

Susceptibility Pattern of Trimethoprim/ Sulfamethoxazole in Methicillin Resistant *Staphylococcus aureus* Isolates of a Tertiary Care Hospital in Karachi

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Abstract

Objective: To comprehend the frequency, resistance and susceptibility pattern of MRSA isolates to Trimethoprimin/Sulfamethoxazole in a tertiary care hospital in Karachi

Methods: A prospective cross-sectional study was performed and a total of 369 clinical specimens, which comprised of eye, ear, wound and pus swabs, blood, urine, sputum samples and tracheal aspirates which were cultured for time frame of 1 year. Standard and specific microbiological techniques were used to identify positive cultures. Out of a total of 369 isolates 165 were found to be MRSA. Spss version 22 was used for statistical analysis.

Results: In study it was deduced that 82.4% of the MRSA strains were resistant to Trimethoprim/Sulfamethoxazole as compared to Clindamycin to which 91.4% of the strains were resistant, while all strains of MRSA were 100% susceptible to Vancomycin. A slightly higher resistance (83%) to Trimethoprim/Sulfamethoxazole of MRSA strains in age group 31-40yrs was noted when compared with other age groups. While resistance to Clindamycin was also slightly increased in age group 20-30yrs.

Conclusions: Trimethoprim/Sulfamethoxazole has maintained its susceptibility to MRSA when compared with Clindamycin but remained inferior to Vancomycin based on data gathered from the antibiogram. Trimethoprim-Sulfamethoxazole can still be considered as a suitable option for treatment of nosocomial MRSA infections in selected cases as an alternative to Clindamycin and Vancomycin.

Keywords: MRSA; Trimethoprim/Sulfamethoxazole, *Staphylococcus aureus*, resistance pattern.

Introduction

Staphylococcus aureus is a Gram positive bacteria that is a small, round shaped, non-motile cocci, found in grape-like (staphylo-) structures. Found commonly in the environment, the mode of transmission for *S. aureus* is via air droplets and aerosols. Approximately a third of healthy individuals carry this bacterium in their pharynx, noses and on their skin [1]. *Staphylococcus aureus* can be termed as one of the predominant pathogens of nosocomial infections – infections occurring within 48 hours of hospitalization, 30 days of an operation or 3 days of discharge. Up to 60% of all nosocomial infections in the ICU are caused by Methicillin-resistant *S. aureus* (MRSA) [2]. In acute care hospital *Staphylococcus aureus* is a major cause of health care associated infections, including nosocomial pneumonia, surgical site infection, skin infections, osteomyelitis, food poisoning, endocarditis and toxic shock syndrome. Other infections such as those of the bloodstream, cardiovascular, and eye, ear, nose, and throat are also included [3,4]. 5% of *Staphylococcus aureus* strains produces a cytotoxin know as Panton-Valentine leukocidin (PVL) which causes tissue necrosis and leukocyte destruction. 85% of these are associated with severe necrotic hemorrhagic pneumonia which

is community acquired. On further investigation, these strains show the following results: 55% of cellulitis strains, 50% of cutaneous abscess strains, 23% of osteomyelitis strains, and 13% of finger-pulp-infection strains [5].

Emerging as a nosocomial pathogen in the 1970s, MRSA has become highly prevalent and is a cause of many community acquired infections. MRSA is primarily an altered form of *S.aureus* that allows it to become resistant to beta lactam antibiotics by expressing a modified version of a penicillin binding protein (PBP2a) [6]. Over many years, *S.aureus* has acquired resistance to several antibiotics by developing a resistance pattern. These patterns include horizontal gene transfer, spontaneous mutations leading to resistance in fluoroquinolones and linezolid and antibiotic trapping for vancomycin [7]. While it is known that *S.aureus* is susceptible to Vancomycin, Trimethoprim/sulfamethoxazole is a viable option for alternative treatment of *S.aureus* infections as it has retained its susceptibility worldwide. For soft tissue infections, Trimethoprim/sulfamethoxazole is recommended but for severe MRSA infections, Vancomycin is preferred [8].

Material and Methods

A prospective cross-sectional study was performed and a total of 369 clinical specimens, which comprised of eye, ear, wound and pus swabs, blood, urine, sputum samples and tracheal aspirates and were cultured for time frame of March 2014 to Nov 2014. Positive cultures for *S. Aureus* were identified. Brain heart infusions Broth were used to process all specimens which were incubated at 35 °C. Macroscopically the cultures were observed for progress for 7 days. On the 7th day Subcultures of all the blood specimens were done before reporting the culture as negative. For 18-24 hours plates were incubated aerobically at 37 °C. All other specimens (wound swabs, ear swabs, eye swabs, sputum, aspirates) were inoculated onto sheep blood, chocolate and Mac Conkey agar plates and incubated at 37 °C for 18-24 hours. In addition all specimens were inoculated on mannitol-salt agar and the incubation was extended to at least 48-72 hours for discernible colony development. Standard procedures were used to identify the isolates

For statistical analysis SPSS version 22 was utilized. Out of total 369 specimens 165 specimens were found to be MRSA. Sensitivity of clindamycin and trimethoprim along with vancomycin to MRSA isolates was deduced as well.

Results & Discussion

Occurring in microscopic clusters that resemble grapes, *Staphylococcus aureus* are spherical, Gram-positive bacteria. Found mainly in the nasal passages, *S. aureus* also colonizes in other anatomical sites like the oral cavity, skin and the gastrointestinal tract. It is a non-motile, non-spores forming bacteria that causes a variety of suppurative, nosocomial infections [9]. Nosocomial infections are those that occur within 72 hours of hospitalization, and were not present or incubating prior to it [10].

Hospitalized patients are at a greater risk of infection by *S. aureus* due to surgical or other wounds. The infection can aggravate if it is resistant to most types of antibiotics, and may require isolation from other patients [11]. Since the infection is only caused by antibiotic resistant strains, it is only treated with vancomycin [12].

In Switzerland, a study was conducted to test the non-inferiority of Trimethoprim/Sulfamethoxazole and Rifampicin against linezolid for treating MRSA infection. 150 adult patients participated in the trial and the authors confirmed with PP analysis that 54/66 (81.8%) showed positive results in the

Linezolid group and 52/59 (88.1%) in the Trimethoprim/Sulfamethoxazole and Rifampicin group (risk difference 6.3%, 95% CI -6.8% to 19.2%). They concluded Trimethoprim/sulfamethoxazole was non-inferior in the treatment of MRSA infection [13].

Another randomized controlled trial studied the effectiveness of Trimethoprim/Sulfamethoxazole against Vancomycin of the 228 intravenous drug users, 101 had proven infections. Out of these, 54 had Methicillin susceptible *S aureus* and 47 had MRSA. Positive results were seen in 57 of 58 Vancomycin recipients and in 37 of 43 TMP-SMZ recipients (P less than 0.02). The authors concluded that Trimethoprim/Sulfamethoxazole could be used to treat certain MRSA cases [14].

However, in another randomized trial conducted in Israel, the authors concluded that Trimethoprim/Sulfamethoxazole did not achieve non-inferiority against Vancomycin. 252 patients took part in the trial (92 had bacteremia). Treatment failure for Trimethoprim-Sulfamethoxazole (51/135, 38%) versus Vancomycin (32/117, 27%)—risk ratio 1.38 (95% confidence interval 0.96 to 1.99) was not significant. However, Trimethoprim-Sulfamethoxazole did not meet the non-inferiority criterion [8].

Four teaching hospitals in Pakistan collected data on 1102 *S.aureus* isolates. MRSA accounted for 41.9% of the *S.aureus* isolates. According to the study nosocomial MRSA was multi-drug resistant whereas community acquired MRSA showed susceptibility to Trimethoprim-Sulfamethoxazole (3.9%) and Clindamycin (63%) [15].

Whereas in our own study we found out 82.4% of the MRSA strains were resistant to Trimethoprim-Sulfamethoxazole as compared to Clindamycin to which 91.4% of the strains were resistant, while all strains of MRSA were 100% susceptible to Vancomycin. This indicates Trimethoprim-Sulfamethoxazole can still be considered as a potential treatment option when compared with Clindamycin. In males 85.9% of the stains were resistant to Trimethoprim-Sulfamethoxazole while in females 79% of the strains were resistant to it as shown in table 1. The age distribution of MRSA and antibiotic resistance pattern is depicted in table 2. It shows that there is a slightly higher resistance (83%) to Trimethoprim-Sulfamethoxazole of MRSA strains in age group 31-40yrs as compared to other age groups. While resistance to Clindamycin was also slightly increased in age group 20-30yrs.

Table 1: Resistance pattern of MRSA according to Gender (n=165)

Resistance pattern of MRSA according to Gender (n=165)			Average Total	P-value
Antibiotics	Male	Female		P-Value=0.997 Chi-square=0.553 Df=6
Oxacillin	100%	100%	100%	
Co-trimoxazole(TMP/SLZ)	85.9%	79%	82.4%	
Clindamycin	89%	94%	91.5%	
Vancomycin	0%	0%	0%	

Table 2: Drug Resistance against MRSA in different Age group

Antibiotics	Age group	Age group	Age group	Age group	Age group
	20-30	31-40	41-50	51-60	>60
Oxacillin	100%	100%	100%	100%	100%
Cotrimoxazole	82%	83%	82.1%	82.2%	82.1%
Clindamycin	94.8%	87.8%	91.9%	90.8%	91.9%
Vancomycin	0%	0%	0%	0%	0%

In 1961 the first strain of *Staphylococcus aureus* that resisted Methicillin was found, marking the beginning of MRSA. Also, resistance was developed against Penicillin, Amoxicillin, Oxacillin, and other beta lactams. In 2002, this also showed resistance against the antibiotic Vancomycin in the United States [16].

Therefore, MRSA needs to be treated as it causes acute infection like boils, cellulitis, impetigo and chronic infections like blood poisoning (sepsis), UTI, pneumonia, septic arthritis, osteomyelitis and endocarditis [17]. Trimethoprim-Sulfamethoxazole may be a useful alternative to Vancomycin for treatment of severe *S. aureus* infections [18].

Treatment of endocarditis, joint infection and meningitis caused due to these infections respond well to Trimethoprim/Sulfamethoxazole. These antibiotics act by inhibiting dihydropteroate synthetase, dihydrofolate reductase, as well as bacterial folic acid synthesis [19]. Hence, due to this unique mechanism this antibiotic can be utilized for the treatment of complicated MRSA infections as an alternative to other antibiotics.

Conclusion

Emerging resistance of MRSA to antimicrobial agents is a growing concern, especially in the developing countries where there is excessive unmonitored usage of antibiotics. This issue has narrowed down our options for usage of different antibiotics to treat severe nosocomial MRSA infections in patients. Despite high resistance pattern of MRSA to antimicrobials agents in our research it was determined that Trimethoprim-Sulfamethoxazole has a comparatively lower resistance to MRSA when compared with Clindamycin, but remained inferior to Vancomycin based on data gathered from the antibiogram. Hence, It can be ascertained that Trimethoprim-Sulfamethoxazole is still a suitable option for treatment of nosocomial MRSA infections in selected cases as an alternative to Clindamycin and Vancomycin which have been used non judiciously in developing countries for the treatment of the aforementioned pathogen.

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