ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY JULY 2017 ISBN 1595-689X VOL18 No. 3
AJCEM/1721 http://www.ajol.info/journals/ajcem
 https://dx.doi.org/10.4314/ajcem.v18i3.1
AFR. J. CLN. EXPER. MICROBIOL. 18 (3): 133-138

STATUS OF RESISTANCE TO ANTIMICROBIAL AGENTS OF *STAPHYLOCOCCUS AUREUS* STRAINS AT THE LABORATORY OF MICROBIOLOGY OF THE HU-JRA ANTANANARIVO

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Introduction: On contact of antibiotics, *S. aureus* has gradually acquired multiple antibiotic resistances, including the methicillin (MRSA) and without lose its virulence. The aim of the present study was to report the evolution of resistance of *S. aureus* to different common antibiotics and to determine the antibiotics active against MRSA.
Materials and methods: This is a retrospective and descriptive study for 10 years from January 2005 to December 2014 at the Laboratory of Microbiology of the HU-JRA Antananarivo, the biggest academic hospital located in the capital of Madagascar. All demands for standard bacteriological examination were registered in the laboratory for various bacteriological exams or from samples taken from hospitalized patients and we included all positive cultures for *S. aureus*. The variables selected and used for the study were community or nosocomial sources of patients and results of susceptibility testing.
Results: A total of 906 results from 282 (31.12%) community-acquired and 624 (68.88%) nosocomial infections were studied an average of 100±25strains by year of study. Overall, the prevalence of MRSA was 13.83% (39 of 282 isolates)for community-acquired strains, and 15.70% (98 of 624) for nosocomial infections (p> 0.05)with a total of 29.53%. Resistance rate to trimethoprim-sulfamethoxazole was significantly higher in nosocomial infection than in community-acquired. No significant difference was observed in other antibiotics. Of the 137 MRSA, except vancomycin, fusidic acid is the antibiotic that worked the most in 114 cases (83.21%) followed by gentamicin in 96 cases (70.07%). Apart from ciprofloxacin and tetracycline that we have noticed an increase in resistance rates in 2012 and 2013, almost all antibiotics tested have a stable rate of resistance.
Conclusion: The antibiotics tested showed extremely high rates of resistance and that the problem of antibiotic resistance in *S. aureus* is effective in our center.
Key words: Resistance -antibiotics- *S. aureus* - HU-JRA Antananarivo

ETAT DES LIEUX DE LA RÉSISTANCE AUX ANTIBIOTIQUES DES SOUCHES DE *STAPHYLOCOCCUS AUREUS* DANS LE LABORATOIRE DE MICROBIOLOGIE DE L'HU-JRA ANTANANARIVO

Résumé
Introduction: Au contact des antibiotiques, *S. aureus* a progressivement acquis de multiples résistances, y compris la méthicilline (SARM) et sans perdre de sa virulence. Le but de la présente étude est de rapporter l'évolution de la résistance de *S. aureus* à différents antibiotiques et de déterminer les antibiotiques actifs contre les SARM.
Matériels et méthodes: C'est une étude rétrospective et descriptive pendant une période de 10 ans allant de Janvier 2005 à Décembre 2014 réalisée au Laboratoire de Microbiologie de l'HU-JRA Antananarivo. Les échantillons ont été prélevés sur des patients hospitalisés ou non et nous avons inclus toutes les cultures positives à *S. aureus*. Les variables étudiés ont été l'origine communautaire ou nosocomiale des échantillons et les résultats de l'antibiogramme.
Résultats: Un total de 906 résultats dont 282 (31,12%) d'origine communautaire et 624 (68,88%) provenant des infections nosocomiales ont été étudiés avec une moyenne de 100±25 souches par année d'étude. La prévalence des SARM était de 13,83% pour les infections communautaires, et de 15,70% pour les infections nosocomiales (p> 0,05). Le taux de résistance à la triméthoprime-sulfaméthoxazole était significativement plus élevé dans les infections nosocomiales que dans les infections communautaires. Sur les 137 SARM, à part la vancomycine, l'acide fusidique est l'antibiotique qui marche le plus dans 114 cas (83,21%), suivie par la gentamicine dans 96 cas (70,07%). La ciprofloxacine et la tétracycline ont montré une augmentation des taux de résistance en 2012 et 2013, alors que presque tous les antibiotiques testés ont un taux de résistance stable.
Conclusion: Les antibiotiques testés ont montré des taux de résistance extrêmement élevés et que le problème de la résistance aux antibiotiques de *S. aureus* est effective à l'HU-JRA Antananarivo.
Mots clés: résistance - antibiotiques - *S. aureus* - HU-JRA Antananarivo

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium that can be apart of the normal flora on the skin and in the nose, but is another of the most important human pathogens. *S. aureus* is an important cause of serious infections in both hospitals and the community. *S. aureus* can cause a variety of infections, most notably skin, soft tissue, bone and bloodstream infections. It is also the most common cause of postoperative wound infections [1].

On contact with antimicrobial agent, *S. aureus* has gradually acquired multiple resistances, including

the methicillin (MRSA) and without lose its virulence. And for the main causes of these resistances, it was reported high consumption of antimicrobial agent, their misuse and the poor implementation of individual and collective hygiene rules within the services including those of intensive care [2].

MRSA infections have always occupied an important place in hospitals and are responsible for a high rate of morbidity and mortality especially in pediatric and surgical services, and is currently admitted that these infections cause a significant lengthening of durations of stay and hospital costs. To this end, *S. aureus* strains resistant to antimicrobial agent represent a major public health problem that Madagascar does not escape and are responsible for a hospital chronic endemic and epidemic globally. They also have the ability to spread like an epidemic in hospitals and care facilities, but it was also shown that they can be more and more frequently from the community[3.4]. Data concerning resistance of *S. aureus* to antimicrobial agent in Madagascar are rare.

The aim of the present study was to report the evolution of resistance of *S. aureus* to different common antimicrobial agent and to determine the antibiotics active against MRSA, to make an update on the susceptibility of *S. aureus* isolates from the laboratory of Microbiology of the University Hospital Joseph Ravoahangy Andrianavalona (HU-JRA) Antananarivo to various drugs and therefore to improve the empirical approaches to the therapy of serious infections.

MATERIALS AND METHODS

This is a retrospective and descriptive study for 10 years from January 2005 to December 2014 at the Laboratory of Microbiology of the HU-JRA Antananarivo, the biggest academic hospital located in the capital of Madagascar. It features several specialized services and a medical laboratory. Patients in the HU-JRA come from all regions of Madagascar.

All demands for standard bacteriological examination were registered in the laboratory for various bacteriological exams or from samples taken from hospitalized patients and we included all positive cultures for *S. aureus.* We excluded from our study the results of susceptibility testing which are not complete, because sometimes, there is a lack of antibiotics discs in the laboratory as procurement was inadequate.

The variables selected and used for the study were community or nosocomial sources of patients and results of susceptibility testing.

Criteria for nosocomial infection were all infections developed in a patient after 48 hours of hospitalization. Strains were considered as community-acquired when isolated from patients thathave not been hospitalized recently or from patients before 48 hours of hospitalization.

Initial identification was based on colony morphology, Gram staining, catalase and agglutination tests with Pastorex Staph. Susceptibility to antibiotics was assessed by the disk diffusion technique on Mueller-Hinton agar. An inoculum was prepared as recommended by the Antibiogram Committee of the French Microbiology Society (CASFM). After 24 hours at 37°C, the zone of inhibition was measured. Concerning the detection of methicillin resistance, we have followed the guidelines of the French Committee for the Antibiogram which recommend to use oxacillin on Mueller Hinton agar + 2% NACL, with incubation at 37°C for 24 hours.

The following 14 antibiotics were tested: oxacillin, penicillin, amoxicillin + clavulanic acid, erythromycin, lincomycin, clindamycin, vancomycin, ciprofloxacin, tetracycline, trimethoprim-sulfamethoxazole, fusidic acid, clarithromycin, gentamicin, chloramphenicol and spiramycin. The breakpoints for resistance were those recommended by the CASFM.

The resistance rate was calculated as the number of non susceptible isolates divided by the total number of isolates. Comparison of resistance rate between nosocomial or community-acquired strains and between MRSA and methicillin-sensitive *S. aureus* (MSSA) was based on Chi square test of Pearson or exact test of Fisher according to the distribution p, significant level considered was $p < 0.05$.

RESULTS

A total of 906 results from 282 (31.12%) community acquired and 624 (68.88%) nosocomial infections were studied an average of 100±25strains by year of study. Strains were isolated from 401 females and 505 males (mean age: 28.10 years old 95%CI [25.9–30.2], range 1– 81 years old, sex-ratio M/F: 1.26).

Concerning the origin of the community-acquired isolates, 256 (92.46%) were from pus, 15 (5.32%) from liquid pleural, 3(1.06%) from genital tract infections, 1(0.35%) from urinary tract infections, 2 (0.71%) from ascites, 3 (1.06%) from LCR, and 2 (0.71%) from sperm.

For nosocomial strains, most (577) were isolated from pus (92.47%), 39 from blood culture (6.25%), 6 (0.96%) from drain and 2 (0.32%) from catheter.

MRSA were found in 130 samples of pus, 2 drains, 4 pleural fluids and one of the genital tract. Overall, the prevalence of MRSA was 13.83% (39 of 282 isolates)for community-acquired strains, and 15.70% (98 of 624) for nosocomial infections ($p > 0.05$)with a total of 29.53%.

The strains of MRSA resistant to other antibiotics tested are represented by Table 1.

TABLE 1: RESISTANCE PHENOTYPE OF MRSA TO OTHER ANTIBIOTICS

Phenotype	CHL	ACF	GEN	TCY	ERY	CLA	LIN	CLI	SPI	CIP	SXT	VAN
Sensitive	90	114	96	33	80	86	90	87	88	72	35	137
Resistant	47	23	41	104	57	51	47	50	49	65	122	0

CHL: Chloramphenicol, ACF: fusidic acid, GEN: Gentamicin, TCY: Tetracycline, ERY: erythromycin, CLA: Clarithromycin, Lincomycin, CLI: Clindamycin, SPI: Spiramycin, CIP: Ciprofloxacin, SXT: Trimethoprim–sulfamethoxazole, VAN: Vancomycin. p=0.14

Of the 137 MRSA, except vancomycin, fusidic acid is the antibiotic that walketh the most in 114 cases (83.21%) followed by gentamicin in 96 cases (70.07%). On the other hand, tetracycline (33 cases) and trimethoprim-sulfamethoxazole (35) were the antibiotics that are most resistant with respectively 75.92% and 74.46% resistance.

Regarding the evolution of the prevalence of patients infected with MRSA, there is a decrease in MRSA rates since 2013 (Figure 1).

Rates of resistance of MSSA and MRSA to the other antibiotics tested for community or hospital acquired infections are shown in Table 2.

TABLE 2: RATES OF RESISTANCE OF MSSA AND MRSA TO THE OTHER ANTIBIOTICS TESTED FOR COMMUNITY OR HOSPITAL ACQUIRED INFECTIONS

Molecules	Phenotypes	Community acquired (n/%)	Nosocomial infection (n/%)	P
Penicillin	Resistant	247 (87.59)	531 (85.10)	>0.05
	Sensitive	35 (12.41)	93 (14.90)	
Oxacillin	Resistant	39 (13.78)	98 (15.73)	>0.05
	Sensitive	244 (86.22)	525 (84.27)	
Fusidic acid	Résistant	12 (4.26)	22 (3.53)	>0.05
	Sensitive	270 (95.74)	602 (96.47)	
Amoxiccilin + clavulanic acid	Résistant	43 (15.25)	113 (18.11)	>0.05
	Sensitive	239 (84.75)	511 (81.89)	
Chloramphenicol	Résistant	25 (8.87)	74 (11.90)	>0.05
	Sensitive	257 (91.13)	548 (88.10)	
Ciprofloxacin	Résistant	68 (24.11)	140 (22.44)	>0.05
	Sensitive	214 (75.89)	484 (77.56)	
Erythromycin	Résistant	42 (14.89)	88 (14.13)	>0.05
	Sensitive	240 (85.11)	535 (85.87)	
Clarithromycin	Résistant	26 (9.25)	55 (8.81)	>0.05
	Sensitive	255 (90.75)	569 (91.19)	
Gentamicin	Résistant	16 (5.67)	41 (6.57)	>0.05
	Sensitive	266 (94.33)	583 (93.43)	
Lincomycin	Résistant	21 (7.50)	57 (9.15)	>0.05
	Sensitive	259 (92.50)	566 (90.85)	
Clindamycin	Résistant	18 (6.38)	49 (7.85)	>0.05
	Sensitive	264 (93.62)	575 (92.15)	
Trimethoprim-sulfamethoxazole	Résistant	211 (74.82)	506 (81.09)	0,02
	Sensitive	71 (25.18)	118 (18.91)	
Tetracyclin	Résistant	166 (58.87)	358 (57.37)	>0.05
	Sensitive	116 (41.13)	266 (42.63)	
Spiramycin	Resistant	20 (7.09)	43 (6.89)	>0.05
	Sensitive	262 (92.91)	581 (93.11)	
Vancomycin	Resistant	0 (0.0)	0 (0.0)	-
	Sensitive	282 (100)	624 (100)	

By table 2, 87.59% of community-acquired strains, and 85.10% of nosocomial strains have penicillinases. Resistance rate to trimethoprim-sulfamethoxazole was significantly higher in nosocomial infection than in community-acquired. Nosignificant difference was observed in other antibiotics (Table 2).Generally, fusidic acid remains the most active antibiotic with only 12% resistance to community-acquired infections and 22% resistance to nosocomial infections followed by gentamicin (16%, 41%), clindamycin (18%, 49%), spiramycin (20%, 43%), chlarythromycin (26%, 55%) and chloramphenicol (25%, 74%) (Table 2).

There were no significant differences in the resistance rates to any antibiotic according to the site of infection, the age group or the year of isolation of the strains (data not shown).

At the outcome of the antibiotic sensitivity testing during all these years, the highest resistance rates were recorded with trimethoprim-sulfamethoxazole where it reached a resistance rate of 98.29% followed by penicillin 95.73 % by the year 2013 in the center (Figure 1).

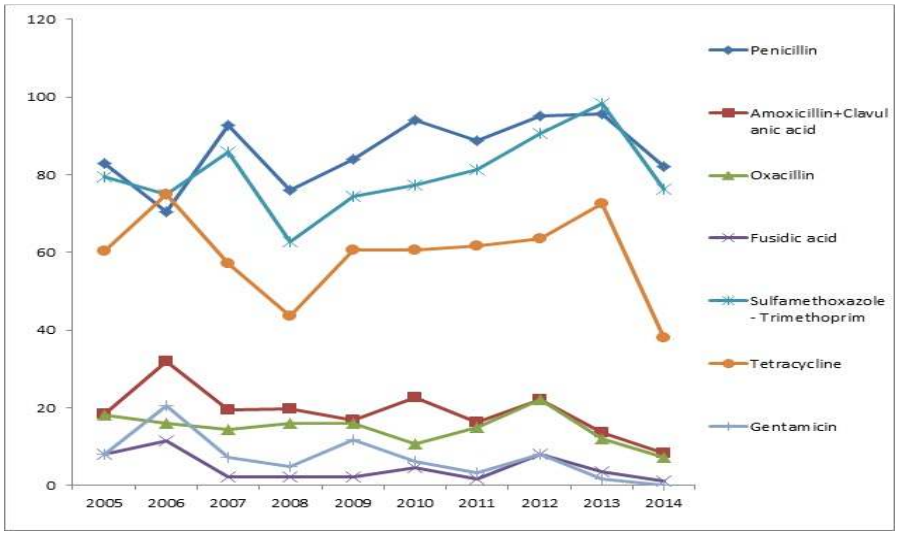

FIGURE 1: CHANGES BY YEAR IN RESISTANCE OF *STAPHYLOCOCCUS AUREUS* TO ANTIBIOTICS

Apart ciprofloxacin and tetracycline that we have noticed an increase in resistance rates in 2012 and 2013, almost all antibiotics tested have a stable rate of resistance. In 2014, we noted a remarkable decline in resistance to all antibiotics tested except for chloramphenicol but this result was not significant (Figure 1 and 2).

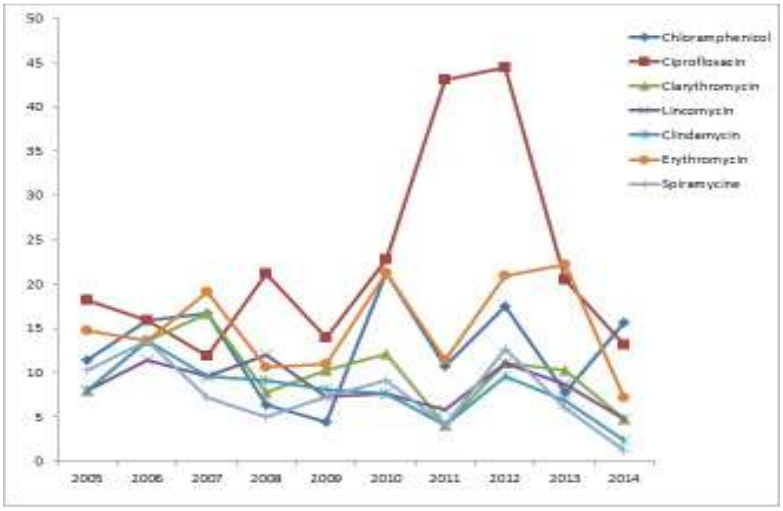

FIGURE 2: CHANGES BY YEAR IN RESISTANCE OF *STAPHYLOCOCCUS AUREUS* TO ANTIBIOTICS

DISCUSSION

We conducted this study to improve the therapeutic approach to infections due to *S. aureus* in Antananarivo. Our results are sometimes incomplete because there are moments when it is a rupture of antibiotic disk stock by lack of supply in our laboratory. So, there is resistance phenotypes that are not listed in our study thus limiting our study. Only 906 results are complete and usable.

Strains were isolated from 401 females and 505 males. The majority of strains isolated from pus of community origin or nosocomial respectively 92.46% and 92.47% followed pleural fluids (5.32%) and blood cultures (6.25%).

MRSA were found in 130 samples of pus. Overall, the prevalence of MRSA is 29.53% which 13.83% (39 of 282 isolates) for community-acquired strains, and 15.70% (98 of 624) for nosocomial infections ($p > 0.05$). Other authors have found MRSA in skin and soft tissue (50.2%) followed by urinary tract (38.2%) collected through a representative sample of French private-sector community-based medical laboratories (Labville network) [5]. The distribution

of MRSA nosocomial infections by infectious site in France in 2006 is dominated by infections of the skin and soft tissues [6], in contrast to other studies, where resistance rates are higher in nosocomial infections [7].

The prevalence of MRSA found in our study is high compared to a study conducted at the Pasteur Institute of Madagascar in 2014 (5.80%)[8]. Although rather low, the rate of resistance to methicillin has increased between 1997–1998 [9]. The very highest rates of methicillin resistance among *S. aureus* isolates have been noted in developed countries and especially in Western Pacific Regions, both in community acquired and nosocomial infections [7]. The prevalence of MRSA has increased worldwide, as it is evident from many surveillance studies [7, 10, 11]. However, there are considerable differences between countries. Most reported MRSA proportions exceed 20% in all WHO regions, and even exceed 80% in some reports [1].
We did not find any significant difference in the rates of resistance to most of antibiotic between strains isolated from nosocomial or community-acquired infections.
Over the past decade, community-acquired MRSA has increased significantly in a number of countries. Fortunately, many of these community-acquired MRSA strains have so far retained susceptibility to a number of non-beta-lactam antimicrobials, whereas most health-care associated MRSA infections are caused by difficult to treat multi resistant strains. For the latter, the treatment of last resort has been glycopeptides such as vancomycin (since the 1950s) and teicoplanin, which can only be given by injection and also needs careful monitoring to avoid adverse side-effects [1].Vancomycin does not yet exist at the pharmacy in Madagascar while its sensitivity is 100% of the tested strains.

Because of their low price and the low rate of resistance, fusidic acid or chloramphenicol in combination with gentamicin may be the more suitable treatment on MRSA strains.
Tetracycline and trimethoprim-sulfamethoxazole do not work that in almost 25% of cases of MRSA.

Resistance rate to trimethoprim-sulfamethoxazole was significantly higher in nosocomial infection than in community-acquired. This rise in resistance has several possible causes, there the free sale of these drugs to Madagascar, self-medication and also their misuse. In general, elevated rates of multidrug resistance may emerge from diverse isolates of *S. aureus* under antimicrobial pressure or as a result of widespread person-to-person transmission of multidrug-resistant isolates [12].

Other authors in Madagascar have found the same rates of resistance (75%) to tetracycline resistance whereas sulfamethoxazole-trimethoprimes is still 38.9% [8]. The resistance rates to cheaper antibiotics such as tetracycline and trimetoprim-sulfamethoxazole are higher than those observed in developed countries and are similar to that observed in African countries [1,7,13].
According to the evolution of antibiotic resistance testing, we noted a decrease in the prevalence of patients infected with MRSA since 2012. The percentage of *S. aureus* isolates reported as MRSA is now stabilizing or decreasing in most European countries, and the European union population-weighted mean MRSA percentage has decreased significantly over the last four years [14].

Sensitivity tests have shown that the problem of antibiotic resistance in *S. aureus* is effective in our center at extremely high rates, averaging 86% for penicillins. Inreality, these results reflect the situation of antibiotic resistance to penicillin in Antananarivo because another study conducted at the Pasteur Institute of Madagascar found a resistance rate to 87.7% [8]. This rate was also brought by other authors[15,16,17,18].
The highest resistance rates during all his years of study have been registered with trimethoprim-sulfamethoxazoles where it reached a 98.29% resistance rate monitoring penicillin 95.73% in the year 2013 in the center.Apart ciprofloxacin and tetracycline that we have noticed an increase in resistance rates in 2012 and 2013, almost all antibiotics tested have a stable rate of resistance. In 2014, we noted a remarkable drop in resistance to all antibiotics tested except for chloramphenicol but this result was not significant. This increase of chloramphenicol resistance may be due to the abuse of this medicine because chloramphenicol is from antibiotics which are still effective against MRSA in recent years in Madagascar. The other tested antibiotics such as spiramycin, clindamycin, lincomycin, and chlarythromycin, quite used to Madagascar were more active with sensitivity rate close to 92% respectively. These antibiotics are available but a fairly expensive cost to the population.

On the national level, real access to drugs began in 2007 as part of national health policy that combines a reinforcement of training of health personnel in quality and quantity with adequate distribution of qualified personnel in all health centers. This measure consists of ensuring at least 85% availability and accessibility of essential medicines, medical consumables, reagents in all health facilities by extending the range of Salama (essential drug supplier) for medicines and consumables hospital. This was accompanied by a rationalization of the prescription and also the rational use of drugs with the objective of improving health system in Madagascar until 2011.The best access to drugs and their rational prescribing in hospitals may explain the decrease in MRSA in our center during 2012. There was also a downward trend in resistance to almost all antibiotics tested but the differences are

not statistically significant. However, private firms, who escape to training and supervision of the Ministry of Health, continued in the irrational prescription of medicines, particularly antibiotics [19].

The decrease in the pharmacological pressure by changes in protocols also probably resulting led to the decline in resistance. Several studies have reported resistance decreases following the suppression or restriction in prescribing certain antibiotics [20].

Conclusion

In summary, methicillin resistance among *S. aureus* isolated at the Laboratory of Microbiology of the HU-JRA Antananarivo are mainly found in pus of community or nosocomial but the rate is still low compared to other countries. Other antibiotics tested showed extremely high rates of resistance and that the problem of antibiotic resistance in *S. aureus* is effective in our center. It is important for Malagasy doctors in case of suspected infection *S.aureus* to do sensitivity tests to antibiotics before prescribing drugs. However a nationwide survey should be undertaken to confirm these results and could be valuable for the selection of therapeutic alternatives and also to control the emergence and dissemination of antimicrobial resistance.

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