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Diversity of *Staphylococcus aureus* in Clinical Isolates, Their Prevalence and Antimicrobial Resistance in District Peshawar, Pakistan

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Abstract: Multiple drug resistant *Staphylococcus aureus* is an important global pathogen and is one of the leading causes of morbidity and mortality among hospitalized patients. The aim of the current study was to investigate the MDR *S. aureus* in hospitalized patients and their appropriate empirical treatment. Total of 400 samples were analyzed in which 76 isolate were positive for the growth of *S. aureus*. Out of 76 samples Methicillin-sensitive *S. aureus* was (31), Methicillin resistant *S. aureus* (37) and Vancomycin resistant *S. aureus* (8). These isolates were confirmed by physiological characteristics and different biochemical tests. Kirby-Bauer method was used for antimicrobial testing. MSSA overall resistance percentage was Clarthromycin 35%, Levofloxacin 33% and Ciprofloxacin 26%. MRSA resistance profile was Ciprofloxacin 86%, Cotrimaxazole 83%, Levofloxacin 82%, Clarthromycin 67%, Doxycycline 33% and Sparfloxacin 31%. While VRSA overall resistance percentage was Levofloxacin 100%, Ciprofloxacin 100% and Cotrimaxazole 100% followed by Doxycycline 33% and Lizenolid 13%. The overall prevalence of MSSA was 40.7%, MRSA 48.6% and VRSA 10.5%. The choice of drug against *S. aureus* was Vancomycin, Lizenolid and Doxycycline respectively. Proper diagnosis and antimicrobial susceptibility testing was recommended to avoid the treatment failure in Multiple Drug Resistance microorganisms causing infections.

Key words: Antibiotics resistance · *S. aureus* prevalence · MRSA · VRSA

INTRODUCTION

Throughout the world multiple drug resistant micro-organisms causing nosocomial infections was one of the leading causes of deaths accounting a major burden on health care systems. *Staphylococcus aureus* is ubiquitous colonizers of human epithelial tissue and are opportunistic pathogens causing serious infection in Hospitalized patients [1]. Emerging Staphylococcus aureus resistance in nosocomial as well as in community isolates is serious problematic because proper and empirical choice of antibiotics must include antimicrobial agents with activity against resistant strains [2]. *S. aureus* causes localized and invasive infections by producing large number of heat stable proteins or toxins [3]. *S. aureus* is the leading isolated pathogen in hospitalized patients causing septic arthritis, pneumonia, abscesses, osteomylitis and endocarditis [4].

Methicillin-Resistant *S. aureus* was first detected in United State of America in the 1970s and considered an endemic by the year of 1990 [5]. MRSA resistance to Methicillin was primarily due to acquisition of mecA gene, which is not natively present, that code for a penicillin-binding protein (PBP2a) which has low affinity for β-lactams antibiotics [6]. MRSA cause most serious infection like pyomyositis [7], purpura fulminans with toxic shock syndrome [8] and Waterhouse-Friderichsen syndrome [9]. Some epidemiological studies shows that MRSA is more virulent than MSSA causing surgical-site infections, pneumonia and bloodstream infections found

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increased morbidity and mortality rate [10]. Mostly MDR shows resistance to only one antimicrobial agent but MRSA and VRSA were frequently resistant to most available antimicrobial agents. The resistance is due to mutation in the genes, antibiotics overdose, animal's husbandry used antibiotics, bacteria persisters, non-specific uses, bacterial biofilm formation and immunosuppressive drugs.

In developing countries like Pakistan, Multi drug resistant bacteria are serious challenge and leading cause of morbidity and mortality and also difficult to control because of unpatented, improper and irrational use of antibiotics. The current study, therefore, has been conducted for the first time to investigate the antibiotic resistance profile of *S. aureus* isolated from Lady Reading Hospital District Peshawar KPK, Pakistan.

METARIALS AND METHODS

The study was conducted at Lady reading Hospital Peshawar, KPK, Pakistan. All the samples were collected from hospitalized patients including different medical wards including ICU. Samples included blood, pus, fluids, HVS and other valuable clinical specimens.

Sample Collection: Total of 400 samples were collected from different hospitalized patients and processed within one to two hours by using proper phlebotomy techniques. Total of 76 specimens were positive for the growth of *S. aureus* as MRSA, VRSA and MSSA.

Specimens Processing: In routine cultures, a loopful of sample was inoculated on MSA media to ensure the growth of organism. Then incubated aerobically at 37°C for 24 hours. Positive samples were streaked on Muller Hinton agar for sensitivity and resistance of antibiotics according to Clinical and Laboratory Standards Institute (CLSI) recommendations. Kirby-Bauer disk diffusion method [11] were used for antimicrobial activity testing. The results were observed after 24 hours of incubation at 37°C.

The antibiotics used in the present study include Levofloxacin (5 µg), Ciprofloxacin (5 µg), Cotrimaxazole (5 µg), Sparfloxacin (5 µg), Oxacillin/Cefoxitin (5 µg), Linezolid (30 µg), Chlorophenicol (30 µg), Fucidic acid (30 µg), Doxycycline (30 µg), Clarthromycin (15 µg) and Vancomycin (30 µg).

Identification of Specimens: Identification and confirmation was done by specific biochemical testing, like gram staining, catalase test, cougulase test and also by phenotypic characteristic. Oxacillin and Vancomycin antibiotic disc resistance confirmed MRSA and VRSA respectively.

Statistical Analysis: Statistical analysis was performed by Microsoft excel and Standard deviation.

RESULT

Resistance and Susceptibility Pattern of Methicillin Sensitive *Staphylococcus aureus* (MSSA) Against Different Antibiotics: Total of 31 isolates were positive for MSSA, the overall resistance percentage towards different antibiotics was Clarthromycin 35%, Levofloxacin 33%, Ciprofloxacin 26%, Fucidic acid 21% and Doxycycline 14%. The susceptibility pattern shows that Vancomycin, Linezolid and Oxacillin was found to be most effective drug against these clinical isolates. Chlorophenicol shows 86%, Doxycycline 80% and Fucidic acid 79% sensitivity. Levofloxacin, Clarthromycin and Sparfloxacin were 67%, 65 % and 38% respectively as shown in Fig. 1.

Methicillin Resistant *Staphylococcus aureus* (MRSA) Resistance and Susceptibility Profile: Total of 37 specimens were positive for MRSA, the overall percentage towards different antibiotics in order of resistance is Ciprofloxacin 86%, Cotrimaxazole 83%, Levofloxacin 82%, Clarthromycin 67%, Doxycycline 33% and Sparfloxacin 31%. The susceptibility pattern shows that, Vancomycin was the most effective drug against these clinical isolates. Vancomycin was recorded as 100% sensitive antibiotic. Linezolid and Chlorophenicol were the second most effective antimicrobial drug with only 92% and 87% resistance respectively. Other effective drug include Fucidic acid with 74% resistance. Doxycycline and Sparfloxacin with a sensitivity of 39% and 31% as shown in Fig. 2.

Vancomycin Resistant *Staphylococcus aureus* (VRSA) Sensitivity and Resistivity Pattern: Only 8 isolates were resistant to Vancomycin out of 76. Linezolid were found to be the most effective drug against VRSA with 87% sensitivity, 2nd effective drug was Doxycycline with the sensitivity of 67%. VRSA were completely resistant to Levofloxacin, Ciprofloxacin and Cotrimaxazole. Sample wise distribution was pus (7) and blood (1). VRSA and MRSA were mostly found in surgical wound infections patients' sample.

Table 1: Sample Distribution

Total samples	Positive for MSSA	Positive for MRSA	Positive for VRSA
Pus (105)	19 (18%)	18 (17.2%)	07 (6.4%)
Blood (103)	09 (8.7%)	16 (15.4%)	01 (0.9%)
Urine (93)	-	-	-
HVS (48)	03 (6.2%)	02 (4.1%)	-
Ear Samples (31)	-	-	-
Body fluid (20)	-	01 (5%)	-
Total (400) 76 (19%)	31(40.7%)	37(48.6%)	08 (10.5%)

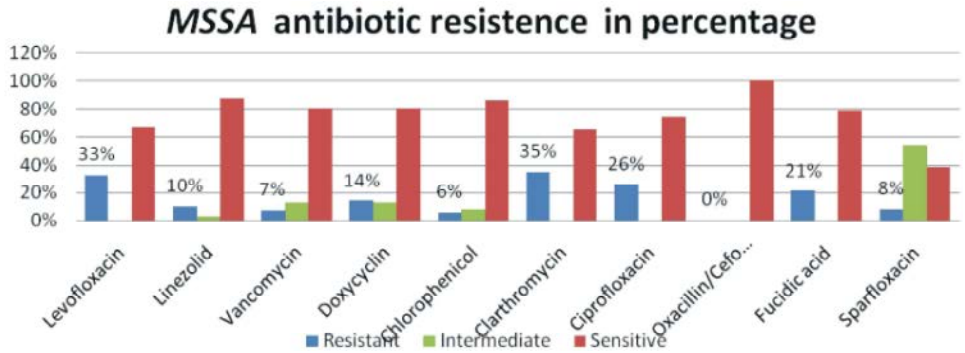

Fig. 1: Resistance and Susceptibility pattern of Methicillin-resistant *S. aureus* (MRSA) against different antibiotics and growth of *S. aureus* on MSA medium

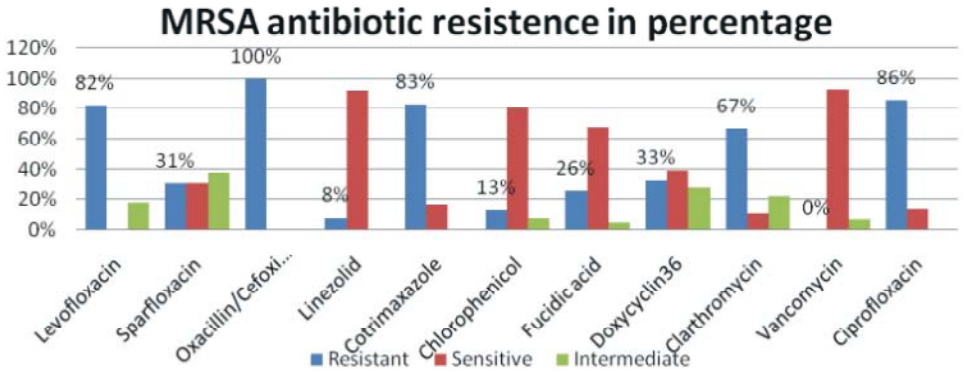

Fig. 2: MRSA antibiotic resistance and sensitivity in graph and on Nutrient agar medium

DISSCUSION

Methicillin Sensitive *Staphylococcus aureus*: The present study showed that Oxazolidinones group antibiotic such as Linezolid is the most effective antibiotic against MSSA with 87% sensitivity; this is slightly low from the study reported by [12] with 100% sensitivity. This was may be due to acquisition of resistance gene from other bacteria. Phenicols group like Chlorophenicol was the 2nd most effective drug with 86% sensitivity; this is slightly greater than the study reported by [13] with 70% sensitivity and correlate with the study of [12] with 93% sensitivity. Overall, Chlorophenicol has a very good activity against Gram-positive bacteria. The difference in resistance rate may be due to mutation in DNA or unprescribed use of antibiotics. The Glycopeptides group antibiotic like Vancomycin was the most effective drug against MSSA but now a days a little bit resistance to Vancomycin is recorded, like the present study shows only 7% resistance, whereas the effectiveness of Vancomycin is also reported by [13] with 9% resistance. Tetracycline group antibiotic like, Doxycycline with a sensitivity of 80%, is slightly lower from the study reported by of [12] with 94% sensitivity. Sparfloxacin with a sensitivity rate of 38% and Intermediate 54% is lower than the study reported by [13] as 30% resistance. Floroquinolones group antibiotic like, Ciprofloxacin in the present study showed 26% resistance to Methicillin sensitive *Staph aureus,* which is correlate with the study reported by [14] with 29% resistance.

Oxacillin shows 100% sensitivity to MSSA. Fucidic acid in the present study shows 21% resistance, which is slightly lower than the study reported by [12] with 35% resistance. Some years ago, Levofloxacin was highly recommended in *Staph* infection due to high in-vitro susceptibility but now a days resistance to Levofloxacin is high as compared to other common antibiotics.

Sample wise distribution percentage was pus 59.4%, blood 29.7% and HVS 11%, this is correlate with the study reported by [16]. Sex wise distribution of our study was female 51% and male 49%, this is slightly correlated with the study reported by [15] with 58% and 42% respectively.

Methicillin-Resistant *Staphylococcus aureus*: The prevalence rate of MRSA in the present study was 49% which is compared to the study reported by [17] 60% at Karachi, [18] Rawalpindi Islamabad 46%, [25] 42% at Islamabad, Pakistan, which is correlate with the present studies. These studies indicate that a specific gene mutation occurs in these strains. Vancomycin which is a choice of drug against MRSA shows 93% sensitivity and 7% intermediate sensitivity which is correlate with the study of [17] with 100% sensitivity and with [19] with 100% sensitivity. Linezolid was the most effective drug in the present study with 92% sensitivity, which is correlate with the study of [12] with 100% sensitivity, this is may be due to different strains of *S. aureus,* or the dose of antibiotics. A tetracycline group antibiotics, Doxycycline in the present study shows 30% sensitivity, that is correlate with the study conducted by [20] with 29% sensitivity and [21] with 37% sensitivity. Levofloxacin, Chlorophenicol and Cotrimaxazole with a resistance rate of 82%, 13% and 83% respectively, is correlate with the study reported by [17] with 80%, 18% and 86% respectively. Sparfloxacin in the present study shows 31% resistance to MRSA, which is compared to the study conducted by [13] with 30% resistance. Fucidic acid in present study shows sensitivity rate of 68%, which is compared with the study reported by [12]. The present study shows more resistance rate to Ciprofloxacin as 86%, which is slightly greater than the study reported by [22] with 56% resistance, this is may be due to mutation in DNA.

Vancomycin-Resistant *Staphylococcus aureus*: VRSA in the present study were recorded as 10.5 which were slightly greater than the studies reported by [23], [24] 7.5% and 6% respectively. This may be due to irrational, excessive and non-patented use of antibiotics.

CONCLUSION AND RECOMMENDATION

The current study conclude that Floroquinolones group antibiotics shows great resistant to multiple Drug Resistance *Staph aureus,* while the drug of choice was Glycopeptide group antibiotics especially Vancomycin and Oxazolidinones group like Lizenolid. The high prevalence of MDR strains was due to unawareness, non patented use of antibiotics and no proper diagnosis and treatment of infections.

It is recommended that the government of Pakistan should banned the non patented sailing of antibiotics, provide facilities in the hospitals for the proper diagnosis and handling of infections and to launch a multidisciplinary program that includes activities to ensure the proper practices for the prevention of hospitals associated infections.

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