

## THE DISTRIBUTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED AT THE NAMIBIA INSTITUTE OF PATHOLOGY IN WINDHOEK, NAMIBIA

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### Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) is a public health problem worldwide which is growing uncontrollably. MRSA are isolates of the bacterium *Staphylococcus aureus* (*S. aureus*) that have acquired genes encoding antibiotic resistance to all beta lactam antibiotics including methicillin. However, the term has increasingly been used to refer to multidrug resistant *S. aureus*. The study aimed to determine the distribution of methicillin resistant *Staphylococcus aureus* isolated from clinical specimens analysed at Namibia Institute of Pathology (NIP), Windhoek, Namibia. Archived *S. aureus* sample records from the year 2010 to 2014 were retrieved from the Laboratory Information System (LIS). Data was analysed using Statistical Package for Social Sciences (SPSS) version 22 and results were presented in cross tabulations. A total of 3727 *S. aureus* isolates were isolated from 2012 to 2014. MRSA was identified in 13.6% of *S. aureus* isolates. It was higher among subjects >30 to ≤50 years of age (45.5%), followed by subjects aged ≤30 years (36.6%). The frequency of MRSA was higher among males (53.9%) than females (46.1%). MRSA isolation was higher from sputum samples (41.3%), followed by pus swabs (35.0%) and blood culture samples (5.9%). Hospitalized subjects had more MRSA isolated from them (65.4%) than non-hospitalized subjects (34.6%). MRSA was high from the spinal cord injury ward (19.7%), followed by ICU (18.3%) and Surgery wards (13.5%). MRSA infections were high among subjects of >30 to ≤50 years of age. Hospitalized subjects had the highest MRSA infection rate with spinal cord injury ward having the highest number of cases. Males were the most affected group by MRSA. MRSA was high from sputum samples compared to other samples.

### Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) are isolates of the bacterium *Staphylococcus aureus* (*S. aureus*) that have acquired genes encoding antibiotic resistance to all beta lactam antibiotics including methicillin. However, the term has increasingly been used to refer to multidrug resistant *S. aureus*.<sup>[1]</sup> MRSA isolates frequently carry resistance genes to other antibiotics that have been used against *S. aureus*. It is a Gram positive, non-motile, spherical, aerobic bacterium.<sup>[2,3]</sup> These bacterial cells are approximately 1 µm in diameter and form clusters which are indicative of their ability to divide in more than one plane. These bacteria are capable of both aerobic and anaerobic respiration and most strains ferment mannitol anaerobically.<sup>[4]</sup>

*S. aureus* produce catalase, coagulase and an extracellular cell clumping factor. By far the most important reservoirs are patients who may be colonized with MRSA without evidence of infection, especially since MRSA may be carried for an extremely long period of time.<sup>[2]</sup> *S. aureus* is found in the nose, throat, mucous membranes and on the skin of humans but it is often harmless.

Methicillin resistant *S. aureus* can be acquired from the hospital or community. Community associated MRSA (CA-MRSA) isolates have become globally pervasive and reports of serious and rapidly progressive fatal disease due to virulent CA-MRSA have alarmed healthcare professionals.<sup>[2]</sup> MRSA is a common bacterial pathogen responsible for a variety of infections both in children and adults.<sup>[5]</sup>

Methicillin resistance was observed in approximately one in three *S.aureus* isolates globally between 2004 and 2011.<sup>[6]</sup> A high MRSA prevalence of 60.40% was reported from Pakistan <sup>[7]</sup> and 54.2% in India.<sup>[8]</sup> The prevalence of MRSA has also been determined in different European countries of which the highest prevalence of MRSA isolates was noted in hospitals in Portugal 54% and Italy (43-58%).The prevalence was only 2% in participating hospitals from Switzerland and the Netherlands.<sup>[9]</sup>

The aim of this study was to determine the distribution of methicillin resistant *Staphylococcus aureus* as well as assess relationships between MRSA infection with age and gender of subjects as isolated from clinical samples at NIP in Windhoek, Namibia.

## Materials and methods

The study population included a total of 3727 records of samples which were analyzed at NIP in Windhoek from which *Staphylococcus aureus* was isolated. Data of all records of *Staphylococcus aureus* isolates from 2010 to 2014 was collected. Both categorical and continuous data was collected from the laboratory information system for the 5 year period.

### Isolation and identification of *Staphylococcus aureus*

The current study used archived patient records for the following clinical specimens: sputum, throat swabs, pus swabs and fine needle aspirates, pleural, synovial, pericardial and peritoneal fluids, urogenital specimens, cerebrospinal fluid, semen, blood culture, catheter tips, CVP tips/lines, endotracheal tubes/tips, bronchial fluid and nasal swabs. The clinical samples were cultured on different media according to the sample type. Isolates were identified as *S. aureus* based on standard microbiological methods which included colonial morphology, Gram staining, coagulase, catalase and DNase tests or using the Vitek™ 2 system (bioMérieux)<sup>[10,11]</sup>. *Staphylococcus aureus* ATCC (American Type Culture Collection) 29213 was used as a control strain for the Vitek™ 2 system and also as a positive control for the DNase test, coagulase test, catalase test and Gram stain.

Methicillin resistance was determined using the oxacillin susceptibility test results. Isolates of *S. aureus* that were resistant to oxacillin were defined as MRSA

### Antibiotic sensitivity testing

#### Disk diffusion method

Antimicrobial susceptibility testing for the isolates of *S.aureus* was performed using the Kirby- Bauer disc diffusion technique on Mueller Hinton agar according to the CLSI.<sup>[14]</sup> Antimicrobial testing was done using: oxacillin (OX) (1 µg), penicillin (P) (10 µg), ofloxacin (OFX) (5 µg), gentamycin (CN) (10 µg), clindamycin (DA) (2 µg), cotrimoxazole (SXT) (25 µg), erythromycin (E) (15 µg), vancomycin (VA) (30 µg), amikacin (AK) (30 µg), tetracycline (TE) (30 µg) and Fucidic acid (FD) (10 µg). The zones of inhibition produced by each antibiotic were measured using a ruler to the nearest millimeter according to the CLSI guideline of 2012 to 2014.

## Results

The sample population consisted of 3727 culture records. There were 2105 males and 1622 females. The subjects were grouped into: subjects ≤30 years old (n=1669), >30 to ≤50 years old (n=1502) and >50 years of age (n= 556). The age grouping was done as described by Khanal *et al*, 2010 and Askarian *et al*, 2008.<sup>[15],[16]</sup>

**Table 1: Study population according to age group and gender**

Age	Frequency ≤30 years	%	Frequency >30 to ≤50 years	%	Frequency >50 years	%	Total
Male	901	42.8	893	42.4	311	14.8	2105
Female	768	47.3	609	37.5	245	15.1	1622
Total	1669	44.8	1502	40.3	556	14.9	3727

The study population had more males (56.5%) than females. Most of the isolates came from subjects less than 30 years old (44.8%).

**Table 2: Distribution of MRSA according to gender of subjects**

Gender	Frequency of <i>S.aureus</i>	%	Frequency of MRSA	%
Male	2105	56.5	274	53.9
Female	1622	43.5	234	46.1
Total	3727	100	508	100

P-value=0.214

There was more *S.aureus* isolated from males (56.5%) than females (43.5%). MRSA was mostly isolated from males (53.9%) compared to females (46.1%).

**Table 3: Frequency of MRSA by setting**

	Frequency of <i>S.aureus</i>	%	Frequency of MRSA	%
Hospitalized	2512	67.7	332	65.4
Non-hospitalised	1215	32.6	176	34.6
Total	3727	100	508	100

P-value= 0.290

The highest frequency of *S.aureus* was obtained from hospitalized subjects with (67.7%) of which (65.4%) of the isolates were MRSA. A total of 32.3% of non-hospitalized subjects had *S. aureus* of which 34.6% of the isolates were MRSA. There was no significant difference in MRSA isolates from hospitalized and non-hospitalized patients.

**Table 4: Frequency of MRSA among different hospitals wards**

Wards	Frequency of <i>S.aureus</i>	%	Frequency of MRSA	%
Spinal cord injury ward	716	28.5	100	25.1
ICU	240	9.6	93	23.4
Surgery	500	19.9	69	17.3

Ophthalmology	331	13.2	41	10.3
Oncology	140	5.6	25	6.3
Paediatric	104	4.1	16	4.0
Gaenaecology,Orthopaedic,Ophthalmology	57	2.3	12	3.0
Urology	42	1.7	11	2.8
*Others	382	15.2	31	7.8
Total	2512	100	398	100

P- value &lt; 0.001

\*Others: wards with less than or equal to 2.0% of MRSA isolates which included cardiac unit, ENT ward, PREM unit, TB ward and wards with a combination of surgery, cardiac, medical and orthopaedic subjects.

The spinal cord injury ward had the highest *S.aureus* frequency (28.5%). PREM unit and TB wards yielded the lowest *S.aureus* frequency (0.7%) and (0.5%) respectively. Highest frequency of MRSA was found in spinal cord injury ward (25.1%), ICU ward (23.4%) and surgery ward (17.3%).

**Table 5: Frequency of subjects with MRSA according to age groups**

Age group	Total <i>S.aureus</i>	%	MRSA	%
≤30 years	1669	44.8	184	36.6
>30 to ≤50 years	1502	40.3	229	45.5
>50 years	556	14.9	95	18.9
Total	3727	100	508	100

P-value &lt; 0.001

The age group >30 to ≤50 years had the highest subjects with MRSA (45.5%). There was a significant difference in subjects with MRSA according to age (P-value < 0.001).

**Table 6: Frequency of MRSA among different clinical specimens**

Specimen	Total <i>S.aureus</i>	%	MRSA	%
Sputum	1540	41.3	210	41.3
Pus swab	1670	44.8	178	35.0
Blood culture	116	3.1	30	5.9
CVP line	32	0.9	19	3.7
Catheter tip	40	1.1	18	3.5
Vaginal swab	75	2.0	16	3.1
Ear swab	68	1.8	9	1.8
ET tube	30	0.8	9	1.8

+Others	156	4.2	19	3.7
Total	3727	100	508	100

P-value < 0.001

+Others: specimens with less than or equal to 1.0 % of MRSA isolates which included pleural fluid, tissue, throat swabs, nasal swabs, synovial fluid, peritoneal fluid, pericardial fluid, CSF, fine needle aspiration and semen. Sputum had the highest frequency of MRSA (41.3%) and there was a significant difference in MRSA isolation among the clinical specimens.

## Discussion

The highest frequency of MRSA isolation was noted from hospitalized subjects (65.4%). This study's findings are in agreement with a study done in South West Nigeria which found hospitalized subjects (73.2%) to have more MRSA isolated from clinical samples compared to non-hospitalized subjects (26.8%).<sup>[17]</sup> A study done in Lahole (2013) found the prevalence of MRSA to be higher in hospitalized subjects (41.1%) than non-hospitalized subjects who had 37.4%.<sup>[18]</sup> A study done in Nepal (2009) reported the rate of MRSA to be 66.9% from hospitalized subjects and 33.1% from non-hospitalized subjects.<sup>[19]</sup> MRSA could be high in hospital settings due to invasive procedures, prolonged hospital stay and contamination of instruments in hospitals.<sup>[15]</sup>

The current study found spinal cord injury, ICU and surgical wards to have the highest frequency of subjects with MRSA with (25.1%), (23.4%) and (17.3%) respectively. The findings were in agreement with a study done in India which noted the rate of MRSA to be higher from surgical and ICU wards respectively.<sup>[20]</sup> A similar study had different findings with MRSA frequency of isolation being high in subjects with burns (68.4%) and surgical wound infection wards (54%).<sup>[21]</sup> The high rate of MRSA among spinal cord injury subjects could be due to the fact that these subjects are at greater risk for developing infections caused by multi-resistant microorganisms because of their prolonged hospital stay. They are also susceptible to developing bedsores due to immobility and these sores are more prone to infections.<sup>[22]</sup>

MRSA was found to be high among subjects >30 to ≤50 years of age. Our findings were similar to those of a study done in Nepal (2010) which found isolation of MRSA to be significantly high among subjects >30 years of age compared to subjects ≤30 years of age.<sup>[15]</sup> Ghamba *et al*, (2012) had different findings as they found that the age group of 41 to 60 years had the highest MRSA frequency of 54.8%, followed by age group 21 to 40 years and 11 to 20 years with 35.7% and 9.5% respectively.<sup>[23]</sup> Madani (2002) found that MRSA affected all age groups, but almost half (45.9%) of the patients were paediatric and geriatric.<sup>[24]</sup>

Most MRSA isolates were from sputum samples (41.3%) followed by pus swabs (35.0%), blood culture specimens (5.9%) and CVP lines (3.7%). Our findings differ from previous studies in India where pus samples and throat swabs were the main source of MRSA.<sup>[25],[26]</sup> Another study in India had different findings from the current study and noted the frequency of MRSA to be more in cutaneous and wound specimens (42.2%) followed by blood cultures, respiratory specimens and urine samples.<sup>[27]</sup> A study done in Delhi showed a high prevalence of MRSA from blood culture of which 35% were from other wards and 43% were from ICU.<sup>[20]</sup> Onelum *et al*, (2015) found highest MRSA isolates from wound swabs (63.4%) followed by urine (24.4%) and (4.9%) from aspirates/pus and uro-genital swabs and this was consistent with a study by Srikanth *et al*, 2013 which found MRSA to be most frequently isolated from pus samples 27.5%, followed by urine 17.2%, cervical swabs 15.2% and blood culture samples 13.7%. Studies done by Anupurba *et al*, 2003 and Vidya *et al*, 2010 also found pus to have the highest rate of MRSA isolates.<sup>[17],[25],[28],[29]</sup> The high rate of MRSA in sputum samples in our study could be due to the fact that there is a connection between lungs and the nasal cavity hence MRSA is mostly isolated from this site.<sup>[30]</sup>

The present study found that MRSA was high in males (53.9%) compared to females (46.1%). This was similar to findings of a study done in South West Nigeria which found MRSA to be higher in males (62.7%) than females (56.9%), although these findings are not statistically significant (p=0.545).<sup>[17]</sup> Another correlating study done in Detroit urban found that MRSA was highest in males with 67% compared to females who had 33%.<sup>[31]</sup> Tiemersma *et al*, 2004 also found MRSA to be more frequent in male (21%) than females (18%)<sup>[32]</sup> and this was similar to the

findings of our study although the findings of the current study were not statistically significant. The high rate of MRSA in males could be due to abuse and tendency of men not completing their antibiotics course.<sup>[15]</sup>

## Conclusion

This study found that MRSA is a major problem in both hospital based and community acquired infections in Windhoek, Namibia. The current study has illustrated that MRSA is a major problem in subjects aged between 30 to 50 years and occurs mostly in male subjects compared to female subjects. The study demonstrated that MRSA was mostly isolated from sputum, pus swabs and blood culture specimens.

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