TRENDS IN ANTIBIOTIC RESISTANCE OF STAPHYLOCOCCUS AUREUS FROM ASYMPTOMATIC NASAL CARRIERS

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ABSTRACT

Objective: To evaluate the trends of antibiotic resistance pattern of *Staphylococcus aureus (S. aureus)* isolated from nares of healthy volunteers.

Methodology: Participants belonging to, community associated (CA) and hospital associated (HA) environment were included in the study. A total of 207 nasal swab samples were processed for the isolation of *S. aureus* using conventional identification procedure. The antibiotic sensitivity testing for the isolates was done using kirbey-baur disk diffusion test.

Results: *S. aureus was found in* 27% of the samples. Maximum resistance (60%) was observed against erythromycin antibiotic. Statistically significant differences were seen in the percentages of *S. aureus* resistance against clindamycin, ciprofloxacin and methicillin between HA and CA. Odds ratio (OR) for clindamycin , ciprofloxacin and methicillin were calculated to be 5.91, 3.84 and 11.9 while the p values were 0.015, 0.040 and 0.001 respectively.

Conclusion: An association of clindamycin and ciprofloxacin resistance with CA S. aureus and a statistically significant association of methicillin resistance with HA S. aureus were observed.

Key Words: *Staphylococcus aureus*, Nasal carriers, Antibiobitcs

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INTRODUCTION

Rapid development of antibiotic resistance in bacteria has gained an extensive attention. Almost every pathogenic bacterial species is known to display antibiotic resistance to at least one commercially launched antibiotic. The bacterial resistance to antibiotics can either be natural or acquired[1]. A widespread and sometimes unsuitable use of antibiotics contributes to the development of resistance[2].

The *S. aureus*, a universal pathogen, is generally regarded as naturally susceptible to virtually every antibiotic that has ever been developed. S. aureus resistance against penicillin was first reported soon after the use of penicillin in 1940s[3], and by the late 1960s about 80% of the S. aureus isolates showed resistance to penicillin[4]. Resistance against beta lactamase resistant beta lactams (methicillin) was reported in 1961. The resistance to quinolones by S. aureus emerged quickly after its use in 1980s[4]. The resistance to almost all kinds of antibiotics commonly employed against S. aureus has now been witnessed.

Apart from bringing clinical manifestation ranging from superficial infections (i.e. abscess)[5,6] to life threating infections (i.e. bacteremia)[7], the *S. aureus* is biologically able to asymptomatically colonize the nares of healthy people[8]. About 30% of human population is the asymptomatic nasal carrier of *S. aureus*[9].

The antibiotic sensitivity profiling for *S. aureus* from nares has been reported by a few studies[10]. However studies to evaluate the association of particular resistance determinant with the carriage are uncommon. The current study was conducted to evaluate the pattern of antibiotic resistance in *S. aureus* isolated from the nose to explore the correlation if any. Healthy volunteers belonging to both community and hospital environment were screened. The overall focus of the study was to explore the trends of resistance pattern between community and hospital associated S. aureus, to possibly aid in the customization of antibiotic therapy.

METHODOLOGY

The media used in this study were of analytical grade. The nutrient broth, manitol salt agar, sheep blood agar, Muller Hinton agar were all purchased from Oxide. The antibiotic discs were from Oxide, while Ames transport medium swabs were purchased from Cito.

Samples were collected from asymptomatic healthy

volunteers belonging to hospital associated and non-hospital associated environment. From hospital environment, the samples were volunteered by doctors, OT technicians, staff nurses and laboratory technicians. Regarding non-hospital associated environment, the samples were collected from volunteers who were not associated to health care profession. The minimum sample size was calculated to be 159 using following parameters; the Z value of standard normal distribution was calculated from 90% (CI) with a margin of error of 5%, the percentage of *S. aureus* nasal carriage of 18.2%[11] and 17.75%[12]. The sample collection procedure was non-invasive and approved by the board of studies of the university. A written consent form was signed by each volunteer. From January 2015 to January 2016 a total of 207 nasal swabs were collected.

The nasal swab samples were aseptically collected from both nares as described in reference[13-15]; and put into transport medium for further processing. The nasal swab sample were inoculated on blood agar and mannitol salt agar. After 24 hours of incubation at 37°C, the suspected *S. aureus* colonies were further identified through microscopic examination and biochemical tests such as catalase and coagulase tests.

The isolated *S. aureus* were tested for their antibiotic sensitivity against commonly used antibiotics using Kirby-Bauer disc diffusion method. For the test *S. aureus* were grown overnight at 37°C in nutrient broth. The culture was diluted to OD_{600} =0.5. About 200ul of the diluted culture was inoculated on Muller Hinton agar and spread evenly with the help of a sterile cotton swab. Commercially available antibiotic discs (Oxide) were placed on the agar surface. The discs were pressed gently to get flat contact with the agar surface. The plates were then incubated at 37°C for 24 hours. Clear zones (zones of inhibition) around the antibiotic discs were observed and the diameters were measured according to Clinical and Laboratory Standard Institute (CLSI).

The percentage of resistance against each antibiotic was calculated for HA population and CA population separately, as well as collectively for both categories. The percent difference of resistance in each category was also calculated.

IBM SPSS version 20 was used for data analysis, using nasal carriage for community associated and hospital associated *S. aureus* and the percentage of antibiotic resistance as the variables of interest. The association of the volunteers was categorized into 02 main environments; HAP and CAP, the bacterial response to antibiotics was categorized into resistant and sensitive. P values were calculated using a chi square independence test. The ORs with 95% CI were calculated manually and using a statistic calculator.

RESULTS

About 60% of the isolated *S. aureus* were resistant to erythromycin antibiotic, while 17% displayed resistance against clindamycin. None of the isolates was resistant to vancomycin (Figure 1).

The resistance levels against erythromycin for CA and HA *S. aureus* were 58% and 59%, while against gentamycin the resistance levels were 42% and 43% respectively, indicating the only differences of about 2% in each case (Figure 2). More than 100% differences in the resistance level were seen for three different antibiotics i.e. ciprofloxacin, clindamycin and methicillin (oxacillin). The percentages of resistance level against ciprofloxacin for CA and HA *S. aureus* were 50% and 20%; in case of clindamycin the percentages of resistance were 42% and 11%, while against methicillin the percentages of resistance were 17% and 71% respectively (Figure 1). The odds ratio (OR) at 95% CI and p values in CA *S. aureus* and HA *S. aureus* for ciprofloxacin, clindamycin, and methicillin are presented in table 1.

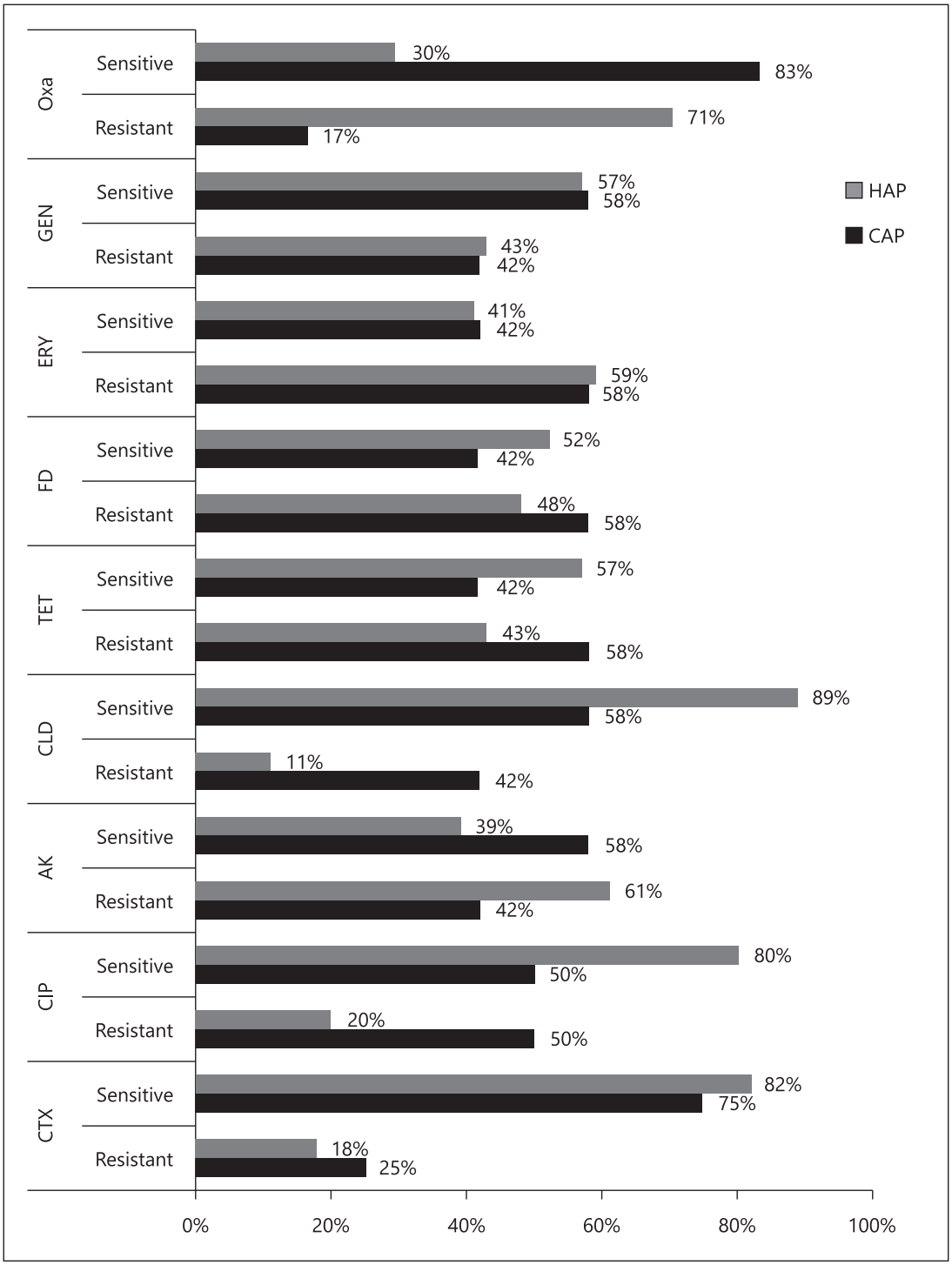
DISCUSSION

Theoretically, a significant non-similar percentage of resistance against a given antibiotic between *S. aureus* isolates from CA and HA population would suggest an association of specific resistance to a particular population. The remarkable differences between the resistance percentage between CA and HA were seen for three different antibiotics tested here i.e. ciprofloxacin, clindamycin and methicillin (oxacillin).

In 2010, a study conducted on the *S. aureus* isolated from the nares of hospital personnel, reported 10% of the isolates to be resistant against ciprofloxacin[16]. Previously only about 8% of *S. aureus* isolated from the nares of HA associated population were reported to be resistant to ciprofloxacin[17]. In the current study the ciprofloxacin resistance in *S. aureus* isolated from HA population was 20%, while for CA it was 50%. The literature survey for clindamycin resistance in *S. aureus* from national and international studies[18-20] revealed that in most cases the focus had been to explore the inducible clindamycin resistance in *S. aureus*. In this study, the focus has been to evaluate the resistance against clindamycin, irrespective of its inducible or constitutive nature. A study involving the clinical isolates of *S. aureus* reported 2.4 % of the *S. aureus* isolates to be resistant against clindamycin[21]. The high percentage of clindamycin resistance in the healthy population not associated to hospital environment as seen in this study is alarming.

The percentage of resistance against ciprofloxacin and clindamycin was found to be higher in CA (i.e. 150% and 282% of difference respectively) than HA (Figure 2). The OR for ciprofloxacin and clindamycin resistance was

Figure 1: Percentages of sensitivity and resistance of the isolated *S. aureus* against various antibiotics.

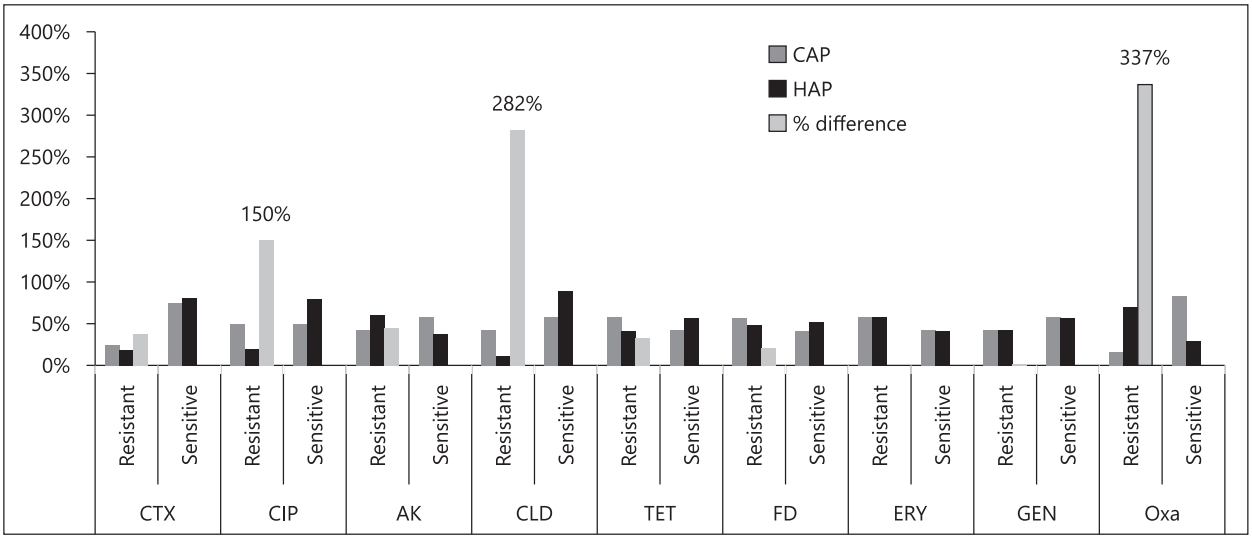


(HAP = Hospital Associated Population, CAP = Community Associated Population, OXA= Oxacillin, GEN= Gentamycin, ERY= Erythromycin, FD= Fusidic Acid, TET= Tetracycline, CLD= Clindamycin, AK= Amikacin, CIP= Ciprofloxacin, CTX= Cefotaxime)

Table 1: The odds ratio (OR) and p values for three antibiotics, using chi square independence test

Antibiotics	OR - (95% CI)	P value
Clindamycin	5.91 (CI=1.27-24.42)	0.015
Ciprofloxacin	3.84 (CI=1.01-14.97)	0.040
Oxacillin	11.9 (CI=2.29-62.12)	0.001

Figure 2: Percentages of sensitivity and resistance of *S. aureus* isolates (From HA and CA population) against various antibiotics with highlighted percentages of differences



3.84 and 5.91 respectively. The p values for ciprofloxacin was 0.04 and for clindamycin it was 0.015. These results suggest an apparent association of ciprofloxacin and clindamycin resistance with CA population.

The percentage of resistance against methicillin was found to be higher in HA (i.e. 337% of difference) than CA (Figure 2). A comparatively higher resistance against methicillin in *S. aureus* isolated from the nares of physicians and staff nurses as compared to sanitary workers and administration staff has been reported[16]. A study involving young people attending secondary schools in urban and rural areas in Kurdistan region, Iraq showed about 22.5% and 2.08% of the *S. aureus* strains to be resistant to methicillin respectively[12]. The OR for methicillin resistance in hospital environment was 11.9 and the p value was .001. These results suggest an association of methicillin (oxacillin) resistance with hospital associated population.

CONCLUSION

An association of clindamycin and ciprofloxacin resistance with CA S. aureus and a statistically significant association of methicillin resistance with HA S. aureus were observed.

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CONTRIBUTORS

BBP conceived the idea, planned the study, and drafted the manuscript. AAP helped acquisition of data and did statistical analysis. DK critically revised the manuscript. All authors contributed significantly to the submitted manuscript.