

ORIGINAL ARTICLE

CO-EXISTENCE OF CIPROFLOXACIN RESISTANCE IN METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM CLINICAL SAMPLES

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Background: Ciprofloxacin is a broad-spectrum, fluorinated quinolone antibiotic. It has been successfully prescribed against a variety of bacteria. Though its use was limited to a last resort therapy against complicated infections, however widespread usage has led to the emergence of ciprofloxacin resistance. The current study was planned to evaluate the existence of ciprofloxacin resistance in methicillin resistant and sensitive *Staphylococcus aureus* from clinical samples. **Methods:** The clinical *S. aureus* isolates from wound, blood, urine and nose, were obtained from various labs of Hyderabad over nine months. The methicillin resistant strains were identified by Kirby baur disc diffusion test using oxacillin (1 µg) discs. The response of *Methicillin Resistant S. aureus* and *Methicillin Sensitive S. aureus* strains against 2nd generation fluoroquinolone, i.e., ciprofloxacin was demonstrated. **Results:** A total of 150 *S. aureus* isolates from various clinical specimens were included in this study. About 14.6 % (n=22) showed resistance against ciprofloxacin while 30% (n=48) were identified as MRSA. About 25% of the Methicillin Resistant *S. aureus* (MRSA) isolates displayed the co-resistance against ciprofloxacin. Among various specimens the maximum co-resistance was seen in case of blood isolates (37.5%) followed by urine isolates (33.3%). Comparatively, ciprofloxacin resistance was found to be lower in Methicillin Sensitive *S. aureus* (MSSA) strains (9.1%). Odds Ratio [OR] was calculated to be 0.30 [95% CI=0.12–0.77]. Statistically significant differences ($p<0.05$) for ciprofloxacin resistance were seen between MRSA and MSSA. **Conclusion:** The results suggest a statistically significant increase of ciprofloxacin resistance in *Methicillin Resistant S. aureus* as compared to *Methicillin Sensitive S. aureus* in clinical samples.

Keywords: *Staphylococcus aureus*, Clinical Specimens, Ciprofloxacin, Methicillin

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INTRODUCTION

The rapid development of antibiotic resistance in bacteria is a global problem and a matter of extensive concern. Currently the known pathogenic bacterial species display antibiotic resistance to at least one commercially available antibiotic.

The *Staphylococcus aureus* (*S. aureus*), is a universal pathogen and generally considered as naturally susceptible to nearly every antibiotic that has ever been developed; however, the resistance to almost all kinds of antibiotics commonly employed against *S. aureus* has now been witnessed.¹ In *S. aureus* the resistance against beta-lactam antibiotics was witnessed soon after its introduction and the resistance against beta-lactamase resistant beta lactams (methicillin) was reported in 1961.² In Pakistan the resistance to methicillin was first reported in 1989.³ Since then a continuous increase in the *Methicillin Resistant S. aureus* (MRSA) infections has been reported in Pakistan.⁴ Methicillin Resistant *S. aureus* generally displays a multiple drug resistance trend and are therefore a serious cause of morbidity and mortality worldwide.⁵ Previously, MRSA infections were known to be hospital acquired; however, the acquisition of MRSA associated infections from community is now a

general trend in Pakistan.⁶ Besides vancomycin, the fluoroquinolones, particularly ciprofloxacin, have been suggested to treat the infections caused by MRSA as well as *Methicillin Sensitive S. aureus* (MSSA).⁷ Ciprofloxacin is a member of 2nd generation fluoroquinolone, which are smaller, more hydrophobic, and less soluble.⁸ Ciprofloxacin inhibits the *S. aureus* growth by targeting the Topoisomerase IV to affect the DNA replication. Topoisomerase IV acts in the decatenation (separation) of interlinked daughter chromosomes to allow in the segregation into daughter cells.⁹ In *S. aureus* the Topo IV is a tetramer composed of GrlA and GrlB, which are the homologous of ParC and ParE, respectively.¹⁰

S. aureus resistance against quinolones emerged soon after its use in 1980s.¹¹ The resistance develops due to the mutations in the topoisomerase IV gene specifically called as quinolone resistance determining region (QRDR).¹²

Resistance to ciprofloxacin in MRSA strains have been widely reported around the globe.¹³ Studies have also suggested the use of ciprofloxacin as a putative risk factor for the emergence of MRSA strains in clinical specimen.¹⁴ Studies have been conducted in various cities of Pakistan to report the Ciprofloxacin resistance in MRSA of clinical origin.^{15,16} A variable level of Ciprofloxacin

resistance in MRSA has been shown. However, the data from Hyderabad, Sindh are not available. This study aimed to evaluate the frequency of ciprofloxacin resistant MRSA and MSSA in various clinical *S. aureus* isolates to possibly aid in the customization of antibiotic therapy against MRSA infections and curtail any further development of MRSA strains.

MATERIAL AND METHODS

This cross sectional comparative study was conducted at department of Microbiology, University of Sindh Jamshoro after getting approval from the university. Duration of the study was from January 2015 to January 2016. Manitol Salt Agar, Muller Hinton Agar and Nutrient broth were purchased from Oxide. Ciprofloxacin and Oxacillin impregnated discs (5 µg and 1 µg, respectively) were from Oxide. Clinical *S. aureus* isolates recovered from blood, wound, Nose, and urine specimens were obtained from various laboratories in Hyderabad. A total of 164 identified *S. aureus* isolates were received. The isolates were sub-cultured on a selective and differential medium, i.e., Manitol Salt Agar. Fourteen (14) of which failed to grow either because of delay in sub-culturing or lower inoculum. The conventional microscopic and biochemical test were performed on these isolates for reconfirmation. One hundred and fifty (150) reconfirmed *S. aureus* isolates were included in this study.

The *S. aureus* were tested for their antibiotic sensitivity against methicillin and 2nd generation fluoroquinolone (i.e., ciprofloxacin) using Kirby-Bauer Disc Diffusion method. The liquid cultures of *S. aureus* were prepared in nutrient broth. The overnight culture was diluted to OD₆₀₀=0.5 to meet the McFarlands standard required for disc diffusion method. With the help of a sterile cotton swab the diluted culture was inoculated on Muller Hinton Agar and spread evenly. Commercially available antibiotic discs (Oxoid) were placed on the agar surface. A gentle pressure was applied on the discs to get flat contact with the agar surface. The plates were then incubated at 37 °C for 24 hours. The diameter of the Clear zones (zones of inhibition) observed around the

antibiotic discs were measured according to Clinical and Laboratory Standard Institute (CLSI).

The resistance to Ciprofloxacin in MRSA and MSSA strains were measured both in terms of absolute and relative values. The percentage of strains expressing ciprofloxacin resistance and their relevance to Methicillin resistance and Methicillin sensitivity were the variable of interest. In order to measure the association between ciprofloxacin resistance/sensitivity and MRSA/MSSA, the Odds Ratios (OR) and 95% Confidence Interval (CI) were calculated manually and using an online statistic calculator where applicable. Fisher's Exact test employing 2×2 contingency table was applied and $p \leq 0.05$ was considered significant.

RESULTS

A total of 150 reconfirmed *S. aureus* isolates were included in this study. Fifty-two (30%) of which were identified as MRSA, while 98 (70%) were MSSA. Both MRSA and MSSA strains were also processed for antimicrobial sensitivity against ciprofloxacin (5 µg) using similar disc diffusion test. Overall 14.6% (n=22) showed resistance against ciprofloxacin. Among the MRSA strains the ciprofloxacin resistance was seen in 25% (n=13) of the isolates, while 9.1% (n=9) of the MSSA isolates showed resistance against ciprofloxacin (Table-1). The OR for ciprofloxacin resistance between MRSA and MSSA was calculated to be 0.30 with 95% CI of 0.12–0.77. A statistically significant level of difference ($p=0.014$) was demonstrated (Table-2).

The data was also processed for evaluating the frequency of ciprofloxacin resistance among MRSA and MSSA for different categories of clinical samples. The mean age (Year) of the patients with standard deviation for each category of specimen was calculated to be blood=32.05±10.38, nose=22.01±8.3, wound=38.7±12.44 and urine=30.86±8.34. Among various specimens the highest co-resistance (i.e., Ciprofloxacin and Methicillin) was seen in case of blood isolates (37.5%) followed by urine isolates (33.3%) (Table-1). Table-2 shows association between MRSA/MSSA and ciprofloxacin resistance/sensitivity along with OR and p -values.

Table-1: Frequency and percentages for ciprofloxacin resistance/sensitivity in MRSA and MSSA from various clinical samples

Specimen	SA	Cip [R]	MSSA	MRSA	MRSA-Cip[R]	MRSA-Cip[S]	MSSA-Cip[R]	MSSA-Cip[S]
	(n)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Blood	19	3 (15.7)	11 (57.8)	8 (42.2)	3 (37.5)	5 (62.5)	0 (0)	11 (100)
Nose	68	10 (14.7)	45 (66.1)	23 (33.8)	4 (17.3)	19 (82.6)	6 (13.3)	39 (86.7)
Wound	27	4 (14.8)	15 (55.5)	12 (44.5)	3 (25)	9 (75)	1 (6)	14 (94)
Urine	36	5 (13.8)	27 (75)	9 (25)	3 (33.3)	6 (66.6)	2 (7.4)	25 (92.6)
Total	150	22 (14.6)	98 (70)	52 (30)	13 (25)	39 (75)	9 (9.1)	89 (90.9)

Key: SA=*Staph. aureus*, Cip[R]=Ciprofloxacin resistant, Cip[S]=Ciprofloxacin sensitive, MRSA=Methicillin Resistant *Staph. aureus*, MSSA=Methicillin Sensitive *Staph. aureus*

Table-2: Association of Ciprofloxacin resistance/sensitivity with MRSA/MSSA in isolates from various clinical specimens

	Cip[R]	Cip[S]	Total	OR [95% CI]	p
Over all					
MRSA	13	39	52	0.30 [0.12–0.77]	0.01
MSSA	9	89	98		
Total	22	128	150		
Blood					
MRSA	3	5	8	NA	NA
MSSA	0	11	11		
Total	3	16	19		
Nose					
MRSA	4	19	23	1.37 [0.34–5.43]	0.72
MSSA	6	39	45		
Total	10	58	68		
Wound					
MRSA	3	9	12	4.67 [0.42–52.12]	0.29
MSSA	1	14	15		
Total	4	23	27		
Urine					
MRSA	3	6	9	6.25 [0.85–46.13]	0.08
MSSA	2	25	27		
Total	5	31	36		

Key: Cip[R]=Ciprofloxacin resistant, Cip[S]=Ciprofloxacin sensitive, MRSA=Methicillin Resistant *Staph. aureus*, MSSA=Methicillin Sensitive *Staph. aureus*, OR=Odds Ratio, CI=Confidence Interval, NA=Not Applicable

DISCUSSION

Ciprofloxacin is one of the fluorinated quinolones and a broad-spectrum antibiotic which is widely prescribed in clinical and hospital settings.¹⁷ However, the widespread use of this antibiotic has led to an emergence of ciprofloxacin-resistant strains.¹⁸ In the current study the overall ciprofloxacin resistance of *S. aureus* was found to be 14%. Categorically about 25% of MRSA strains displayed co-existence of Ciprofloxacin Resistance from various clinical specimens. The Ciprofloxacin Resistance in MSSA was determined to be significantly lower (9.1%). By the early 1990s, ciprofloxacin resistance in many MRSA isolates around the globe was frequently reported.¹⁴ MRSA expressing Ciprofloxacin resistance has also been reported from various cities of Pakistan.^{1,16,19} In 2010 a study conducted in Karachi reported about 67% of Ciprofloxacin Resistance in MRSA.²⁰ An increased ciprofloxacin resistance in MRSA (79%) was reported²¹ from Peshawar through data collected in 2012–2013. Further studies from Peshawar reported increased incidences of ciprofloxacin-methicillin co-existence, i.e., 86% in 2014¹⁵ and 80% in 2016⁵. In 2015 Hizbullah *et al*¹⁹, from Islamabad reported about 25% of MRSA to be resistant to ciprofloxacin. The studies conducted from Hyderabad are scarce however, in 2017 about 37% Ciprofloxacin resistant MRSA was reported.²² The current study also investigated the differences of ciprofloxacin-methicillin co-existence in various clinical

specimens. The highest percentage of such a co-existence was seen in case of blood isolates (37.5%) followed by urine isolates 33.3%. The statistical analysis for the determination of association between ciprofloxacin resistance/sensitivity and MRSA/MSSA did not show any significant associations for various clinical specimens. The overall analysis suggests a variable trait of the ciprofloxacin-methicillin co-existence for different cities of Pakistan. This is perhaps due to the different trends in the prescription, consumption and over the counter availability of ciprofloxacin antibiotic, leading to the selection of resistant mutants among MRSA strains. In recent investigations fluoroquinolones themselves have been suggested to be the risk factors for the emergence of MRSA. A significant correlation between the isolation of MRSA and ciprofloxacin prescriptions has also been reported.²³

CONCLUSION

The results suggest a statistically significant increase of ciprofloxacin resistance in Methicillin Resistant *S. aureus* as compared to Methicillin Sensitive *S. aureus* in clinical samples.

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