Original Article

OCCURRENCE AND ANTIBIOTIC SUSCEPTIBILITY PROFILES OF STAPHYLOCOCCUS AUREUS IN SPUTUM OF TB PATIENTS

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Abstract

Patients with tuberculosis are easily exposed to other infections especially of staph aureus due to lack of immunity.

Aims and Objectives: Study was designed to identify the presence of staph in sputum of patients as this micro-organism may interfere with the treatment of patients. Study also tried to find out the most suitable antibiotic effective against staph aureus.

Methodology: Sputum samples of 50 patients with tuberculosis were taken for the presence of staph aureus from Feb 2014 – May 2014. Patients were taken from out Door Department of Chest Clinic of Sir Ganga Ram Hospital. Patients provided consent prior to assessment. Identification of *S. aureus* was confirmed by a positive catalase and coagulase test. Susceptibilities were carried out by standard disk diffusion method using antibiotic multidisc containing augmentin (30 ug), gentamicin (10 ug) and ciprofloxacin (5 ug). At the end of incubation, the diameters of the zones of inhibition were measured by ruler.

Results: Mean age of male patients was 32.22 and of female was 36.41. BMI of male was 19.66 kg/m^2 and of female patient was 17.5 Kg/m^2. 100% male and female patients showed the presence of catalase positive *staph aureus*. Sensitivity of ciprofloxacin against the strains of *Staph* aureus was 27.7% in male and 41.6% in female patients. Sensitivity of gentamicin against the strains of Staph aureus was 50% in male and 25% in female patients. Among three antibiotics augmentin showed highest resistance against the *staph aureus* i.e. 78% in case of male and 66% in female patients.

Conclusion: A high prevalence of *staph aureus* in sputum of TB patients is alarming which may be due to polluted environment and week immunity. Effective antibiotic against the strains of *staph aureus* is gentamicin in male and ciprofloxacin in female sputum sample. Further studies are needed to confirm the high carrier rate as well as to evaluate the impact of staph

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aureus on morbidity and mortality among large number both sexes of TB patients.

Key Words: Sputum, Staph aureus, tuberculosis.

Introduction

One – third of the world's population is thought to have been infected with M. tuberculosis. In 2013 there was 1.3 to 1.5 million due to tuberculosis especially in developing countries[1] because of a poor immune system in patients with tuberculosis[2]. Patients with tuberculosis are easily exposed to other infections especially of staph aureus due to their week immune defence system.[3]

Humans are a natural reservoir of Staphylococcus aureus. About 30 – 40% of healthy adults are colonized, with 10 – 20% being colonized thoroughly.[4] Patients with impair leucocyte function are therefore at increased risk of infection of mucous membrane barrier associated with staphylococci.[5]

Staphylococcus aureus is notorious for its ability to develop resistance to antibiotics. It is proposed that Protein A is a surface protein of S aureus which binds with Fc region of immunoglobulin G molecules and alters the mode of action of IgG. Infections are preceded by colonization.[6]

Ciprofloxacin is a second-generation fluoroquinolone active against both Gram – positive and Gram – negative bacteria. It functions by inhibiting DNA gyrase, topoisomerases necessary to separate bacterial DNA, thereby inhibiting cell division.[7,8]

Augmentin is a combination of amoxicillin and clavulanate potassium. It is a semisynthetic antibiotic with bactericidal activity against both Gram-positive (staph aureus) and Gram – negative bacteria (Escherichia coli, Haemophilus influenzae etc). It overcomes antibiotic resistance in bacteria that secrete β-lactamase, which otherwise inactivates most penicillins.[9]

Gentamicin is an aminoglycoside antibiotic active against both Gram – negative bacteria (Pseudomonas, Proteus, Serratia), and the Gram – positive Staphylococcu. It irreversibly binds the 30S subunit of the bacterial ribosome, disrupt protein synthesis.[10]

In spite of the development of curative medicine, tuberculosis continues a leading cause of human mortality in the developing world because of immune deficiency in patients. There is little information about the presence of Staphylococcus aureus in tuberculous patients, an important nosocomial pathogen. Study was therefore designed to identify the presence of staph in sputum of patients as this micro-organism may interfere with the treatment of patients. Study also tried to find out the most suitable antibiotic acting against staph aureus.

Methodology

Sputum sample of 50 patients including both male and female with tuberculosis were taken for the presence of *S aureus* from Feb 2014 – May 2014. Patients were taken from out Door Department of Chest Clinic of Sir Ganga Ram Hospital. Patients provided consent prior to assessment.

Sputum was taken from the patients and transported at room temperature to the laboratory, plated for culture and incubated at 37°C. The next day, gram stain was performed. Identification of *S. aureus* was confirmed by a positive catalase and coagulase test. Susceptibilities were carried out by standard disk diffusion method (Kirby – Bauer disk diffusion test) using antibiotic multidisc (Abitek, Liverpool) containing augmentin (30 ug), gentamycin (10 ug) and ciprofloxacin (5 ug). At the end of incubation, the diameters of the zones of inhibition were measured. The results were assessed based on the 2002 American National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

Table 1: Comparison of demographic characteristics of male with female patients.

Values are Expressed as Mean ± SD	No. of Cases are in Parenthesis	
Characteristics	Male patients (18)	Female patients (12)
Age (year)	32.22 ± 5.8	36.41 ± 6.7
BMI (Kg/m^2)	19.66 ± 4.5	17.5 ± 5.2
Area of Residence	Shahdara (07) Station (06) Mozang (05)	Shahdara (06) Station (05) Mozang (01)

Table 2: Comparison of sensitivity of drugs for staph aureus in sputum of male and females patients.

Values are Expressed as Percentages	No of Cases are in Parenthesis	
Drugs	Male Patients (18)	Female Patients (12)
Ciprofloxacin	27.7%	41.6%
Augmentin	22.2%	33.3%
Gentamicin	50%	25%

Statistical Analysis

The data was entered and analyzed by using SPSS – 16.0. All quantitative variables of males like age, basal mass index were expressed as mean ± SD and compared these variables with females using student 't' test. P values of ≤ 0.05 will be considered statistically significant. Sensitivity of antibiotics was expressed in percentages.

Results

Mean age of male patients was 32.22 and of female was 36.41. BMI of male was 19.66 kg/m^2 and of female patient was 17.5 Kg/m.^{2} Most of the patients had no history of any other disease. On the other hand only six patient had a family history of diabetes and two had a family history of tuberculosis. In female family history of diabetes was observed in 04 and of tuberculosis in 03 whereas10 patients showed no history of any disease (data not shown). Area of residence for 07 males and 06 females was Shahdara (near the river Ravi). Station is the area of residence of 06 males and of 05 females and Mozang the area of residence was of 05 male and 01 female patients.

Comparison of sensitivity of drug for staph aureus in sputum of male and females is tabulated as table 2. Sensitivity of ciprofloxacin against the strains of Staph was 27.7% in male and 41.6% in female patients. . Sensitivity of Augmentin against the strains of Staph was 22.2% in male and 33.3% in female patients. Sensitivity of Gentamicin against the strains of Staph was 50% in male and 25% in female patients.

Discussion

S aureus is the most hazardous of all staphylococcal bacteria. These bacteria are spread by having direct contact with an infected person by contaminated object, or by inhaling infected droplets etc. The antibiotics which are used by carriers kill the strains that are not susceptible, leaving resistant strains.[11]

According to our study mean age of male patients was 32.22 year and of female was 36.41 with BMI 19.66 kg/m^2 and 17.5 kg/m^2 respectively. However it is reported that there was no significant gender difference in TB patients who were younger than 45 years.[12]

Present study showed that 50% of both male and female patients live near river Ravi, 40% live near station of Lahore city (Areas of big pollutant). It is reported that aerobiology plays a fundamental role in the transmission of infectious diseases especially in very young and in old age patients with pulmonary diseases. Study found secondary bacterial infection in these patients may be due to S aureus, H influenzae, Strept pneumoniae, or Strept pyogenes.[13]

According to our study sputum samples of all male and female patients showed the presence of staph aureus (100%). However a study reported that 7.8% of Staphylococcus aureus were present in sputum sample of patients with lung disease.[14] Another study stated that 21% of patients were carriers of staph aureus.[15] A study found that pathogen like S. aureus effect on the immunity of patient by producing protein A which binds with Fc region of immunoglobulin and the Fab regions of the B-cell receptor and cause B cell death[6]. Additionally it is reported that patients have been exposed to S. aureus, among them 30% may be asymptomatic and most of the patients fail to develop or have no serious S. aureus infections. Reason of developing infection is that either patient fails to develop immunity to S. aureus.[16]

Present study found that highest sensitivity of ciprofloxacin to S. aureus was 41.6% in female patients. On the other hand gentamicin showed highest sensitivity (50%) to S. aureus in male patients. Among three antibiotics augmentin showed least sensitivity or highest resistance against the S aureus i.e. 78% in case of male and 66% in female patients. Multidrug resistance is common in pathogens including staph, E. coli

etc is mainly due to environmental resistome genes which may be transferred from non-disease – causing bacteria to infected bacteria leading to clinically significant antibiotic resistance.[17]

Staph. aureus may cause resistance to drug due to many enzymes like it produces coagulase which clots plasma and coats the bacterial cell to prevent phagocytosis. It produces Hyaluronidase which helps in spreading of S aureus. It also produces DNAse which breaks down the DNA, lipase to digest lipids, staphylokinase to dissolve fibrin and beta-lactamase for drug resistance.[18,19]

According to our study ciprofloxacin showed ~70% and augmentin showed ~78% resistance against staph aureus present in sputum of male patients. It is reported that many bacteria showed resistance against broad spectrum antibiotic like ciprofloxacin leaving it significantly less effective than it would have been.[20] It is observed that an increase in antioxidant capacity of S aureus plays an important role in developing resistant against ciprofloxacin.[21]

According to our study augmentin showed highest resistance (78%) against the strains of staph aureus. Our study is in line with a study who observed 70% resistant of S aureus against augmentin[22] and in contrast to the studies who found that the pathogenic staphylococcus strains were sensitive to augmentin 61.0% and 54.7%.[23,24] It is reported by a group of workers that difference in the pattern of the organism's resistance may be due to the changing nature of the pathogen in the different environmental conditions and therefore not advocated for the treatment of staph infections.[25,26]

We observed that gentamicin showed 50% resistance in male and 75% resistance against S aureus present in sputum of female. A study stated that the resistance to gentamicin may be due the activity of enzyme aminoglcyoside N acetyltransferase and phosphotransferase present in s aureus.[20,27]

Our study is in agreement with studies who concluded that resistance recorded against augmentin, amoxicillin and cloxacillin is worrisome because these drugs are used routinely to treat a myriad of human diseases. Studies also reported that location from where the pathogens were isolated had an effect on the sensitivity patterns noted.[28,29]

Conclusion

A high prevalence of staph aureus in sputum of TB patients is alarming and suggested that it may be due to polluted environment and week immunity. Increasing resistance of staph aureus against antibiotic demands coordinated monitoring of its activity and rational use of the antibiotics. Further studies are needed to validate the high carrier rate as well as to evaluate the impact of staph aureus on morbidity and mortality among large number of gender based TB patients.

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