

Antimicrobial Susceptibility Patterns of *Staphylococcus aureus* Strains Isolated at the Namibia Institute of Pathology from 2012 to 2014

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Received 24 May 2016; accepted 5 September 2016; published 8 September 2016

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Abstract

Staphylococcus aureus (*S. aureus*) is known to acquire resistance to new drugs and continues to defy attempts to control it. Infections caused by antibiotic resistant strains of *S. aureus* have reached epidemic proportions globally and the increasing rates of antimicrobial resistance are resulting in fewer treatment options. Methicillin resistant *S. aureus* (MRSA) has also emerged as a serious therapeutic problem worldwide. However, data on the antimicrobial susceptibility patterns of this bacterium over a period of time in Namibia are not available. A descriptive retrospective study was therefore conducted to investigate the antimicrobial susceptibility patterns of 600 *Staphylococcus aureus* strains isolated at the Namibia Institute of Pathology (NIP) from January 2012 to December 2014. The results showed that a high proportion of isolates were resistant to penicillin (92.4%) and cotrimoxazole (44.9%), while the antibiotics to which the isolates were least resistant included vancomycin (0%), fusidic acid (0.3%) and ciprofloxacin (4.4%). Methicillin resistance was observed in 13.5% of the staphylococcal isolates. Apart from clindamycin (P value = 0.039) and cotrimoxazole (P value = 0.030), the susceptibility patterns of the antibiotics did not differ significantly over the three years. Moreover, wound swabs and sputum were the clinical samples from which *S. aureus* was most commonly isolated at NIP. The results from this study suggest that continuous local surveillance on the resistance patterns of *S. aureus* should be performed on regular basis in Namibia, in order to have adequate information for the empirical treatment of *S. aureus* infections.

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Keywords

***Staphylococcus aureus*, MRSA, Antimicrobial Susceptibility, Specimens, NIP**

1. Introduction

Staphylococcus aureus is a versatile human pathogen which causes a broad spectrum of significant opportunistic infections, ranging from a relatively mild involvement of the skin and the soft tissue, to life threatening systemic illnesses such as sepsis, pneumonia, meningitis, pulmonary infections, bloodstream infections and endocarditis, as well as toxin-mediated syndromes like toxic shock, scalded skin syndrome and food poisoning [1]-[4]. The importance of *S. aureus* as a persistent nosocomial and community acquired pathogen has become a global health concern [5].

Staphylococcus aureus can exemplify the adaptive evolution of bacteria in the antibiotic era better than any other human pathogen, as it has demonstrated a unique ability to quickly and successfully respond to each new antibiotic with the development of a resistance mechanism [5] [6]. As rapidly as new antibiotics are introduced, *S. aureus* has developed resistance mechanisms [2]. *Staphylococcus aureus* is capable of producing many antibiotic resistant strains [7] and shows acquired resistance to many structurally unrelated antibiotics [2]. *Staphylococcus aureus* resistance to antibiotics is a worldwide problem [8] and sub-Saharan Africa is no exception [5] [9].

Methicillin resistant *S. aureus* (MRSA) infections are additional to the burden of methicillin susceptible *S. aureus* (MSSA) [10]. Also known as “a superbug”, MRSA has become a major problem in most medical institutions, because it is creating life-threatening situations [11]. MRSA has also been found to be associated with multiple-drug resistance in *S. aureus* [2] [4] [12] and certainly, MRSA possesses a visible threat in many African countries [13].

Infections caused by antibiotic resistant strains of *S. aureus* have reached epidemic proportions globally [8]. It appears that the widespread and indiscriminate use of antibiotics without prescriptions in the developing countries has rendered the commonly used antibiotics completely ineffective against treatment of *S. aureus* [5].

The mutual practice in managing common infections in Namibia is to treat empirically [14]. The sale of medicines is regulated; antibiotics are controlled drugs and can therefore only be sold upon prescription by an authorized prescriber [14]. In the public sector, medicine prescribing is guided by the Namibia Essential Medicines List (NemList) and the Namibia Standard Treatment Guidelines (STGs), which includes guidelines for common illnesses in Namibia [14]. However, several studies in Namibia have revealed that there is a problem with antibiotic usage in the country and that there is a very high non-compliance of prescribing with the Namibia STGs [14] [15].

Despite these alarming facts, to the best of our knowledge, no studies have been done to investigate the antimicrobial susceptibility patterns of this bacterium from a variety of clinical samples over a period of time at NIP. It is against this background that this study is carried out.

2. Methodology

Study Design

This research was a descriptive retrospective study, carried out to establish the antimicrobial susceptibility patterns of *Staphylococcus aureus* strains isolated at the NIP central reference laboratory in Windhoek, Namibia from 2012 to 2014. Routinely collected antimicrobial susceptibility data retrieved from the NIP laboratory information system was used. The study included 600 non-duplicate *S. aureus* records collected between January 2012 and December 2014, which made up a representative sample size. The sample size was calculated using Fisher's formula for sample size determination.

$$\begin{aligned} \text{Formula: } n &= Z^2 pq/d^2 \quad [16] \\ &= (1.96)^2 (0.5)(0.5)/(0.04)^2 = 600 \text{ records} \end{aligned}$$

The 600 antibiograms were then allocated amongst the three years (2012, 2013 and 2014) proportionally,

based on the population for each year in order to make up the study representative.

3. Materials and Methods

3.1. Specimen Analysis and Antimicrobial Susceptibility Testing

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 [17] or using the Vitek™ 2 system (bioMérieux).

Antibiotic susceptibility testing (AST) of some of the *S. aureus* isolates was performed using the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. Mueller-Hinton agar media was utilized and was prepared using Mueller-Hinton agar powder (Laboratorios Conda S.A./Pronadisa) consisting of acid casein peptone (H), beef infusion, starch and bacteriological agar, which was dissolved in distilled water. The AST results were interpreted as sensitive, intermediately resistant and resistant according to the CLSI guidelines [18]. For some isolates, AST was performed using the Vitek™ 2 system (bioMérieux). A purity plate was also employed to ensure that a pure culture was used for testing.

Staphylococcus aureus ATCC (American Type Culture Collection) 29213 was used as a control strain for the Vitek™ 2 system (bioMérieux) 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, while those that were susceptible were classified as MSSA [11]. Amongst the antibiotics tested against the *S. aureus* isolates were ciprofloxacin, erythromycin, clindamycin, oxacillin (for cloxacillin), cotrimoxazole, fusidic acid, vancomycin, tetracycline and penicillin. These antibiotics were included in the study, because they are among the antimicrobial agents that should be considered for routine testing and reporting according to the Performance Standards for Antimicrobial Susceptibility Testing for *Staphylococcus* species [18] and because they are the most commonly tested antibiotics against *S. aureus* at NIP.

3.2. Methods of Data Analysis

Data was analyzed using the IBM SPSS version 22. Descriptive statistics were used to determine the frequencies of *S. aureus* isolates from various clinical samples and their susceptibility to various antimicrobial agents for the period of 2012 to 2014. Results were displayed in frequency tables.

The Pearson Chi-square *P* value was used to determine whether a statistically significant association existed between variables. Using a 95% confidence level, a *P* value of less than or equal to 0.05 ($P \leq 0.05$), was considered to be statistically significant.

4. Ethical Considerations

Ethical clearance and approval to conduct the research was granted by the research committees of the Ministry of Health and Social Services (MoHSS), NIP and the Namibia University of Science and Technology (NUST). Individual patient consent was not required, as all records used were fully de-identified.

5. Results

Of the 600 specimens harbouring isolates of *Staphylococcus aureus*, pus and wound swabs had the highest frequency of isolates; 250 (41.7%), closely followed by sputum with 243 isolates (40.5%). Ear, nasal and throat (ENT) swabs had a frequency of 34 (5.7%) isolates, while blood culture had 25 (4.2%). Body fluids, central venous lines, catheter tips, endotracheal tubes, eye swabs, tissue, urethral swabs, vaginal swabs and cerebrospinal fluid each contributed less than 3% to the total number of isolates (Table 1).

A high proportion of isolates were resistant to penicillin (92.4%) and cotrimoxazole (44.9%) in this study, while moderate resistance rates to tetracycline (17.4%) and cloxacillin (oxacillin) (13.5%) were observed. No resistance to vancomycin was observed. Resistance to erythromycin was 10.2%, while ciprofloxacin, clindamycin and fusidic acid showed less than 7% resistance. Table 2 is a summary of the overall antibiogram for the nine (9) antibiotics used.

Apart from clindamycin and cotrimoxazole, the antimicrobial susceptibility patterns for the majority of the antibiotics (ciprofloxacin, erythromycin, fusidic acid, penicillin, cloxacillin, tetracycline and vancomycin) did not vary significantly over the three years (as indicated by the *P* values). A decreasing trend in resistance to clindamycin (statistically significant) and tetracycline (not statistically significant) over the three years was observed, as is depicted in **Table 3**.

The prevalence of MSSA was higher than that of MRSA. The overall prevalence of MRSA for this study was 13.5%. The difference in the prevalence of MRSA between the three years was not statistically significant. **Table 4** represents the prevalence of MRSA and MSSA.

Table 1. Frequency of clinical isolates of *Staphylococcus aureus* in various clinical specimens, Windhoek, 2012 to 2014 (n = 600).

Clinical specimens	Frequency
Body fluids	6 (1.0%)*
Blood culture	25 (4.2%)
Central venous lines	5 (0.8%)
Catheter tips	3 (0.5%)
Endotracheal tubes	4 (0.7%)
Pus and wound swabs	250 (41.7%)
Eye swabs	1 (0.2%)
Tissue	11 (1.8%)
Urethral swabs	2 (0.3%)
Vaginal swabs	14 (2.3%)
Ear, nasal and throat swabs	34 (5.7%)
Sputum	243 (40.5%)
Cerebrospinal fluid	2 (0.3%)
Total	600 (100.0%)

*The category body fluids includes: ascitic fluid (two specimens), pleural fluid (one specimen) and synovial fluid (three specimens).

Table 2. Overall antibiogram of the *S. aureus* isolates for the nine antibiotics, from 2012 to 2014 (n = 600).

Antibiotics	Isolates tested	Sensitive isolates	Resistant isolates	Intermediately resistant isolates
Ciprofloxacin	585	535 (91.5%)	26 (4.4%)	24 (4.1%)
Erythromycin	596	535 (89.8%)	61 (10.2%)	0 (0%)
Clindamycin	582	545 (93.6%)	37 (6.4%)	0 (0%)
Cloxacillin	600	519 (86.5%)	81 (13.5%)	0 (0%)
Cotrimoxazole	572	315 (55.1%)	257 (44.9%)	0 (0%)
Fusidic acid	591	581 (98.3%)	2 (0.3%)	8 (1.4%)
Penicillin	595	45 (7.6%)	550 (92.4%)	0 (0%)
Tetracycline	598	494 (82.6%)	104 (17.4%)	0 (0%)
Vancomycin	397	396 (99.7%)	0 (0%)	1 (0.3%)

Table 3. The antimicrobial susceptibility patterns of *S. aureus* against the nine antibiotics over the three years (2012, 2013 and 2014).

Antibiotics	2012			2013			2014			P value			
	No. Tested	S	R	I	No. Tested	S	R	I	No. Tested		S	R	I
Ciprofloxacin	204	183 (89.7%)	11 (5.4%)	10 (4.9%)	180	166 (92.2%)	6 (3.3%)	8 (4.4%)	201	186 (92.5%)	9 (4.5%)	6 (3.0%)	0.736
Erythromycin	211	184 (87.2%)	27 (12.8%)	0 (0%)	180	167 (92.8%)	13 (7.2%)	0 (0%)	205	184 (89.8%)	21 (10.2%)	0 (0%)	0.194
Clindamycin	198	179 (90.4%)	19 (9.6%)	0 (0%)	179	168 (93.9%)	11 (6.1%)	0 (0%)	205	198 (96.6%)	7 (3.4%)	0 (0%)	0.039
Cloxacillin	215	182 (84.7%)	33 (15.3)	0 (0%)	180	160 (88.9%)	20 (11.1%)	0 (0%)	205	177 (86.3%)	28 (13.7%)	0 (0%)	0.469
Cotrimoxazole	214	133 (62.1%)	81 (37.9%)	0 (0%)	180	90 (50.0%)	90 (50.0%)	0 (0%)	178	92 (51.7%)	86 (48.3%)	0 (0%)	0.030
Fusidic acid	212	209 (98.6%)	1 (0.5%)	2 (0.9%)	180	177 (98.3%)	1 (0.6%)	2 (1.1%)	199	195 (98.0%)	0 (0%)	4 (2.0%)	0.733
Penicillin	213	13 (6.1%)	200 (93.9%)	0 (0%)	180	17 (9.4%)	163 (90.6%)	0 (0%)	202	15 (7.4%)	187 (92.6%)	0 (0%)	0.457
Tetracycline	215	169 (78.6%)	46 (21.4%)	0 (0%)	179	151 (84.4%)	28 (15.6%)	0 (0%)	204	174 (85.3%)	30 (14.7%)	0 (0%)	0.149
Vancomycin	102	102 (100%)	0 (0%)	0 (0%)	130	129 (99.2%)	0 (0%)	1 (0.8%)	165	165 (100%)	0 (0%)	0 (0%)	0.357

Table 4. The prevalence of MRSA and MSSA.

Year	MRSA	MSSA	Total
2012	33 (15.3%)	182 (84.7%)	215 (100.0%)
2013	20 (11.1%)	160 (88.9%)	180 (100.0%)
2014	28 (13.7%)	177 (86.3%)	205 (100.0%)
Total	81 (13.5%)	519 (86.5%)	600 (100.0%)

P value = 0.469.

6. Discussion

Pus and wound swabs (41.7%) were the most common specimens from which *S. aureus* was isolated, as shown in **Table 1**. This is in agreement with several other studies which also found that the highest frequency of isolates was recovered from wounds, abscesses and exudates [4] [5] [10] [11]. This signifies the important role of *S. aureus* in abscess formation [19] and confirms *S. aureus* as an important etiologic agent in wound infections.

A significant number of *S. aureus* isolates (40.5%) were recovered from sputum, in this study. This finding was however not consistent with other studies that reported the frequency of *S. aureus* in sputum to be moderate (13.1%) [19] to very low (4.0%) [10] and (3.8%) [2]. The reporting structure of *S. aureus* in sputum (when and when not to report it as a pathogen in sputum) in the different settings, could account for the differences in the findings.

The frequency of *S. aureus* bacteraemia was low in this study (4.2%), which was in agreement with a study conducted in South Africa which also noted a low frequency (2.6%) of *S. aureus* from blood [10]. In contrast, high rates of *S. aureus* isolates from blood have been reported from other studies in sub-Saharan Africa; 18.7% [5] and 35.5% [12]. This finding illustrates that there is variance amongst different nations in the types of infec-

tions that *S. aureus* causes.

The low rate of *S. aureus* isolates from the nose in this study (ear, nasal and throat (ENT) swabs only accounted for 5.7% of the isolates), may be due to the fact that our study only used routinely collected clinical laboratory data and patients were not being screened for nasal carriage. This is worth mentioning, because the organism is found in the anterior nares of 20% - 40% of the adults [4].

Of the 600 isolates included in this study, only 2 (0.3%) were retrieved from cerebrospinal fluid (CSF), revealing a low level of *S. aureus* meningitis in this setting. This agrees with a recent study conducted in Namibia which also found that *S. aureus* was not a common cause of meningitis in Namibia [20]. Apart from CSF, other normally sterile fluids such as synovial fluid, pleural fluid and ascitic fluid and indwelling medical devices also yielded low quantities of *S. aureus* isolates.

Staphylococcus aureus did not show 100% susceptibility to any antibiotic in this study (Table 2). It showed resistance or intermediate susceptibility to all the antibiotics. The antibiotics to which a high overall resistance was seen in this study were penicillin (92.4%), cotrimoxazole (TMP/SMX) (44.9%) and tetracycline (17.4%), while the antibiotics to which the isolates were least resistant to were vancomycin (0%), fusidic acid (0.3%) and ciprofloxacin (4.4%).

The high rates of penicillin resistance obtained from the invitro susceptibility tests were expected, as it has been recognized that only a small proportion of the *S. aureus* lineages do not produce beta-lactamases [19]. The results of this current study are in line with several studies done in Africa that also reported a greater than 90% resistance to penicillin [5] [10].

The high rate of *S. aureus* resistance to cotrimoxazole (TMP/SMX) noted in this study was comparable with data from other African settings which also found high rates of resistance to this antibiotic. These resistance rates ranged from 30.8% to 84.5% [5] [7] [9] [10] [21] [22].

While literature attributes the high rates of resistance of *S. aureus* to cotrimoxazole to over the counter availability in some countries [9] [19], this cannot be held responsible for the high rates of resistance in Namibia, as antibiotics are only sold upon prescription [14]. The reason for the high TMP/SMX resistance rate could be due to its use as prophylaxis for opportunistic infections, particularly in HIV patients [23]. The prevalence of HIV in Namibia is quite high; with an estimated 13.1% of the adult population living with HIV in 2013 [24] and thus this could indicate high use of cotrimoxazole in Namibia. The high rates could also possibly be attributed to the fact that cotrimoxazole is indicated for the management of a wide range of common conditions in Namibia [23], which may also indicate wide use.

These high rates of *S. aureus* resistance to penicillin and cotrimoxazole, make the drugs ineffective against *S. aureus* infections in this setting and should therefore not be used as empirical therapy.

Moderate resistance to tetracycline (17.4%) was observed, indicating that this broad spectrum antibiotic is still effective (although not highly) against *S. aureus* infections in Namibia. The resistance to tetracycline observed in this current study, was lower than that reported in other African nations which ranged from 30.0% to 68.8% [5] [7] [9] [10] [21] [22], which is an encouraging finding.

Resistance to ciprofloxacin was low (4.4%), suggesting its effectiveness against the *S. aureus* isolates. This is keeping in agreement with some studies in Africa which have also revealed low resistance of *S. aureus* to ciprofloxacin [7] [10].

In this study, resistance to the macrolide, erythromycin was low (10.2%). This is consistent with results from other studies conducted in Uganda and Nigeria which also found low resistance rates of 7.8% and 11.8% respectively [7] [9]. Resistance to clindamycin was also low (6.4%), indicating its effectiveness against infections caused by *S. aureus* strains. This finding was also in agreement with several studies that also found resistance to clindamycin to be low (less than 10%) [2] [9]. This is a major finding, because in Namibia this lincosamide has been recommended for the management of soft tissue infections, bacterial skin infections and osteomyelitis, amongst other conditions [23] [25].

It should however be noted that several studies in Africa also found moderately high resistance to clindamycin [10] [22] and erythromycin [5] [10], indicating that they are not highly effective against infections caused by this bacterium in some other countries. Knowledge of the local antimicrobial resistance patterns of bacterial pathogens is therefore very essential [10].

The overall high degree of susceptibility to fusidic acid observed in this study (98.3%) is likely related to the fact that fusidic acid is not widely recommended as treatment for infections in Namibia [23], indicating possible low exposure to the drug. Reduced susceptibility to fusidic acid (resistance and intermediate resistance), was

however also observed in this study and although the proportion of isolates that showed overall reduced susceptibility was low; 2 isolates (0.3%) showed resistance, while 8 isolates (1.4%) were intermediately resistant (**Table 2**), this finding is of importance, because it is in contrast with several studies in Africa which showed full sensitivities to fusidic acid for both MSSA and MRSA [9] [10] [12].

It is important to note here that the two isolates that were resistant to fusidic acid were both MRSA and that of the eight that were intermediately resistant, only one was an MSSA; therefore fusidic acid is still highly effective against MSSA infections in this environment. A relationship between methicillin resistance and resistance to fusidic acid was noted in the present study; also intermediate resistance to fusidic acid appears to be on the increase, as shown in **Table 3**.

It is encouraging to report that in the present study, no isolates (MRSA and MSSA) were resistant to vancomycin (**Table 2**). This makes it the drug of choice for treatment of infections caused by *S. aureus*. The results obtained from this study show good correlation with several studies which found full sensitivity to vancomycin for both MSSA and MRSA [9] [10] [19]. However one vancomycin intermediate *S. aureus* (VISA) was observed in the study, as shown in **Table 2** and **Table 3**. This was an MRSA.

The enormous activity of vancomycin against *S. aureus* observed in this study, confirms that this glycopeptide should be used as empiric therapy for serious staphylococcal infections, while waiting for susceptibility testing results to come through [19]. However, to avert further emergence of VISAs and possibly vancomycin resistant *S. aureus* (VRSA), vancomycin should be reserved for serious cases where it is clearly needed and should be used as a last resort.

Our findings indicate that there was no statistically significant difference in the antimicrobial susceptibility patterns for the majority of the antibiotics in the three years studied (**Table 3**). The stability in the performance of these antibiotics against *S. aureus* infections over the years may indicate that misuse of antibiotics is not that bad or that there is an acceptable rational use and prescription of antibiotics by clinicians.

The patterns of antibiotic susceptibility of *S. aureus* to cotrimoxazole differed significantly across the three years (P value = 0.030), as shown in **Table 3**. Of interest was the fact that from 2012, resistance increased by 12.1% from 37.9%, so that by 2013 there were equal proportions of isolates that were resistant and sensitive to this antibiotic (50% resistant and 50% sensitive).

A decreasing trend in resistance to clindamycin and tetracycline over the three years was observed in this study (**Table 3**). This is particularly surprising, because due to its broad spectrum activity, tetracycline has been recommended in Namibia for the management of a wide range of conditions [23] and this feature makes it more prone to misuse which causes an increase in resistance. However, it is important to note that this finding for tetracycline was not statistically significant (P value = 0.149).

Although the exact reason for the decreasing trend for clindamycin is not clearly understood, this finding may point to a controlled and rational use of antibiotics by clinicians, since the introduction of the STGs in 2011.

The overall MRSA prevalence of 13.5% noted in this study was comparable with data from several other studies conducted in Africa which also found low to moderate resistance to methicillin, ranging from 1.6% to 16.2% [7] [9] [22]. Cloxacillin (oxacillin) prescription should however be tightly controlled and the misuse and extensive use of this antibiotic should be prevented, in order to avoid an increase in the prevalence of MRSA, which will pose a great therapeutic challenge in the treatment of *S. aureus* infections in Namibia. There was no statistically significant difference in the prevalence of MRSA in the three years (2012-2014); the P value was 0.469, as shown in **Table 4**.

7. Conclusions

The current study has provided important data on the antimicrobial susceptibility patterns of *S. aureus* to several antibiotics, including the prevalence of methicillin resistance. The findings from this study can be used to guide the choice of empirical treatment of *S. aureus* infections and may also inform the review of treatment guidelines by the relevant authorities.

We concluded that there was a high level of resistance to penicillin and cotrimoxazole among *S. aureus* isolates at NIP and in view of these high resistance rates, treatment of *S. aureus* infections with these antibacterial agents, without antibiotic sensitivity testing would therefore be unrealistic. The susceptibility patterns of *S. aureus* to most of the antibiotics did not differ significantly over the three years. Moreover, cloxacillin prescription in Namibia should be tightly controlled in order to avoid an increase in the prevalence of MRSA. The isola-

tion of a VISA and the emerging decline in the susceptibility of the isolates to fusidic acid, particularly amongst MRSA observed in this study, indicate that stronger measures to tighten antibiotic use in Namibia must be put in place. A national antibiotic resistance surveillance of this organism is recommended.

Acknowledgements

The authors would like to thank the MoHSS and NIP for assistance and approval of this study. We would also like to acknowledge Mr Innocent Maposa and Mr Owen Mtambo of the Department of Mathematics and Statistics (NUST) for their help with the statistics.

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