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# CAPSULAR TYPING OF COAGULASE POSITIVE (COPS) COMMUNITY ASSOCIATED METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (CA-MRSA) ISOLATED FROM ANTERIOR NARES OF SCHOOL CHILDREN FROM LUSHOTO, KOROGWE, MUHEZA AND TANGA DISTRICTS IN TANZANIA

**Neel R.**[1]* and Ragini Deshpande[2]

[1]* Department of Science, Sebastian Kolowa University College, Magamba, Lushoto, Tanzania
[2] Department of Science, University of Dodoma Dodoma, Tanzania

## ABSTRACT

Nasal colonization with community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) is being increasingly reported, especially in places where in school children are in close contact and in reduced hygiene. Present study was carried out to find out the capsular typing of *Staphylococcus aureus* (*S. aureus*) coagulase positive (CoPS) in school children in few Districts in Tanga region, Tanzania. Total 1574 (784 from boys and 790 from girls) nasal swabs collected were subjected to standard bacteriological culture. *S. aureus* isolates were identified by mannitol fermentation, coagulase positivity. Antimicrobial susceptibility test was performed on muller-hinton agar (MHA) by modified Kirby-Bauer disc diffusion method with methicillin antibiotic. There was a high rate of *S. aureus* nasal colonization in boys (50.25%) and girls (51.01%) in the 5-15 year age group. An alarming rate of incidence of MRSA in boys (32.39) and girls (30.37) of community acquired methicillin resistant *S. aureus* nasal colonization in the community. Isolates resistant to methicillin was resistant to penicillin. In our findings the predominant capsular type in the region was type-8, it was 49.18% and T5 192 (29.05%) and for non T5/T8 200 (25.09%) for (CoPS) *S. aureus* and T8 for 261 (52.83%), T5 was 113 (22.87%) and non T5/T8 was 120(24.29%) for MRSA. This implies that type-5 strains may be less invasive than type-8 strains.

**Keywords:** *Staphylococcus* isolates (Co PS), Nasal carriage, Capsular typing and School children.

## INTRODUCTION

*Staphylococcus aureus* is a known colonizer in humans and has been implicated in community acquired soft tissue infections. *Staphylococcus aureus* is ubiquitous in nature and a known colonizer in humans. Community acquired soft tissue infections due to *S. aureus* is quite common.[1] Recently, community acquired *S. aureus* has raised concerns due to increasing methicillin resistance. Microorganisms that cause invasive disease commonly produce extracellular capsular polysaccharides.[2] Capsules enhance microbial virulence by rendering the bacterium resistant to phagocytosis. Capsule production by *S. aureus* was first described in 1931 by

Gilbert.[3] However, since capsule detection methods were crude (India ink negative staining, colony morphology on agar plates and in serum-soft agar, and lack of cell-associated clumping factor), very few strains of *S. aureus* were recognized as capsule positive. These highly encapsulated strains (typified by strains M and Smith diffuse) produced mucoid colonies, resisted phagocytosis, and were virulent for mice.[4-8]

Bacterial capsular polysaccharides (CP) are carbohydrate polymers comprised of repeating saccharide units. Several of these CP have side chains attached to their backbone structures. The side chains may include O-acetyl, phosphate, sialic acid, and other moieties. Those moieties represent the immune dominant epitopes and the most functional ones. The clinically significant *Staphylococcus aureus* type 5 CP (CP 5) and type 8 CP (CP 8) are comprised of a trisaccharide repeat unit with one O-acetyl group attached to each repeat unit. The immunogenicity of these CP and the functionality of antibodies to the backbone and the O-acetyl moieties were investigated.[9]

The role of the S. aureus capsule in the pathogenesis of staphylococcal infections has been examined in a number of test systems. Serotype 5 and 8 strains of *S. aureus* resist opsonophagocytic killing by human polymorphonuclear leukocytes. In addition, CP5 enhanced virulence in mouse models of lethality, bacteremia, and septic arthritis. CP5 expression also promoted renal abscess formation, subcutaneous abscess formation, and long-term nasal colonization in mice.[10]

## MATERIALS AND METHODS

The isolates of "CA-MRSA" as a patient without previous hospitalization within 1 year, including the absence of antimicrobials or treatment clinical visits within the prior 6 months. The samples were collected from students in Primary schools, age between 5 to 15 in different parts different parts of Tanga region from October 2011 to February 2012 from 784 (49.80%) male individuals and 790 female students (50.19%) of School children who were not taking any antibiotics during the sample collection. The samples were mainly collected from anterior nares (AN) for the resident flora. A swab from both anterior nares was obtained from each student. Swabs were carefully inserted into each nostril so that the tip is entirely at the nasal ostium level (about 2 Cm. from the edge of the nare) and gently rolled 5 times. Cotton swabs were dipped in peptone water and then used for swabbing the selected sites. After swabbing the site, the swabs were immediately dipped into peptone water and transported to the laboratory and inoculated on to Mannitol Salt Agar (MSA) plates. The plates were incubated overnight at $37^0$ C with 5% $CO_2$. All strains were further tested for the production of free coagulase enzyme using tube coagulase test based on standard methods. *Staphylococcus aureus* ATCC-25923 of known coagulase production was included as control strain. The coagulase positive Staphylococci was subsequently confirmed as *Staphylococcus aureus* by morphological and biochemical studies such as microscopic examination, Gram's staining, motility, catalase, coagulase, Mannitol fermentation test, phosphatase and Novobiocin resistant test using standard methods.[11]

### Kirby-Bauer Disc Diffusion Method

These *Staphylococcus aureus* were screened for methicillin resistance by modified Kirby-Bauer disc diffusion method using 8 antibiotics discs were further used to find out the antibiotic sensitivity pattern of these MRSA. These antibiotics disc were Penicillin (10 Units), methicillin (30ug).

### *Preparation of specific capsular polysaccharide suspension of S. aureus prototype strains for immunization*

From overnight brain heart infusion broth culture, 10 fold dilutions are made in phosphate buffered saline and spread onto Mueller Hinton Salt Agar plates. The plates were incubated

overnight at $37^0$C in candle jar (5%$CO_2$). The plates showing growth just below confluence ($10^{-3}$ – $10^{-4}$) were used.

- Colonies were scraped into 5ml of PBS.
- The suspension is heat inactivated in water bath at $70^0$ C for one hour.
- The suspension is washed once by centrifugation, the pellet resuspended in 5ml of PBS, tested for sterility by plating 100µl on the Tryptone Soya Agar plates, and incubated at $37^0$ C overnight. If the growth was > 10 CFU then they were discarded.
- This suspension is stored in the refrigerator and used for immunizing the New Zealand White rabbits within one week. Every week fresh suspension with the same protocol was prepared and used.

### *Preparation of the antiserum*

- Female New Zealand White rabbits, ≈ 3kg on arrival are allowed to acclimatize for at least one week prior to immunization.
- A pre-immune serum is obtained by bleeding 30 ml blood from the central artery of the ear.
- The rabbits are inoculated through the peripheral ear veins for four weeks on every Monday, Wednesday and Friday as follows as shown in Table 1.
- During the fifth week, the rabbits are bled on three consecutive days – Tuesday, Wednesday, and Thursday. The central artery is used to get at least 40-60 ml blood per bleed into 50 ml conical polypropylene centrifuge tubes. The blood is allowed to clot at room temperature for one hour, and centrifuged at 1000-1500 rpm for 20 minutes and the serum is collected.
- The rabbits are allowed a rest for two months and boosted by immunizing the animals with 0.4ml of the suspension on three alternate days and on the following week the animals are again bled and the serum collected.

### *Preparation of Capsular specific antiserum*

The serum obtained is absorbed with their respective mutant strains as follows: The mutant strains are processed as for the preparation of the capsular polysaccharide suspension for the prototype strains and then the serum was absorbed by adding 2 volumes of serum to one volume of packed cells and then dispersed by gently stirring. After 18hrs at $4^0$C, the cells are removed by centrifugation and the serum is used for typing. The serum is stored with 0.02% sodium azide in aliquots at $4^0$C was shown by Karakawa.[12] This serum is tested for specificity by the agglutination test with an against the prototype strains, and their mutants.

### *Capsular Typing*

Capsular typing is done by simple agglutination test by mixing one drop of the specific antiserum with one drop of bacterial suspension in saline. For every isolate, test is done using both the antisera to rule out any cross-reactions. The standard strains are used as controls. The pre-immune serum is used as a negative control.

### *Ethical considerations*

The Ethical Review Board of Sukoine Agriculture University (SUA), Morogoro, Tanzania approved the study. Sampling was performed after obtaining oral consent from the subjects.

## RESULTS

There was a high rate of *S. aureus* nasal colonization in School boys (50.25%) and School girls (51.01%) in the 5-15 year age group. An alarming rate of incidence of MRSA in boys (32.39) and girls (30.37) of community acquired methicillin resistant *S. aureus* nasal colonization. Isolates resistant to methicillin was resistant to penicillin. In our findings the predominant capsular type in the region was type-8, it was 49.18% and T5 192(29.05%) and for non T5/T8 200(25.09%) for (CoPS) *S. aureus* and T8 for 261(52.83%), T5 was 113(22.87%) and T5/T8 was 120(24.29%) for MRSA.

In our findings, Swab samples were collected from 784 (49.80%) boys (children) and 790 (50.19%) from girls age group 5 to 15. Total 394 (50.25%) Staphylococci (CoNS) were isolated out of which 784 boy's student's anterior nares (AN).

Samples 446 (49.22%) were coagulase positive (CoPS) and 254(32.39%) were MRSA from school children age between 5 to 15 in Table 2.

Total 561(71.51) Staphylococci (CoNS) were isolated out of which 790 girls students anterior nares (AN). Samples 403(51.01%) were coagulase positive and 240(30.37%) were MRSA in Table 3.

Average colonization of Staphylococci (CoNS) from male and female students 64.24%, positive (CoPS) were 50.63% and MRSA were 31.38 %. Colonization of Staphylococci (CoNS) was found to be more in girls in Tanga, Korogwe and Muheza Districts, but more or less similar in Lushoto District. coagulase positive (CoPS) was found to be more or less similar in boys and girls resident individuals in all parts of 4 districts. MRSA colonization was more in boys than girls in Tanga region, where as in Muheza Korogwe and Lushoto Districts MRSA colonization was found to be more in school girls than boy's anterior nares.

### *Antibiotic resistance*

All isolates 494 isolates of School boys and girls resistant to methicillin were resistant to penicillin.

### *Capsular polysaccharide*

Capsular polysaccharide antigen types in *Staphylococcus aureus* isolates from School children of both sex in Table 4.

## DISCUSSION

There was a high rate of *S. aureus* nasal colonization in boys (50.25%) and girls (51.01%) in the 5-15 year age group similar result found with Devjyoti Majumdar.[13] Isolates resistant to methicillin was resistant to penicillin. Similar report found with K.V.Ramana.[14]

In our findings the predominant capsular type in the region was type-8, it was 49.18% and T5 192(29.05%) and for non T5/T8 200(25.09%) for (CoPS) *S. aureus* and T8 for 261(52.83%), T5 was 113(22.87%) and T5/T8 was 120(24.29%) for MRSA in agreement with the findings of Paul et al.[15]

This implies that type-5 strains may be less invasive than type-8 strains. Nasal carriage shows highest incidence of *S. aureus* with CPS T8 isolates. The capsular polysaccharides expressed by *S. aureus* are clearly important in the pathogenesis of staphylococcal infections. They enhance staphylococcal virulence by impeding phagocytosis, resulting in bacterial persistence in the bloodstream of infected hosts.

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**Table1:** Preparation of the antiserum

| Week | Days | Volume |
|---|---|---|
| 1 | Mon, Wed, Fri | 0.1 ml |
| 2 | Mon, Wed, Fri | 0.2 ml |
| 3 | Mon, Wed, Fri | 0.3 ml |
| 4 | Mon, Wed, Fri | 0.4 ml |

**Table 2:** Colonization of Staphylococci isolates from anterior nares (AN) of high school students age between 5 to 12 (male)

| Area and Male Individuals 784 | Staphylococcal isolates | Anterior nares | |
|---|---|---|---|
| | | n | % |
| Tanga district 185 | CoNS | 99 | 53. 51 |
| | *S.aureus*(CoPS) | 81 | 43.78 |
| | MRSA | 61 | 32.97 |
| Muheza District (201) | CoNS | 111 | 55.22 |
| | *S.aureus*(CoPS) | 80 | 39.80 |
| | MRSA | 50 | 24.87 |
| Korogwe District(222) | CoNS | 153 | 68.91 |
| | *S.aureus*(CoPS) | 120 | 54.05 |
| | MRSA | 63 | 28.37 |
| Lushoto District 176) | CoNS | 120 | 68.18 |
| | *S.aureus*(CoPS) | 113 | 64.20 |
| | MRSA | 80 | 38.63 |
| 784 | CoNS | 483 | 61.60 |
| | *S.aureus*(CoPS) | 394 | 50.25 |
| | MRSA | 254 | 32.39 |

**Table 3:** Colonization of Staphylococi isolates from anterior nares (AN) of high school students age between 5 to 12(female)

| Area And Female Individuals 790 | Staphylococcal isolates | Anterior nares | |
|---|---|---|---|
| | | n | % |
| Tanga District (205) | CoNS | 135 | 65.85 |
| | *S.aureus*(CoPS) | 86 | 41.95 |
| | MRSA | 44 | 29.75 |
| Muheza District (168) | CoNS | 121 | 72.02 |
| | *S.aureus*(CoPS) | 66 | 39.28 |
| | MRSA | 54 | 32.14 |
| Korogwe District (190) | CoNS | 151 | 79.47 |
| | *S.aureus*(CoPS) | 111 | 58.42 |
| | MRSA | 53 | 30.52 |
| Lushoto District (227) | CoNS | 154 | 67.84 |
| | *S.aureus*(CoPS) | 140 | 61.67 |
| | MRSA | 89 | 39.20 |
| 790 | CoNS | 561 | 71.01 |
| | *S.aureus*(CoPS) | 403 | 51.01 |
| | MRSA | 240 | 30.37 |

{AN: Anterior nares. CoNS: Coagulase negative Staphylococci. CoPS: Coagulase negative Staphylococci}.

**Table 4:** Capsular polysaccharide antigen types in *Staphylococcus aureus* isolates from School children of both sex

| Capsular antigen types | | | | | | | |
|---|---|---|---|---|---|---|---|
| Isolates | Total no. of isolates 1291 | T5 | | T8 | | Non T5/T8 | |
| | | n | % | n | % | n | % |
| *S. aureus* (CoPS) | 797 | 192 | 24.09 | 392 | 49.18 | 200 | 25.09 |
| MRSA | 494 | 113 | 22.87 | 261 | 52.83 | 120 | 24.29 |

T5 type 5, T8 type 8, MRSA: mithicillin resistant *Staphylococcus aureus*

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