not so many treatment alternatives.⁵ Several resistant strains can be a source of clinical situations shifting between superficial infections and serious life-threatening infections.⁶ Hence, *S. aureus* should be frequently isolated from the community and Hospital acquired infections. Cell envelope, ribosome and nucleic acids are the targets for antibiotics in *Staphylococci*. These days antibiotic resistant strains of *S. aureus* raised in hospital settings. Developing antibiotic resistance is a major problem with *S. aureus*.⁷

The present study is to assess non atypical pathogen particularly *S. aureus* in clinically suspected atypical pneumonia cases.

METHODS

This is a cross sectional study conducted in Department of Microbiology, Sri Venkateswara Institute of Medical Sciences,

a tertiary care teaching hospital, Tirupati. Clinically suspected atypical pneumonia cases were selected as per case definition: fever without chills, headache, myalgia and cough without sputum production.^{8,9,10} Total 250 cases of clinically suspected atypical pneumonia were analysed. Institute Ethics Committee approval was obtained before the starting of the study. Clinical samples of Sputum, Bronchoalveolar lavage and pleural fluid were collected as explained by Isa S. Touhali et al¹¹ after obtaining the informed written consent.

Before processing samples; centrifugation was done at 6000 rpm for bronchoalveolar lavage and pleural fluid. Sputum samples were mixed with equal amount of Dithiothreitol (DTT) for lysis of mucus and centrifuged at 13000 rpm for 10 minutes, supernatant fluid was discarded. Pellet was collected and divided into two portions for staining and culture. First portion of pellet was used for gram staining and Z-N staining by standard method.¹² Gram staining was done for identification of gram-positive organisms and gram-negative organisms. Z-N staining was done to identify acid-fast bacilli.

Second portion was used for culture to isolate typical organisms. A loopful of second portion of pellet was cultured on Nutrient agar, blood agar and MacConkey agar by standard method.¹³ Typical organisms were identified by based on growth, colony characters and biochemical reactions. Antibiotic sensitivity test (Hi media) was performed for each identified organism to know the sensitivity and resistant pattern. *S. aureus* was identified by based on growth, colony characters and biochemical tests.

Statistical Analysis

Data was recorded, managed and analysed using Microsoft Excel 2007 (Microsoft Corp, USA).

RESULTS

Of the total of 250 cases, 19 isolates of *S. aureus* were isolated in culture (Table 1). *S. aureus* showed highest resistance to Ciprofloxacin 11 (57.89%). No resistance was seen in Linezolid, Netilmicin, Tetracycline, Vancomycin, Levofloxacin (Table 2). *S. aureus* showed highest sensitivity to Vancomycin 19 (100%) and Tetracycline 19 (100%). No sensitivity was seen in Nitrofurantoin (Table 2).

Total Cases	Organism	Culture Positive	Percentage
250	<i>S. aureus</i>	19	7.6%

Table 1. Total Culture Positive Cases of *S. aureus*

Antibiotics	Sensitive (%)	Resistant (%)
Amikacin (30 mcg/disc)	3 (15.79)	1 (5.26)
Ciprofloxacin (5 mcg/disc)	7 (36.84)	11 (57.89)
Cefoxitin (30 mcg/disc)	10 (52.63)	8 (42.11)
Clindamycin (2 mcg/disc)	14 (73.68)	4 (21.05)
Cotrimoxazole (25 mcg/disc)	14 (73.68)	5 (26.32)
Erythromycin (15 mcg/disc)	12 (63.16)	7 (36.84)
Nitrofurantoin (300 mcg)	0	1 (5.26)
Gentamicin (10 mcg/disc)	12 (63.16)	7 (36.84)
Linezolid (30 mcg/disc)	16 (84.21)	0
Netilmicin (30 mcg/disc)	1 (5.26)	0
Tetracycline (30 mcg/disc)	19 (100)	0
Vancomycin (30 mcg/disc)	19 (100)	0
Levofloxacin (5 mcg/disc)	1 (5.26)	0

Table 2. Antibiotic Resistance and Sensitivity Pattern in *S. aureus*

DISCUSSION

S. aureus is a gram positive typical pathogen causing pneumonia mainly in hospital settings.¹ *S. aureus* in hospitalized patients with pulmonary infections involved in three main subsets of pneumonia: hospital-acquired pneumonia (HAP), health care associated pneumonia (HCAP) and Ventilator-associated pneumonia (VAP).¹⁴ HAP- Infection of lung tissue occurs in 48 hours or longer following the hospitalization of a patient with non-intubation. VAP-Hospital acquired infection of lung tissue that generally occurs in 48 hours or longer following intubation for mechanical ventilation. HCAP-Acute infection of lung parenchyma occurring from health care settings includes dialysis centres, nursing homes, Outpatient clinics or hospitalized patients within the past 3 months period. This was previously incorporated in HAP later became a separate group because of some cases appearing as outpatients with pneumonia had been infected by way of multidrug-resistant (MDR) strains previously associated with HAP. Untreated pneumonia can lead to extensive damage of the lung finally impairment of lung function occurs and also mortality rates are raised up to 25%.¹

S. aureus is an extremely versatile pathogen in humans, which readily acclimatize to changing environment and attain antibiotic-resistance genes through a number of diverse mechanisms. Recently, World Health Organisation classified global resistance threats and with the organisms of high precedence is *S. aureus*. *S. aureus* is a considerable opportunistic pathogen that inhabits in healthy humans and lead to one of the main causes of bacterial infections like pneumonia in the developed world.¹⁵

We evaluated patients with suspected atypical pneumonia and reported typical pathogen *S. aureus* in 19 cases among 250 cases. Ruling out whether a patient is infected with atypical or typical pathogen merely by observing the symptoms is difficult; because co infections by *S. aureus* are common. Therefore, thorough investigations for both typical and atypical pathogens are required.

This study results were almost similar to the study conducted by Shah BA et al,¹⁶ where *S. aureus* is the second most common pathogen causing pneumonia. Our study results are in discordance with other Indian study Para et al¹⁷ and also various parts of the country,¹⁸⁻²¹ where *S. pneumoniae* was the most common pathogen causing pneumonia.

S. aureus showed highest resistance to Ciprofloxacin 11 (57.89%), Cefoxitin 8 (42.11), Erythromycin & Gentamicin 7 (36.84%), Cotrimoxazole 5 (26.32%), Clindamycin 4 (21.05%), Amikacin and Nitrofurantoin 1 (5.26%) (Table 2). Highest sensitivity was shown to Vancomycin 19 (100%) and Tetracycline 19 (100%) followed by Linezolid 16 (84.21%), Clindamycin & Cotrimoxazole 14 (73.68%), Erythromycin & Gentamicin 12 (63.16%), Cefoxitin 8 (42.11%) Amikacin 3 (15.79%), Netilmicin and Levofloxacin 1 (5.26%) (Table 2). Vancomycin was the most effective drug against *S. aureus* which was in concordance with other studies from Turkey and in Europe.²²

In recent times multi drug resistant *S. aureus* strains are developing in pneumonia cases. Different mechanisms are involved in *S. aureus* antimicrobial resistance (AMR).

Resistance was acquired through basic mechanisms like drug inactivation, enzymatic drug modification, drug efflux, drug binding site modification, displacement of drug and bypass mechanisms concerning acquirement of a new drug-resistant target. Increasing of resistance is also by resistance determinants transformed through transportable genetic elements by way of plasmids, transposons and the staphylococcal cassette genetic material or by mutations in chromosomal genes.²³

Vancomycin resistance was acquired through conjugation process. Other suggested mechanisms are alterations of cell wall and increased cell wall synthesis that may stop Vancomycin from binding to cell wall which results in continuation of cell wall synthesis. Increase in usage of Vancomycin, directed to emergence of two categories of glycopeptide-resistant *S. aureus*. In the first category Vancomycin intermediate-resistant *S. aureus* (VISA) incessant exposure of glycopeptides lead to thickening of the cell wall and poorly cross-linked cell wall. In the second category Vancomycin-resistant *S. aureus* (VRSA) - High level of resistance is due to acquisition from *Enterococcus* species of the VanA operon.²⁴

Vancomycin is imperative for management of severe pneumonia infections.²⁵ One of the study stated emergence of multidrug resistance strains of *S. aureus* comprising resistant to Vancomycin all over the world in such strains noticeable variations observed in physiology, colony morphology and growth characteristics because of high reductive circumstances with depressed acetate metabolism.²⁶ In 1980s, first synthesized Ciprofloxacin belongs to the Fluoroquinolones and extensively used in gram positive organisms. Generally, this drug is helpful in extermination of MRSA. Various parts of the world reported MRSA resistance to Ciprofloxacin.²⁷ Neeta D Gade et al.,²⁸ reported highest resistance to Ciprofloxacin which is in concordance with our study. Other studies from India^{29,30} they also reported 90% of resistance to Ciprofloxacin. Mehta AP et al., reported gradual increase in Ciprofloxacin resistance over years, which was 39% in the year 1922 and steadily raised to 68% in the year of 1996.³¹ In health care settings, Ciprofloxacin cannot be helpful as a good empirical choice for treating *S. aureus* infections because of its highest resistance. Strains showing resistant to Ciprofloxacin tend to show increased resistance to other antibiotics also.^{32,33}

42.11% Cefoxitin resistance was observed in the current study. Generally, Cefoxitin resistance denotes that it is Methicillin resistant *Staphylococcus aureus* (MRSA).³⁴ MRSA infections are common in hospital settings. As stated, earlier Ciprofloxacin is commonly administered drug in such infections.²⁷ Inappropriate treatment management may also be one of the reasons in developing resistant strains. Hence screening must be needed for pneumonia causing pathogens both typical and atypical along with their antibiogram.

CONCLUSIONS

Multidrug resistant *Staphylococcus aureus* can be seen in clinically suspected atypical pneumonia patients. Simply based on clinical symptoms we cannot identify the organism causing the infection. It is important to determine as to whether it is caused by atypical pathogen or typical pathogen.

Sometimes co-infections might be the reason. That's the reason why screening is essential for both atypical and typical pathogens. Clinicians must follow the authentication-based guidelines and proper patient management which will help in reducing the severity of the disease. Mainly screening will guide clinicians to give proper treatment as well as decrease the drug resistance. Continuous surveillance is necessary to find out the progression of resistance and mechanisms of resistance. Cautious use of antimicrobials is essential to stop the emergence and spread of resistant microbes.

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