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Genetic polymorphism of agr Locus and antibiotic resistance of Staphylococcus aureus at two hospitals in Pakistan

Sadia Khan¹, Faisal Rasheed², Rabaab Zahra³

ABSTRACT

Objective: The accessory gene regulator (agr) locus in Staphylococcus aureus (S. aureus) is a global regulator of quorum sensing and controls the production of virulence factors. This study was carried out to investigate the agr specific groups both in methicillin resistant and sensitive Staphylococcus aureus (MRSA and MSSA) and their relation with antibiotic resistance.

Methods: A total of 90 clinical S. aureus isolates were studied from two tertiary care hospitals. The isolates were identified by standard biochemical tests. Methicillin resistance was confirmed by oxacillin and cefoxitin resistance. Multiplex PCR was used to determine the agr groups.

Results: MRSA prevalence was found to be 53.3%. The agr groups’ distribution in MRSA was as follows: 22 (45.8%) belonged to group I, 14 (29.1%) belonged to group III and 2 (4.1%) belonged to group II. agrIV was not detected in MRSA. For 17 isolates, the agr group was not detected. agr III isolates showed higher antibiotic resistance than agrI isolates except in case of oxacillin and linezolid.

Conclusions: Strict infection control policy and antibiotic guidelines should be adopted to control the problem of MRSA. Higher prevalence of agr I and agr III shows that they are dominant agr groups of our area.

KEY WORDS: S. aureus, agr, MRSA, MSSA.

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INTRODUCTION

Staphylococcus aureus is an extracellular Gram positive pathogen, which causes a number of infections such as pneumonia, endocarditis, and septic arthritis.¹ In many cases, the infection originates from hospital derived antibiotic resistant bacteria, among which the most common is MRSA whose prevalence varies markedly between different regions and hospitals.² Within hospitals, MRSA accounts for 40–70% of infections in Intensive care units³ and overall it is responsible for 50% or more of hospital acquired infections in many countries. Normal nasal carriage of S. aureus is 25-30% whereas less than 2% of normal individuals are colonized with MRSA.⁴

In Pakistan, the prevalence of MRSA has increased tremendously over the years. It was reported as 5% in 1989 and since then has increased up to 51%. It is reported to range from 42 to 51%, increasing from the 1990 to 2000⁵ and from 19.5% in 2001 to 40% in 2008.⁶

S. aureus produces many virulence factors comprising of toxins and enzymes, regulated by agr and sar systems.⁸ The accessory gene regulatory (agr) system down regulates the expression of surface proteins while up regulates the expression

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172 Pak J Med Sci 2014 Vol. 30 No. 1 www.pjms.com.pk
of expoproteins. It encodes two transcripts, RNAII and RNAIII where RNAII encodes for \textit{agr}A, \textit{agr}B, \textit{agr}C and \textit{agr}D. \textit{S. aureus} isolates can be divided into four \textit{agr} groups on the basis of the specificity of the auto-inducing peptide (AgrC). Further, \textit{S. aureus} strains belong to specific \textit{agr} groups implicating the importance of the knowledge of \textit{agr} gene groups.

The current study was designed to analyze the genetic polymorphism of \textit{agr} locus among \textit{S. aureus} isolates and to assess its relationship with antibiotic resistance profile.

**METHODS**

**Bacterial Isolates:** The study includes a total of 90 \textit{S. aureus} clinical isolates, out of which 35 were collected from Holy Family Hospital, Rawalpindi whereas, 55 isolates were collected from Microbiology Laboratory, Pakistan Institute of Medical Sciences, Islamabad during the months of April to Oct 2011. These isolates were taken from different sources where 18 were from nasal swab, 50 from pus, 3 from peri rectal swab, 10 from blood, 4 from tracheal secretion, and one each from the following sources: tissue, prostatic secretion, throat swab, semen and CVP tip. The culture media for isolation of \textit{S. aureus} were blood agar, mannitol salt agar and brain heart infusion (BHI) broth/agar.

**Identification of \textit{S. aureus} Isolates:** Identification of isolates was performed by Gram staining and routine biochemical tests including catalase, coagulase, mannitol salt fermentation, and DNase tests.

**Antibiotic Susceptibility Testing:** Susceptibility testing was conducted by disk diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI). Fusidic acid (susceptibility and resistance were ≥22 mm and <22 mm) and tigecycline susceptible breakpoints ≥19 mm zone size were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and US FDA clinical breakpoints respectively. \textit{S. aureus} ATCC 25923 was used as quality control strain.

**DNA Extraction:** Extraction procedure was followed according to manufacturer’s instructions using Wizard Genomic DNA Extraction Kit (Promega Inc., Madison, USA).

**PCR Amplification and Detection of \textit{agr} Groups:** Primers provided by Integrated DNA Technologies (California, USA) were chosen from published sequences. The PCR assay was performed using green master mix (Promega Inc., Madison, USA). Amplified samples were analyzed by electrophoresis on a 1% agarose gel and stained with ethidium bromide.

**Statistical Analysis:** Statistical analysis was performed using the software SPSS 17.0 (SPSS Inc, Chicago, USA). Differences among different groups were analyzed using \(\chi^2\) test. \(p\) value less than 0.05 was considered as significant.

**RESULTS**

Ninety clinical isolates were confirmed as \textit{S. aureus} by Gram staining and standard biochemical tests. All isolates were mannitol fermenters and positive for catalase, DNase and coagulase. Resistance to oxacillin and cefoxitin or both according to CLSI presented the prevalence of MRSA to be 53.3%.

**Prevalence and Association of MRSA and MSSA with Gender and Age:** In our study, MRSA isolates were comparatively more prevalent in males 55.6% (35/63) than females 48.1% (13/27) whereas MSSA isolates were more prevalent in females 51.9% (14/27) than males 44.4% (28/63) with no significant statistical difference (\(p = 0.519\)). Age was categorized into three groups i.e., 1-18 years, 19-44 years and 44+ years. Higher prevalence 68.4% (13/19) and 60.9% (14/23) of MRSA was observed in age groups 44+ years and 1-18 years, respectively. Whereas, comparatively low prevalence (21/48) of MRSA was found in 19-44 years age group. Prevalence of MSSA was found to be 56.2% (27/48), 39.1% (9/23) and 31.6% (6/19) among groups aged 19-44, 1-18 and 44+ years, respectively. Association of MRSA and MSSA with age was found non-significant (\(p = 0.133\)).
## Antibiotic Resistance Profile of MRSA and MSSA Clinical Isolates

Table I shows the antibiotic resistance of MRSA and MSSA isolates. Majority of MRSA isolates exhibited high level of resistance to penicillin, cefoxitin and fusidic acid and comparatively low resistance to vancomycin. MSSA isolates were also highly resistant to penicillin. Tigecycline against MRSA and vancomycin and cefoxitin against MSSA clinical isolates were found to be the most effective antibiotics.

## Prevalence of agr Specific Groups in MRSA and MSSA

Using multiplex PCR, MRSA and MSSA clinical isolates were grouped in four agr specific groups (Fig. 1). Among all groups, agrI was the most prevalent followed by agrIII and agrII. agrIV was absent in MRSA while 4.7% MSSA isolates were positive for it (Table II). Non-type able agr group among MRSA and MSSA were 20.8% and 16.6%, respectively.

### Association between Antibiotic Resistance and agr Specific Groups

MRSA and MSSA isolates showed high resistance against penicillin with statistically significant difference ($p = 0.002$) in all agr groups as shown in Table III. However, all agr containing MRSA and MSSA isolates were sensitive to tigecycline.

## DISCUSSION

In this study, prevalence of MRSA was found to be 53.3% which is consistent with the results reported by other researchers.\(^5,12\) Although higher rate of MRSA was observed in males with non-significant difference that is similar to the earlier reports.\(^2,3\)

Higher prevalence of MRSA was observed in case of patients aged 44+ years (68.4%) and 1-18 years (60.9%) which is close to the data,\(^3\) where it was 61.4% in 41-80 years age group. Similar results were reported in studies conducted in Malaysia and India.\(^13,14\)

Previously, it has been shown that more cases of MRSA are reported from patients staying in intensive care units\(^2,3\) and the same was observed in

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**Table I: Antibiotic resistance of MRSA and MSSA clinical isolates.**

| Antibiotic          | MRSA (Resistance %) | MSSA (Resistance %) | Overall Resistance (%) |
|---------------------|----------------------|----------------------|------------------------|
| Cefoxitin           | 45 (95.8)            | 0                    | 51.1                   |
| Penicillin          | 47 (97.9)            | 37 (88.1)            | 93.3                   |
| Trimethoprim-       | 11 (22.9)            | 16 (38.1)            | 30.0                   |
| sulmethoxazole      |                      |                      |                        |
| Chloramphenicol     | 7 (14.6)             | 1 (2.4)              | 8.9                    |
| Erythromycin        | 17 (35.4)            | 9 (21.4)             | 28.9                   |
| Tetracycline        | 8 (16.7)             | 3 (7.1)              | 12.2                   |
| Levofloxacin        | 9 (18.8)             | 4 (9.5)              | 14.4                   |
| Vancomycin          | 3 (6.3)              | 0                    | 3.3                    |
| Linezolid           | 10 (20.8)            | 7 (14.6)             | 21.1                   |
| Fusidic acid        | 31 (64.6)            | 10 (21.4)            | 45.5                   |
| Tigecycline         | 0                    | 0                    | 0                      |

**Table II: Distribution of agr groups in MRSA and MSSA clinical isolates.**

| agr group | MRSA n (%) | MSSA n (%) | Total n (%) |
|-----------|------------|------------|-------------|
| I         | 22 (45.8)  | 20 (47.6)  | 42 (46.7)   |
| II        | 2 (4.1)    | 4 (9.5)    | 6 (6.7)     |
| III       | 14 (29.1)  | 9 (21.4)   | 23 (25.6)   |
| IV        | 0          | 2 (4.7)    | 2 (2.2)     |
| Non-typeable | 10 (20.8) | 7 (16.6) | 17 (18.9) |

**Table III: Antibiotic resistance pattern of S. aureus isolates in agr-specific groups.**

| agr-specific groups | Oxacillin | Cefoxitin | Penicillin | SXT* | Chloramphenicol | Erythromycin | Tetracycline | Levofloxacin | Vancomycin | Linezolid | Fusidic acid | Tigecycline | Non-typeable | Total n (%) | p value |
|---------------------|-----------|-----------|------------|------|----------------|--------------|--------------|--------------|------------|-----------|--------------|------------|---------------|-------------|---------|
| agrI n (%)          | 25 (59.5) | 21 (50)   | 41 (97.6)  | 11 (26.1) | 4 (9.5)       | 12 (28.5)    | 6 (14.2)     | 4 (9.5)      | 0          | 9 (21.4)  | 20 (47.6)    | 0          | 7 (41.1)      | 48 (53.3)   | -       |
| agr II n (%)        | 3 (50)    | 2 (33.3)  | 4 (66.6)   | 1 (16.6) | 0              | 2 (33.3)     | 0            | 2 (33.3)     | 0          | 1 (16.6)  | 2 (33.3)     | 1 (16.6)   | 7 (41.1)      | 1 (4.1)     | 0.471   |
| agr III n (%)       | 13 (56.5) | 13 (56.2) | 23 (100)   | 10 (43.4) | 4 (17.3)      | 7 (30.4)     | 4 (17.3)     | 5 (21.7)     | 1 (50)     | 1 (50)    | 11 (47.8)    | 2 (8.9)    | 0              | 8 (8.9)     | 0.214   |
| agrIV n (%)         | 0          | 0         | 1 (50)     | 1 (50)   | 1 (50)        | 1 (50)       | 0            | 1 (50)       | 0          | 1 (16.6)  | 1 (50)       | 0          | 0              | 0 (0)        | 0.635   |
| Non-typeable n (%)  | 7 (41.1)  | 10 (58.2) | 15 (88.2)  | 4 (23.5) | 0              | 4 (23.5)     | 1 (50)       | 1 (50)       | 1 (50)     | 1 (50)    | 7 (41.1)     | 1 (16.6)   | 8 (8.9)       | 8 (8.9)     | 0.511   |

* SXT: Trimethoprim-sulfmethoxazole; n: No. of isolates.
earlier findings. These groups in our locality that is opposite to the
as
Only 6 isolates were typed as agr
clinicians in Pakistan. Against it would be a serious concern for the
MRSA is vancomycin so the emerging resistance
Currently the drug of choice for treating life
influenced by any of the resistance mechanisms
tigecycline is a good choice and has not yet been
mutations of multiple gene copies, chloramphenicol-
structuring or sampling bias.
In conclusion, agrI was the most prevalent group
in all the hospital departments, all type of sources
MRSA isolated except agr IV and all agr IV isolates were resistant
to penicillin in this study similar to the
report by other study.22
In conclusion, agrI was the most prevalent group
in all the hospital departments, all type of sources
and age groups followed by agrIII. The uniform
fitness of S. aureus agr groups in some cases suggests
that they also have comparable competitive ability
within the host. The allocation of agr groups in this
study perhaps reflects ecological and geographical
structuring or sampling bias.

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Conflicts of interest: Nothing to declare.

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this study. Since the patients in ICUs are acutely ill
and immune-compromised, it generates more risk
for the infections.
A higher percentage (94%) of MRSA was found in
nasal swabs than other specimens in contrast to the
pus and sputum samples reported by others.3,13 This
is due to the reason that nasal swabs constitute the
major portion of samples coming from ICU.
For all the antibiotics tested, MRSA isolates
showed great resistance than MSSA. Most of MRSA
isolates presented multiple drug resistance. All
isolates indicated 21.1% resistance to linezolid while
MRSA presented 20.8% resistance. These results
are in contradiction with other reports3,16 and up to
10% has been reported from Iran.17 This high level
of resistance observed in isolates could be due to
mutations of multiple gene copies, chloramphenicol-
flofrenicol resistance (cfr) gene carriage or misuse
of this effective drug. Levofloxacin and tetracycline
presented the resistance in the range of 16%-19% in
MRSA isolates while 14.6% isolates were resistant
to chloramphenicol which is similar to other
studies.17,18
This study also identified 3% MRSA isolates
which were resistant to vancomycin. This finding is
in contrast with other studies from the region.3,14,19
Currently the drug of choice for treating life
threatening infection caused by multidrug resistant
MRSA is vancomycin so the emerging resistance
against it would be a serious concern for the
clinicians in Pakistan.
In this study, tigecycline was the only drug with
100% sensitivity showing similarity with the earlier
findings.3 Reports from other studies suggest that
tigecycline is a good choice and has not yet been
influenced by any of the resistance mechanisms
which are involved in other antimicrobials.19,20

By amplification of the hyper-variable domain of
the agr locus, we assigned agr groups to our clinical
isolates. Agr I group was the most prevalent group
in both MRSA and MSSA clinical isolates followed by
agr III which is similar with other studies.10,21,22
Agr group I and III are closely related having
80% sequence homology that would propose an
exclusive genetic characteristics of our isolates and
selection for the coexistence of S. aureus strains in
the population.
No agr group was identified for 17 isolates
which is in accordance with a previous report.21
Only 6 isolates were typed as agr II and 2 isolates
as agr IV which represents reduced prevalence of
these groups in our locality that is opposite to the
earlier findings.21 agr IV was only detected in case of
MSSA while it was absent for MRSA isolates which
is similar to results of few other studies.10,21 Their
absence shows that competition does not favor
these strains.
Although agr I was dominant in all sources,
hospital wards and age groups, it was higher in nasal
swabs (50%), OPD (50%) and 44+ years age group
(57.8%) respectively. agrIII was more prevalent in
sources other than pus or nasal swabs (31.8%), in
44+ years age group (10.4%) as compared to other
age groups and in outpatients (28.2%) as compared
to hospitalized patients. Resistance profile suggests
that agr III isolates are more resistant than agr I.
Resistance to oxacillin is almost similar in all agr
groups except agr IV and all agr IV isolates were resistant
to penicillin in this study similar to the
report by other study.22
In conclusion, agrI was the most prevalent group
in all the hospital departments, all type of sources
and age groups followed by agrIII. The uniform
fitness of S. aureus agr groups in some cases suggests
that they also have comparable competitive ability
within the host. The allocation of agr groups in this
study perhaps reflects ecological and geographical
structuring or sampling bias.

175
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Pak J Med Sci 2014 Vol. 30 No. 1
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Authors Contribution:

SK: Performed the experiments and prepared draft of paper. FR: Drafting and revising the paper for intellectual concept. RZ: Conception and design of research, analysis and interpretation of the data, approval of final version.