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METHICILLIN-CIPROFLOXACIN CO-RESISTANCE IN *STAPHYLOCOCCUS AUREUS* FROM ASYMPTOMATIC NASAL CARRIER

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ABSTRACT

The current study was aimed to evaluate the co-existence of methicillin and ciprofloxacin resistance in *Staphylococcus aureus* (*S. aureus*) isolated from the nares of asymptomatic healthy volunteers. Two hundred and seven (207) nasal swab samples were collected from participants belonging to, Community Associated and Hospital Associated environment and processed for the isolation of *S. aureus* using conventional identification technique. *S. aureus* was isolated from a total of 56 samples. 32 of these were from Male volunteers while 24 were from Female volunteers. The isolates were assessed for their antibiotic resistance potential against ciprofloxacin and methicillin using conventional Kirbey-baur disk diffusion test. 59% (n=33) of these showed resistance against methicillin while 26.7 % (n=15) were identified as ciprofloxacin resistant strains. About 21.2% of the Methicillin Resistant Strains displayed the co-resistance against ciprofloxacin. Categorically almost similar co-resistance was seen in case of isolates from both genders (i.e. Male: 21%, Female: 21.4%). None of the *S. aureus* strains isolated from community associated population showed co-resistance while co-resistance frequency among *S. aureus* isolated from hospital associated population was found to be 22.5%. The *S. aureus* isolated from the volunteers who consume tobacco products showed 63% reduced co-resistance than the *S. aureus* isolated from the volunteers who do not consume the tobacco products. Co-existence of ciprofloxacin and methicillin resistance was found comparatively higher (23.5%) in the isolates recovered from volunteers having ages below or equal to 25 years of age.

1. INTRODUCTION

S. aureus being common inhabitants of various body sites [1], is most prevalent in anterior nares. In-effect the nares are known to be the principal reservoir for these organisms [2]. It has been established that nasal carriage of *S. aureus* poses a high risk of acquiring an infection with this pathogen [3-5]. Studies report that *S. aureus* are disseminated from the nares via hands [6] to other body sites where infections can occur [7].

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S. aureus is known as universal pathogen and causes a wide variety of diseases in humans as well as animals, affecting public health and the livestock industry[8,9]. *S. aureus* is one of the major causes of mastitis in lactating cows, sheep and goats [10].

The development of bacterial resistance against antibiotics is a global problem. At present the known pathogenic bacterial species display antibiotic resistance to at least one available antibiotic. The co-existence of resistance against more than one group of antibiotics is now a common phenomenon and a matter of extensive concern [11]. In Pakistan the *S. aureus* resistance against methicillin was first

reported in 1989 [12]. Since then *Methicillin Resistance S. aureus* (MRSA) has been continuously reported [13]. Resistance against various groups of antibiotics is a general trend of MRSA, these strains therefore are a serious cause of morbidity and mortality [14]. Besides vancomycin as an alternate antibiotic option, the ciprofloxacin, is also prescribed to treat the infections caused by *Methicillin Sensitive S. aureus* (MSSA) as well as MRSA [15].

Ciprofloxacin is a member of quinolone group of antibiotics. It is among the 2nd gerenartion of quinolones [16]. Being fluorinated it is generally called as flouroquinolone antibiotic. Ciprofloxacin is a broad spectrum antibiotic targeting Topoisomerase IV to thereby affecting the DNA replication in bacterial cells. In *S. aureus* the Topo IV is composed of GrlA and GrlB [17] and acts in removing the superhelicity by separating the interlinked daughter chromosomes at the time of segregation into daughter cells [18]. Initially the use ciprofloxacin was restricted for complicated infections; however widespread usage has led to the emergence of ciprofloxacin resistance.

Previously we evaluated the co-existance of methicillin and ciprofloxacin resistance in *S. aureus* from various clinincal samples [19], the current study is however aimed to evaluate the co-existence of ciprofloxacin resistance in methicillin resistant *S. aureus* isolated from the nares of asymptomatic healthy volunteers. A comparative analysis based on the current data is presented herein to explore the current trends in the co-existence of these two groups of antibiotics.

2. METHODOLOGY

All the media and antibiotics used in this study were from oxide, while Ames Transport Medium Swabs were purchased from Cito. Asymptomatic healthy volunteers belonging to hospital associated and non-hospital associated environment were selected for sample donation. The doctors, staff nurses, OT technicians, laboratory technicians etc. all belonging to the hospital associated environment were approached for sample donation, through a non-invasive collection procedure [20-22]. In case of non-hospital associated environment the sample donors were not affiliated to any kind of health care profession. The project was approved by the board of Research and Graduate Studies, University of Sindh, Jamshoro. A verbal consent was sought from each volunteer. Using following parameters such as; Z value of standard normal distribution calculated at 90% Confidence level with up to 5% margin of error and 25% of the methicillin and ciprofloxacin co-resistance in *S. aureus* [19] the minimum sample size was calculated to be 203. The duration of sample collection was from January 2015 to January 2016. Blood Agar and Manitol Salt Agar were inoculated with the nasal swab sample were. After of incubation period of 24 hours at 37°C the suspected *S. aureus* colonies were further identified through microscopic examination and biochemical tests. The *S. arueus* isolates were tested for their antibiotic sensitivity/resistivity against methicillin and ciprofloxacin using *Kirby-Bauer* Disc Diffusion method as described in reference No. 23 [23]. The analysis of the data was performed based on host and environment related risk factors for the prevalence of methicillin-ciprofloxacin co-resistance.

3. RESULTS

Two hundred and seven (207) nasal swab samples collected from healthy volunteers belonging to community and hospital associated environments were processed for the isolation of *S. aureus* during this study. Samples from volunteers of both gender and various ages were collected. A total of 133 male and 74 female volunteers were engaged for this study. The mean ages along with standard deviations of volunteers in both genders, populations, tobacco consumers/ non-consumers and *S. aureus* carriers / non-Carriers are presented in the Table 1. *S. aureus* was isolated from a total of 56 (27%) samples. The identification of the *S. aureus* isolates was confirmed though traditional microscopic and biochemical tests.
The potential to resist methicillin was assessed by traditional Kirby baur disc diffusion test using oxacillin (1µg) discs. Thirty three [33 (58.9%)] of which were identified as MRSA, while 23 (41.1%) were MSSA (Table 2). The existence of ciprofloxacin resistance in both MRSA and MSSA strains was assessed using ciprofloxacin (5µg) discs through similar disc diffusion test. Overall 26.7 % (n=15) *S. aureus* strains showed resistance against ciprofloxacin. Among the MRSA strains the ciprofloxacin resistance was seen in 21.2% (n=7) of the isolates, while 34.7 % (n=8) of the MSSA isolates showed resistance against ciprofloxacin. Categorically, the percentages of co-resistance for male and female were calculated to be 21% and 21.4% respectively (Table 2). The co-resistance of methicillin and ciprofloxacin was not seen in the *S. aureus* isolated from community associated population, while 22.5% of the methicillin resistant strains from the hospital associated environment showed co-resistance with ciprofloxacin (Table 2).
The methicillin resistant *S. aureus* isolates from the volunteers who consume tobacco products showed 12.5% co-resistance while 24% of co-resistance was

observed in MRSA isolates form the volunteers who do not consume the tobacco products (Table 2). The data was also categorized in to two broad age groups i.e. volunteers having ages below or equal to 25 years of age and volunteers having ages above 25 years of age. The co-resistance of methicillin-ciprofloxacin was found to be 23.5% (n=4) in the isolates recovered from volunteers having ages below or equal to 25 years and 18.7% (n=3) from the volunteers having ages above 25 years (Table 2). A comparative diagram displaying the percentages of methicillin-ciprofloxacin co-resistance in various categories is expressed through Figure 1. We also calculated the percentage differences for both categories using an online calculator. The percentages of differences in each category is displayed in Figure 2. About 182% of difference in the prevalence of co-resistance was seen between Hospital Associated *S. aureus* isolates and Community associated *S. aureus* isolates. The *S. aureus* isolated from volunteers that regularly consume the tobacco showed about 63% of difference for co-resistance (Figure 2).

Table 1

The mean ages along with standard deviations of volunteers in genders, populations, tobacco consumers/non-consummers and Staph aureus nasal carriers / non-carriers. SAC = *S. aureus* carrier, SAnC = *S. aureus* non-Carrier, M = Male, F = Female, HAP = Hospital Associated Population, CAP = Community Associated Population, TC = Tobacco Consumer, TnC = Tobacco non-Consumer.

Age	**SAC**	**SAnC**	**M**	**F**	**HA**	**CA**	**TC**	**TnC**
Mean	25	26.6	29.03	20.7	26.5	25.4	31.12	23.10
Standard Deviation	8.6	10.5	10.03	7.7	7.3	12.9	8.6	9.7
Total	56	151	133	74	117	90	76	131

Table 2

Table showing the absolute and relative values of methicillin-ciprofloxacin co-resistance in *S. aureus* isolated from the nares of healthy volunteers. MRSA = Methicillin Resistant *S. aureus*, MSSA = Methicillin Sensitive *S. aureus*, CIP = Ciprofloxacin.

	Male		Female		Total (%)
	CIP		CIP		
	Resistant	Sensitive	Resistant	Sensitive	
	n (%)	n (%)	n (%)	n (%)	
MRSA	4 (21)	15 (79)	3 (21.4)	11 (78.6)	33 (58.9)
MSSA	4 (30.7)	9 (79.3)	4 (40)	6 (60)	23 (41.1)
	Hospital Associated Population		Community Associated Population		
MRSA	7 (22.5)	24 (77.5)	0 (0)	2 (100)	33 (58.9)
MSSA	2 (15.3)	11 (84.7)	6 (60)	4 (40)	23 (41.1)
	Tobacco Consumers		Tobacco Non-Consumers		
MRSA	1 (12.5)	7 (87.5)	6 (24)	19 (76)	33 (58.9)
MSSA	1 (25)	3 (75)	7 (36.8)	12 (63.2)	23 (41.1)
	Below 25 Years		Above 25 Years		
MRSA	4 (23.5)	13 (87)	3 (18.7)	13 (81.3)	33 (58.9)
MSSA	7 (58.8)	10 (40.2)	1 (16.6)	5 (83.4)	23 (41.1)

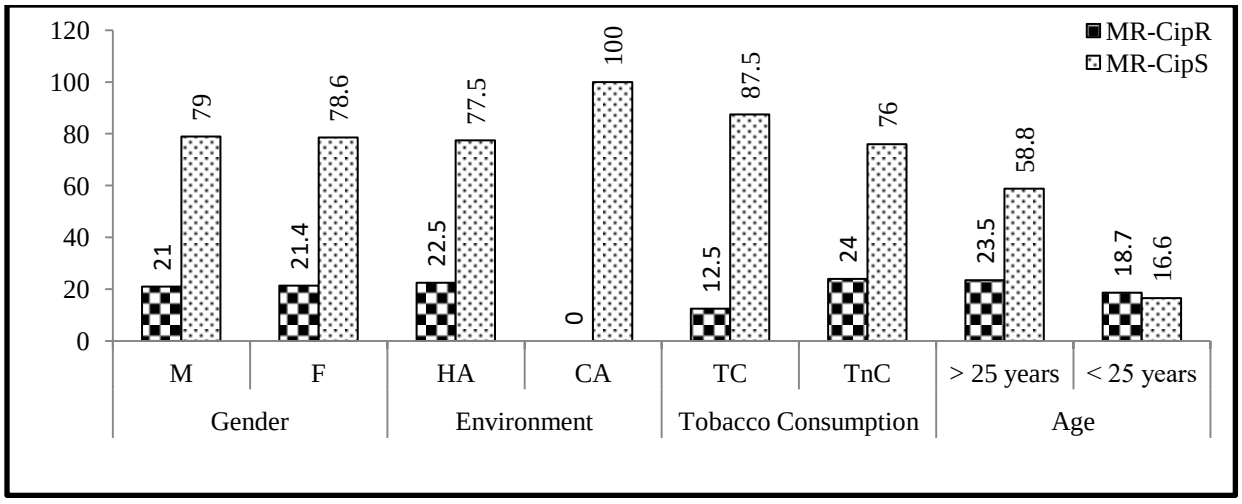

Figure 1 A bar diagram displaying the categorical percentages of methicillin-ciprofloxacin co-resistance in *S. aureus* isolated from the nares of healthy volunteers. (MR-CipR = Methecillin-ciprofloxacin co-resistance, MR-CipR = Methecillin Resistant and ciprofloxacin Sensitive, M = Male, F = Female, HAP = Hospital Associated Population, CAP = Community Associated Population, TC = *Tobacco Consumers*, *TnC = Tobacco Non-Consumers.*).

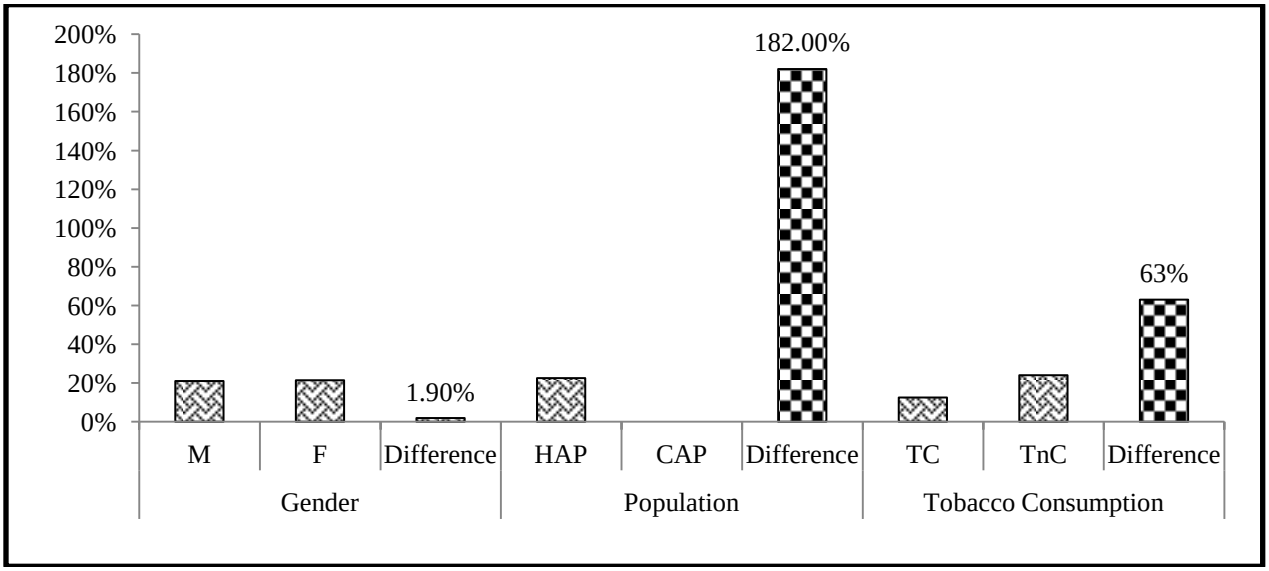

Figure 2 A bar diagram displaying the percentages differences for methicillin-ciprofloxacin co-resistance between various categories. (MR-CipR = Methecillin-ciprofloxacin co-resistance, M = Male, F = Female, HAP = Hospital Associated Population, CAP = Community Associated Population, TC = *Tobacco Consumers*, *TnC = Tobacco Non-Consumers)*

4. DISCUSSION

This cross-sectional study was designed to evaluate the prevalence of methicillin-ciprofloxacin co-resistance in *S. aureus* isolated from the nares of healthy volunteers at Mirpurkhas, Sindh. This is probably the very first report of this kind from Mirpurkhas. Previously we reported the said phenomenon for *S. aureus* from clinical origin [19] at Hyderabad, Sindh. Ciprofloxacin a member of 2nd generation quinolones is widely prescribed in clinical and hospital [24] settings. Its valuable use as an alternate therapy for MRSA strains seems to be limited due to the indiscriminate and widespread use of this antibiotic leading to the emergence of ciprofloxacin-resistant [25] strains. In the current study we evaluated the frequency of methicillin-ciprofloxacin co-resistance of *S. aureus* isolated from the nares of healthy volunteers. Overall among the MRSA strains the ciprofloxacin co-resistance was seen in 21.2% (n=7) of the isolates. Previously we reported about 25% of methicillin-ciprofloxacin co-resistance in clinical *S. aureus* isolates. In 2010 Naeem Akhtar working on the *S. aureus* nasal carriage in Health Care Workers reported 71.4% of the Methicillin-ciprofloxacin co-resistance [26]. In the current study we report about 22.5% of Methicillin-ciprofloxacin co-resistance in the *S. aureus* isolates from the nares of people working at hospital environment, while none of the *S. aureus* expressing Methicillin-ciprofloxacin co-resistance was isolated from the nares of people associated to community. About 182 percentage of difference was calculated for Methicillin-ciprofloxacin co-resistance between these two environments, indicating the association of this co-resistance with hospital associated environment. In the current study we report the categorical percentages of Methicillin-Ciprofloxacin co-resistance in male and female genders as; Male: 21%, Female: 21.4% respectively which comparatively seems almost similar. The percentage of differences calculated for this was only 1.9%, indicating no influence of gender on the prevalence of this phenomenon.

The methicillin resistant *S. aureus* isolates from the volunteers who consume tobacco products showed 12.5% co-resistance while 24% of co-resistance was observed in MRSA isolates form the volunteers who do not consume the tobacco products. The percentage of difference calculated for this category turned out to be 63%. The prevalence of the Methicillin-Ciprofloxacin co-resistance for *S. aureus* isolated from the volunteers having ages below or equal to 25 years was calculated to be 23.5% while for the ones which were isolated from the volunteers having ages above 25 years of age was 18.7% (n=3).

5. CONCLUSION

Infections due to Gram negative and Gram positives organisms have successfully been treated with ciprofloxacin. The emergence of ciprofloxacin resistance especially in MRSA strains is a matter of serious concern. In 2010 about 71.4% of co-resistance is *S. aureus* isolates from the nares of healthy volunteers was reported for Rawalpindi [26], comparatively lesser prevalence (21.2%) of Methicillin-Ciprofloxacin co-resistance in is reported herein. The higher percentage of co-resistance difference between isolates from Hospital associated environment and community associated environment in Mirpurkhas region seems alarming and requires reasonable remedial strategies.

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8. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interest regarding publication of this article.

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