ANTIBIO-RESISTANCE STUDY OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED IN ALGERIAN HOSPITALS

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Abstract. During 06 months (January-June 2013), 109 strains of *S. aureus* were isolated from 4270 specimens (pus, blood, puncture, CSF) from patients aged from 1 day to 76 years hospitalized in majority (89%). 58.7% of these strains from pus, and 53 strains (48.7%) were MRSA (multi-resistant). The 56 MSSA, less resistant, expressed in the majority of cases, a separated penicillin resistance. Finally, between these 109 strains of *S. aureus*, none is wild-type. Thus, antibiotic resistance seems still not overcome and the therapeutic arsenal is shrinking.

Keywords : *Staphylococcus aureus*, multi-résistances, MRSA, MSSA

INTRODUCTION

The starting point of this work is to achieve a phenotypic analysis of *S. aureus* strains samples isolated from various pathological products for 6 months, in the microbiology laboratory of the CHU Mustapha Bacha (Algiers). For phenotypic analysis of strains, sensitivity against a range of antibiotics (antibiogram) is used routinely in the laboratory. A comparison of phenotypic *S. aureus* strains resistant (MRSA) and methicillin-sensitive (MSSA) was performed.

The objective of this study is the isolation and the identification of *S. aureus* strains in various pathological products of hospitalized and outpatients; the resistance study of *S. aureus* strains against certain antibiotics and, the resistance study ofstrains phenotypes and the study of bacteriological characteristics of staphylococcal infections through a prospective study.

MATERIAL AND METHODS

During 06 months (January to June, 2013), 4270 Samples from outpatients and hospitalized in different services at CHU Mustapha Bacha(Algiers) were analyzed.

Material. Samples consist of pus, urine, cerebrospinal fluid (CSF), blood, punctures fluidsand care equipmenst. Internal samples from medical services, emergency and surgical units; external specimens done by private doctors, were made by non-hospitalized patients. Each sample is accompanied by a detailed information sheet.

Methods:

- *Macroscopic examination* is done at the reception of specimens and during incubation (10 days).

- *Cyto-bacteriological examination*allows microscopic identification of live bacteria.The cell count is performed directly by counting under a microscope using a Malassez slide. Finally, staining with bleu of methylene, fast and simple, used to assess the bacterial morphology.

- *Bacteriological examination* is performed after cultivation by seeding the surface in Petri dishes (GSF medium, GSC and Chapman).

- The susceptibility testing is performed for each strain isolated by the method of diffusion on Mueller Hinton with antibiotic discs of Bio-Mérieux Laboratory (Penicillin, Oxacillin, Cefoxitin, Gentamycin, Kanamycin, Tobramycin, Tetracyclin, Erythromycin, Clindamycine, Pristinamycin, Ciprofloxacin, Levofloxacin, Vancomycin, Rifampicin, Acid Fusidic and Cotrimoxazol). If the diameter of inhibition is greater than the diameter of the critical concentration, the bacterium is sensitive (S) if less, it is resistant (R) and, if between the two diameters, it is intermediate (I) [23].

- *Detection of inducible phenotype MLSb*: testing Erythromycin, Clindamycin and Pristinamycin allows to know the resistance phenotypes and interpret the results. It couldbe identified by agar diffusion. Indeed, when anErythromycin disc (inducible) is placed next to a Clindamycin disc, Lincomycin, Spiramycin or Josamycin (non-inducible), a flattening of the inhibition zone of the disc containing the inducing antibiotic is not observed compared with the Erythromycin disk shaped region forming a "D". The distance between the Erythromycin and Clindamycin disks usually equal to 24 mm from edge to edge, is generally sufficient to observe interactions.

- *The weekly quality control of susceptibility testing* use the reference strain ATCC 25923. The inhibition diameter of the reference strain is compared with the limits of inhibition diameters described by the standards (CLSI) values (Clinical Laboratory Standards Institute). It must be in perfect agreement with those standards.

RESULTS AND DISCUSSION

Exams concerned the urine (32.2%), blood (21.6%), CSF (12%), pus (10.63%) and care equipment (2.9%). On 4270 samples analyzed, 1093 (25%) allowed bacterial growth (positive). After *E.coli* (34.9%), *Staphylococcuus spp.*are among the most germs isolated (18.9%). Our results are almost similar to those obtained in western Algeria (12%) [7, 8]. *S. aureus* was frequently isolated in samples of superficial pus (58.72%). This was expected because *Staphylococcus spp.*are capable of causing the formation of "collected suppuration". These pyogenic germs are also found in the drain (pus deep) at a remarkable rate of 22.94 %. However, it is not always easy to distinguish between simple colonization of a true infection, given the lack of information collected history. In addition, 143 samples (3%) were contaminated either by the patient during sampling or by the laboratory during the analysis (nosocomial infection). A total of 207 isolates, 109 that represent 54.5% pure, were identified as *S. aureus* (coagulase-free) or 10% of all positive samples and 2.6% of the samples examined (Table 1).

Table 1

Isolation frequency of*S. Aureus*

Pvts	PP	*Staphylococcus spp.*	*S.aureus*	% Vs Pvts	% Vs CP	% Vs *Staphylococcus spp.*
4270	1093	207	109	2.55%	9,97%	54,5%

In addition, 89.9% of the strains are hospital-based and 10.1% of Community origin. *S. aureus* was isolated mainly from pus (58%). The preferred site is skin infections. This frequency is even more significant in relation to the total number of samples of pus

(11% Vs. 23% for aspiration). Pathologies involving *S. aureus* were grouped in standardized conditions. They are highly polymorphic but largely dominated by skin infections (53%), osteoarticular (13.7%) and pneumonia (9.2%). Although the terrible nosocomial meningitis are rare (0.92 %). In the majority of skin and soft tissue infections, *S. aureus*is the pathogen of the first skin infections [17, 18]. High bacteremia (13.76%) are responsible for a high mortality rate. *S. aureus*and *E. coli* share with the first row of germs that cause bacteremia [12]. Osteoarticular infections represent 9.17% and *Staphylococcus spp.*are involved in 50-60% of these infections; responsible for prolonged hospital stay. Pneumonia are also common (7.34 %) and *S. aureus* is often implicated [5]. Urinary tract infections are less frequent (2.75 %) and *S. aureus* is rarely involved [18]. Endocarditis (0.92 %), is a serious infection and *S. aureus* is responsible for half of the cases. The literature confirms the rarity of the location of this germ in the brain [6]. Detection of Oxacillin resistance allowed from 109 isolates, group them into 2 categories: sensitive (MSSA) and resistant to Oxacillin (MRSA) (Table 2).

Table 2

Rate of strains MSSA and MRSA

Strain	Effective	Percentage
MSSA	**56**	51,38 %
MRSA	**53**	48,62 %
Total	**109**	**100 %**

Between 109 strains of *S. aureus* isolated, 53 (48.62%) are resistant to Oxacillin (MRSA) and 56 (51.38%) are sensitive (MSSA). All strains are resistant to at least one antibiotic (absence of wild-type strain). The 40 strains isolated Penicillin-resistance represents the majority phenotype. For MRSA, 35 different phenotypes are resistant to at least one antibiotic. In addition to the family of β-lactam antibiotics, all strains are resistant to Kanamycin (multi-resistant). Resistance rates of *S. aureus*againts different families of antibiotics are highly variable (Table 3); 100% to Penicillin, 54.13% to Kanamycin, 48.62% forOxacillin, 31.2% to Tetracycline, 28.4% to Erythromycin and 25.7% to Clindamycin.

The resistance rate is less than 20% for others. Any resistance was observed for more recent molecules (Rifampicin, Fosfomycin and Teicoplanin). The rate of resistance to Penicillin G is close to that found by several authors due to the production of penicillinase [11, 19]. Genetic resistance holder belongs to a transposon, usually located on a large plasmid. It can also be integrated into the chromosome [17]. The detection of methicillin-resistance using the disc Cefoxitin or Oxacillin showed the same results, in contrast to some studies [14, 21]. The frequency of resistance to Oxacillin (48.62%) is comparable with that found in other studies [15, 22].

This rate is higher than that found in Morocco in 2008 (07.1%). Methicilin remains the treatment of choice in Morocco. The percentages of resistance of MSSAagainst different families of antibiotics are highly variable. MSSA strains were resistant to Penicillin (P) (100%), Gentamycin (GN) (26.8%), Tetracycline (TE) (12.5%), Kanamycin (K) (10.7%), Erythromycin (E) (7.1%) and Fusidic acid (FA) (5.4%). However, no strain is resistant to Vancomycin (VA) to Tobramycin (TOB) and Levofloxacin (LVX).

Table 3

Distribution of *S. aureus*strains according to their sensitivity to ATB

Antibiotics	Resistant strains	(%)	Sensitive strains	(%)
Penicillin (P)	109	100	0	0
Oxacillin(OX)	53	48,62	56	51,38
Kanamycin(K)	59	54,13	50	45,87
Gentamycin(GM)	15	13,76	94	86,24
Erythromycin(E)	31	28,44	78	71,56
Ciprofloxacin (CIP)	13	11,93	96	88,07
Clindamycin (CM)	28	25,69	81	74,31
Pristinamycin (PT)	3	2,75	106	97,25
Vancomycin (VA)	1	0,92	108	99,08
Tétracyclin (TE)	34	31,19	75	68,81
Rifamycin (RIF)	0	0	109	100
Fusidiqueacid (FA)	21	19,27	88	80,73
Tobramycin (TOB)	10	9,17	99	90,83
Sulfamethoxazol +	13	11,93	96	88,07
Triméthoprim (SXT)	/	/	/	/
Levofloxacin (LVX)	11	10,09	98	89,91
Fosfomycin (FOS)	0	0	109	100
Teicoplanin (TEIC)	0	0	109	100

As far as, percentages of MRSA resistance against various families of antibiotics are highly variable (Table 4 and 5).

Table 4 - 5

Distribution of MSSA and MRSA by their resistance to ATB

antibiotics	resistant strains MSSA	P (%)	resistant strains MRSA	P (%)
Pénicilline (P)	56	100	53	100
Oxacilline(OX)	00	0	53	100
Kanamycine(K)	06	10,71	53	100
Gentamycine(GM)	15	26,79	15	28,30
Erythromycine(E)	04	7,14	27	50,94
Ciprofloxacine (CIP)	01	1,79	12	22,64
Clindamycine (CM)	03	5,36	25	47,17
Pristinamycine (PT)	00	/	03	5,66
Vancomycine (VA)	00	/	01	1,89
Tétracycline (TE)	07	12,5	27	50,94
Rifamycine (RIF)	00		00	
Acide fusidique (FA)	03	5,36	18	33,96
Tobramycine (TOB)	00		10	18,87
Sulfaméthoxazole + Triméthoprime (SXT)	00	/	13	24,53
Levofloxacine (LVX)	00	/	11	20,75
Fosfomycine (FOS)	00	/	00	/
Teicoplanine (TEIC)	00	/	00	/

There is a total resistance of all strains (100%) to Penicillin (P), Oxacillin (OX) and Kanamycin (K), followed by a remarkable resistance to Erythromycin (E) and Tetracycline (TE) (51%), resistance rate is the same for Clindamycin (CM) (47%), to Fusidic acid (FA), Gentamicin (GM), Sulfamethoxazol+Trimethoprim and (SXT), the

resistance is respectively 34%, 28% and 24%, a small percentage is recorded for Pristinamycin (PT) (5%) and Vancomycin (VA) (1.9%).

Concerning Aminoglycoside, it is important to detail resistance phenotypes because of their variety and their epidemiological importance.The wild type has a large majority, the K phenotype was found in 11% of strains, the KTG in 13 % and 21% for KT. No MRSA strain has expressed a wild-type againstAminoglycosides. For all strains, wild Aminoglycosides phenotypes represent the majority, followed by K phenotype (38%) and KT (10%) and KTG (6%). For Macrolides (MLS), the wild type is also the majority (89%), followed by the MLSB (c) (5%), the MLS B (i) (4%). For all strains, the phenotype is wild MLS majority (64%), the MLSb is inducible expression (09%) and 21% of cases are constitutive. In Africa, the prevalence of MRSA is changing. It was 36% in Benin, in 2006 [1], before reaching 14.5% in 2008 [1], while in Algeria, this rate is increasing with 4.5% in 2002 [24], 33.2% in 2008 [25] and 45% in 2009 [8]. The results of the study on MRSA prevalence in Tlemcen, giving a rate of 51%, much lower than what was reported in Senegal (72%) [2, 26]. However, it is close to that reported in Egypt in 2007 but remains significantly higher than those reported for the Côte d' Ivoire (25%), Morocco (19.3%) and Tunisia (15.3%) [2, 9, 20].

Indeed, data may change over time and space [9]. The resistance phenotypes of *S. aureus* are highly variable. This is explained by the fact that it is a multi-resistant bacterium to all β -lactams. At the molecular level, the cassette chromosome carrying the *mecA gene* coding for Methicillin-resistance, vehicle copies of plasmids responsible for resistance to other antibiotics.

For 53 MRSA resistance is associated in 100% of cases with resistance to Kanamycin, Gentamicin (28.3%), Erythromycin and Tetracycline (50.94%),Clindamycin (47.17%), Fusidic acid (33.96 %),Cotrimoxazole (24.53%) and Levoflaxine (20.75%). Antibiotic resistance of Aminoglycoside is mainly associated to Methicillin resistance and 11% of MSSA were phenotype A, while all MRSA were resistant to at least one of the 3 antibiotics of this family. Methicillin resistance is associated in 66% of cases with a phenotype K , 21% to KT phenotype and 13% to KTGphenotype. These results are similar to those obtained by the CHU of Constantine where all MRSA are resistant to Kanamycin and 37.5% have a phenotype KTG and 2.5 % with KT phenotype[4]. In our study, the MLSbphenotype is well represented (33strains) and most often constitutif (21%) than inductible (09 %), while in another study [22],MLSbphenotype is often constitutif (17 %) than inductible(14 %).

The incidence of 2 resistance phenotypes MLSb (i) and MLSb (C) in *S. aureus*differs by geographic region. They cannot predict resistance or sensitivity for Meticellin [11]. One strain resistant to Vancomycin, may have a sensitivity of 100 % againstTeicoplanine, thus confirming the role of Glycopeptides as treatment of choice against MRSA, *S. aureus* decreased sensitivity to Glycopeptides poses a topical issue and various studies report the detection of *S. aureus* strains with reduced susceptibility to these antibiotics. These strains have been described in Tunisia. Other strains having a high level of resistance to Glycopeptides have been described more recently. In Algeria, 3Vancomycin-resistant strains were isolated at the University Hospital of Tlemcen in 2012. Emergence of these strains requires increased vigilance. This resistance may be due to the kinetics of bactericidal Glycopeptides is slow, the tolerance phenomena and the low tissue penetration. These necessitate a bactericidal treatment in combination where antibiotics are often abusive [3].

CONCLUSION

Under our work done in CHU Mustapha Bacha, Algiers, during 06 months and 109 strains of *Staphylococcus aureus* from 1093 bacteria isolated from 17 services, divided into 3 categories (medicine, intensive care and surgery), we can conclude that *Staphylococcus spp.*are among the most frequently isolated microorganisms, phenotypic analysis of the samples showed strains of S.aureus Methicillin-sensitive (MSSA) and Methicillin-resistant strains (MRSA) . The study of the resistance phenotype shows that all strains are resistant not only to β -lactams and Kanamycin, but almost all strains are multidrug resistant. Resistance rates of 109 strains of *S. aureus* isolated counter 16 antibiotics and determining the prevalence of Methicillin-R strains show that 48.62% of the analyzed strains are resistant to Methicillin and 100% to Penicillin. The average rate of resistant strains is 54.13% to Kanamycin, 13.7% to Gentamicin, 31.2% to Tetracycline, 28.4% to Erythromycin, 25.7% to Clindamycin, and 11.9% to Trimethoprim-Sulfamethoxazole . For Glycopeptides, only a strain is resistant to Vancomycin. No strain is resistant to Teicoplanin. This confirms that this antibioticrepresentsthe reference antibiotic in the treatment of MRSA infections. This molecule must be preserved and used only in severe MRSA infections. The evolution of resistance in recent years remains high, which should push harder. The basic hygiene measures to prevent the spread of *S. aureus* are simple:

- Good hygiene (washing hands, treating wounds, use your own towel and antiseptic soap, clean toilets and body).
- Rational use of antibiotics (antibiogram, isolate patients with MRSA, must sterilization of surgeryinstruments).

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