Trends in the antibiotic resistance of *S. aureus* clinical isolates: a 4 years retrospective study in a teaching hospital in South Italy

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SUMMARY

Staphylococcus aureus is responsible for life-threatening conditions, while in the meantime it has rapidly acquired resistance to several antibiotic classes. In the context of an effective empirical antibiotic therapy, an accurate evaluation of the resistance rates of *S. aureus* may be critical. The aim of this study was to determine the resistance rates of *S. aureus* in the years 2015-2018 and to assess the impact of specimen stratification on the resistance rates.

We have retrospectively analysed *S. aureus* strains isolated from blood, bronchial aspirate, pus, sputum and urine collected from hospitalized and ambulatory care patients. The comparison between resistance rates from 2015 to 2018 and among different specimens was assessed by Fisher's exact test followed by Benjamini and Hochberg's correction of the *p-values*.

Higher resistance rates were detected for penicillin followed by oxacillin, levofloxacin, erythromycin and clindamycin. Differences in the annual resistance rates were not statistically significant after the BH's correction. The comparison between cumulative *S. aureus* resistance rates stratified by specimens showed some statistically relevant differences among the five specimen types. In particular, *p*-values were statistically significant for clindamycin, erythromycin, gentamicin, levofloxacin, oxacillin, penicillin and vancomycin.

Annual resistance rates of *S. aureus* clinical isolates remained constant over the course of time. Moreover, the stratification of the data by specimen may significantly impact on the evaluation of the resistance rates, at least for some antibiotics. Therefore, if the number of data is high, stratification by specimens may be recommendable to better approach an empirical antibiotic therapy.

Keywords: *Staphylococcus aureus*, cumulative antibiogram, resistance, antibiotic.

INTRODUCTION

Staphylococcus aureus is a Gram-positive, non-motile, coagulase-positive coccoid bacterium normally present in the human nasal mucosa and skin. It may colonize a general population in 20-40% of cases [1]. In particular, about 15% of the general population is permanently colonized [2]. It is known that colonization is an important risk factor for disseminated infections. In fact, the disruption of the cutaneous or mucosal barrier can allow *S. aureus* to enter the underlying tissues or the bloodstream with subsequent infection [3]. Moreover, people with invasive medical devices or immunodeficiency are more vulnerable to *S. aureus* infections [4].

S. aureus infections may range from mild to life-threatening conditions like endocarditis, chronic osteomyelitis, pneumonia, or bacteraemia,

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which are associated with significant morbidity and mortality [5]. Despite the initial antibiotic efficacy such as penicillin and methicillin, acquired resistance to these antibiotics quickly spread. In particular, methicillin-resistant *S. aureus* (MRSA) has become an important health threat in both health care and community settings [3]. In recent years, MRSA was also detected in livestock, companion animals and some other farm animal species [6]. Moreover, in the last 20 years, the emergence of *S. aureus* clinical isolates with reduced susceptibility or complete resistance to vancomycin was observed [5]. These isolates provided a clinical challenge to microbiologists to detect, in particular, vancomycin-intermediate *S. aureus* (VISA) and heterogeneous vancomycin-intermediate *S. aureus* (hVISA) [7]. In Europe, MRSA prevalence increases from the North to the South. In particular, less than 5% of *S. aureus* isolates from invasive infections in northern Europe are MRSA. On the contrary, the prevalence of MRSA is around 25-50% in southern Europe. In particular, MRSA isolates proportion in Italy was 33.9% in 2017, whereas in 2000 it was 44.3% [8].

In this context, an accurate evaluation of the resistance rates of *S. aureus* may be critical in guiding the empirical antibiotic therapy. Nevertheless, the simple aggregation of the hospital and unit-based data may be insufficient to evaluate the epidemiology in all clinical scenarios.

For example, Islam and colleagues reported that a proportion of methicillin-susceptible *S. aureus* was significantly higher in wound specimens than in aggregate data (63% vs. 53%, *p*-value < 0.01) [9].

The aim of this study has been to evaluate the cumulative antibiograms of *S. aureus* clinical isolates stratified by specimen at the Policlinico of Bari University Hospital. We specifically assessed the yearly resistance rates from 2015 to the end of 2018. Moreover, the resistance rates of isolates collected from different specimens have also been compared.

MATERIALS AND METHODS

Bacteria collection and susceptibility testing

1229 samples (339 blood cultures, 321 bronchial aspirates, 225 pus, 241 sputa, 103 urines) from hospitalized (1087) and ambulatory (38) care patients were retrospectively evaluated between January 2015 and the end of December 2018. In particular, the first isolates per specimen per patient were retained for the analysis. Multiple specimen types were obtained from 98 patients. Resistance rates were analysed for the following antibiotics: fusidic acid, clindamycin, daptomycin, erythromycin, gentamicin, levofloxacin, linezolid, oxacillin, penicillin, rifampicin, cotrimoxazole, teicoplanin, tetracycline, tigecycline and vancomycin.

All procedures in this study were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Sample information (date of sampling, ward, type of specimen, testing results) together with the data of patients (*i.e.*, sex) were recorded in an anonymous database by transforming sensitive data into alphanumeric codes. No clinical data associated with these specimens were available.

Biochemical identification and antibiotic susceptibility were performed using the automated VITEK MSTM and VITEK 2 SystemTM (bioMérieux Marcy-l'Étoile, France) according to the manufacturer's instructions. The interpretative breakpoints of the MIC values were based on the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The isolates of *S. aureus* with the intermediate susceptibility values have been classified as resistant.

Statistical analysis

The Fisher test was performed to evaluate the independence of the annual resistance rates of each antibiotic for each specimen. The *p*-values were then corrected for multiple comparisons with the Benjamini -Hochberg's (BH) procedure with False Discovery Rate (FDR) <5% [10].

To evaluate the independence of the resistance rates of each antibiotic among the selected specimens, the Fisher test was performed on a 5x2 matrix, and the *p*-values were corrected by BH's procedure with FDR<5%. Moreover, evaluation of effect size was only performed on the statistically different groups by Cramer's V.

Cramer's V is a measure of association between two nominal random variables, which is also appropriate for tables larger than 2x2. The coefficient ranges between 0 (no relationship) and 1 (perfect relationship). Cramer's V is computed by:

$\sqrt{X^2/[nobs * (min(ncols, nrows) - 1)]}$

$X^2 =$ derived from the Pearson's chi − square test

$nobs$ = number of observations

$ncols$; $nrows$ = number of columns and rows, respectively

Specifically, Cramer's V effect size was considered small (if Cramer's V was <0.3), medium (if Cramer's V was=0.3 or <0.5) or large (if Cramer's V was ≥0.50).
Calculations of all statistical tests were performed by the open source environment R 3.5.1 [11].

RESULTS

In total, 1229 strains of *S. aureus* were isolated from the evaluated specimens collected from 1124 patients (660 males and 464 females, M:F=1.42).
In general, the higher resistance rates to *S. aureus* isolated from all specimens were detected for penicillin followed by oxacillin, levofloxacin, erythromycin and clindamycin (Figures 1-5). On the contrary, the lower resistance rates were detected for vancomycin, tigecycline, teicoplanin, linezolid, fusidic acid and cotrimoxazole. Some vancomycin resistant isolates were detected in bronchial aspirates (1 in 2015 and 1 in 2016), pus (2 in 2018), sputum (2 in 2017) and urine (2 in 2015, 1 in 2016 and 2 in 2017). In general, a slight decrease of the resistance rates was observed in 2018, but no significant *p-values* were detected after the BH's correction. As a result of the comparison of the resistance rates among *S. aureus* isolates collected from the different specimens, it was possible to highlight some differences. Notably, higher resistance rates to clindamycin were detected in isolates collected from sputum (58.30%) and urine (51.55%). Moreover, isolates collected from sputum and urine exhibited higher resistance rates to erythromycin, 65.56% and 53.40%, respectively. Isolates from sputum and pus showed higher resistance to gentamicin (15.45% and 13.76%, respectively) while isolates from urine and pus had a higher resistance to levofloxacin (46.60% and 33.63%, respectively). *S. aureus* isolates resistant to oxacillin were more common in pus and urine (43.12% and 37.18%, respectively) while more resistant isolates to penicillin were detected in pus and sputum (86.76% and 82.76%, respectively). Finally, higher resistance rates for vancomycin were identified in isolates collected from urine (4.90%), pus (0.91%) and sputum (0.84%). All the differences reported were statistically significant after BH's correction (Figure 6). However, despite the significance of the results, the effect size estimations by Cramer's V may be considered small. Specifically, the erythromycin and clindamycin exhibited higher values (0.24 and 0.21, respectively), while the lowest value was detected on gentamicin (0.09). The values of the Cramer's V for each significant group are hereby reported:
– Clindamycin: 0.21
– Erythromycin: 0.24
– Gentamicin: 0.09

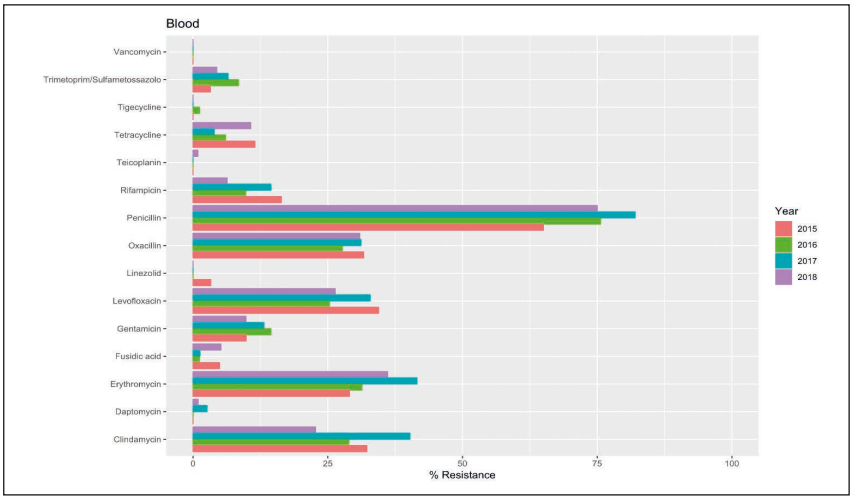

Figure 1 - Evaluation of the yearly resistance rates of *S. aureus* isolates collected from blood from 2015 to 2018. All *p*-values were not statistically significant after BH correction.

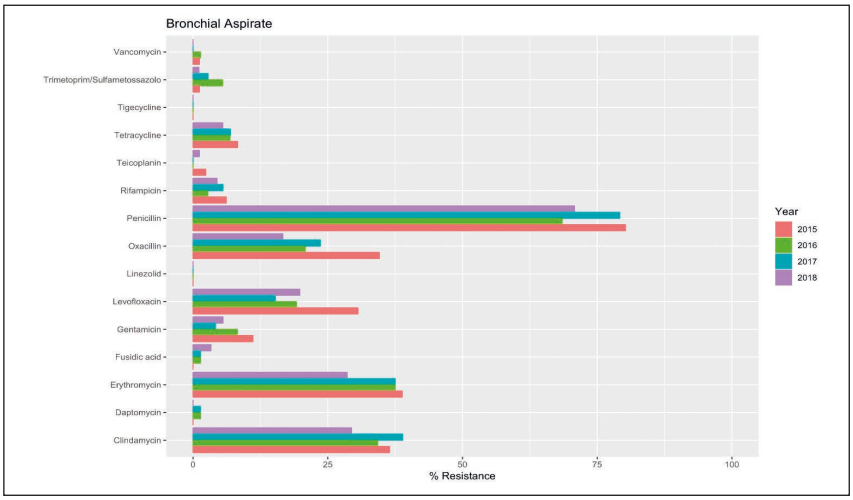

Figure 2 - Evaluation of the yearly resistance rates of *S. aureus* isolates collected from bronchial aspirates from 2015 to 2018. All *p-values* were not statistically significant after BH correction.

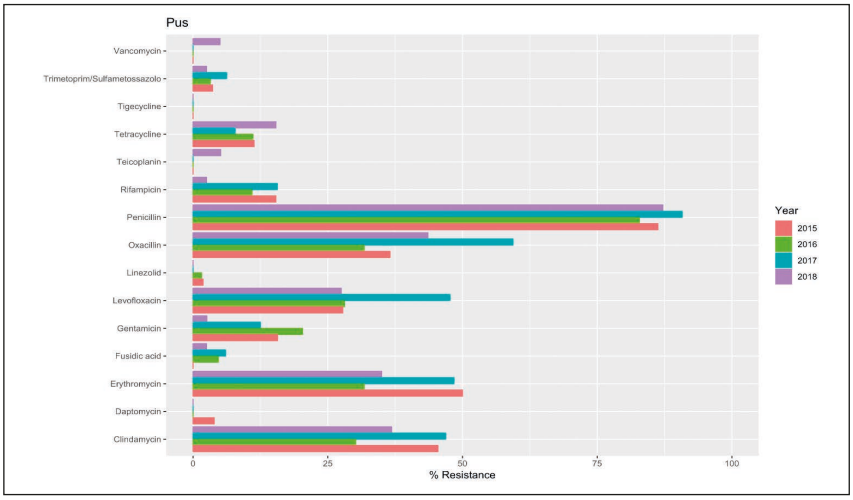

Figure 3 - Evaluation of the yearly resistance rates of *S. aureus* isolates collected from pus from 2015 to 2018. All *p*-values were not statistically significant after BH correction.

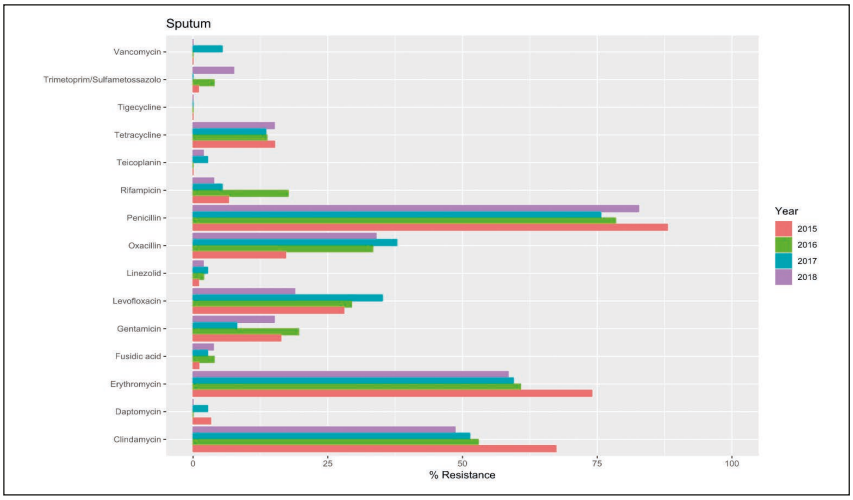

Figure 4 - Evaluation of the yearly resistance rates of *S. aureus* isolates collected from sputum from 2015 to 2018. All *p*-values were not statistically significant after BH correction.

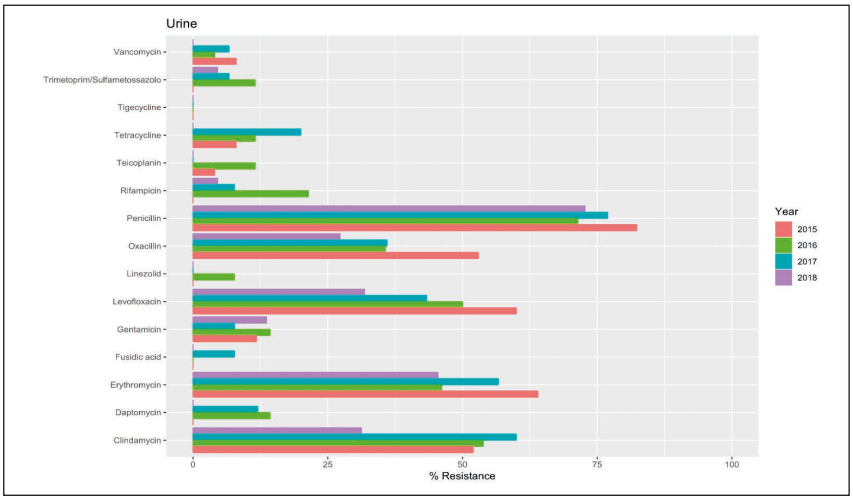

Figure 5 - Evaluation of the yearly resistance rates of *S. aureus* isolates collected from urine from 2015 to 2018. All *p*-values were not statistically significant after BH correction.

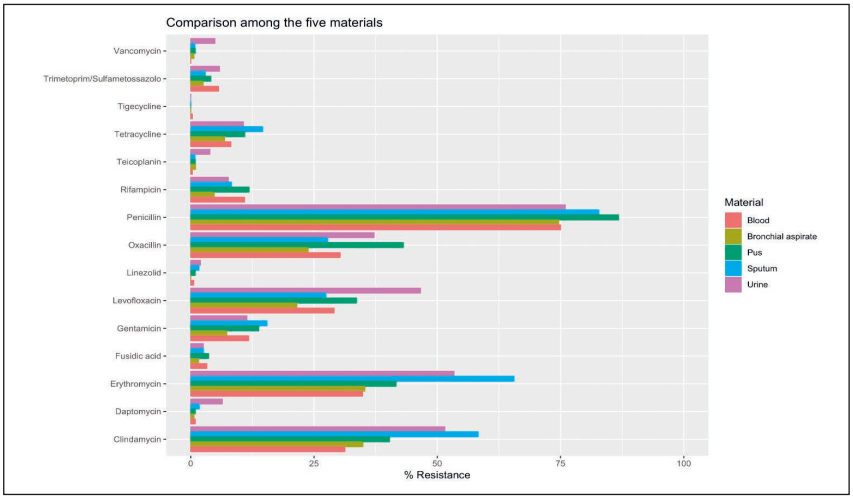

Figure 6 - Comparison of the cumulative *S. aureus* resistance rates stratified by specimens. *P*-values corrected by BH procedure were statistically significant for clindamycin, erythromycin, gentamicin, levofloxacin, oxacillin, penicillin and vancomycin.

– Levofloxacin: 0.15
– Oxacillin: 0.15
– Penicillin: 0.12
– Vancomycin: 0.13

DISCUSSION

Worldwide, the antimicrobial resistance has become a considerable threat and it is responsible for negative clinical outcomes and increased health costs. *S. aureus* isolates accounted for 29% of all bacterial isolates reported in Europe and it is estimated that 72,444 cases of invasive MRSA infections occurred in the United States in 2014. MRSA alone is estimated to cause almost half of all deaths caused by antibiotic-resistant organisms [12-13]. MRSA isolated from blood cultures in Italy decreased from 44.3% in 2000 to 33.9% in 2017 but the decrease was less pronounced in the last years: 33.6% in 2014, 34.1% in 2015 and 33.9% in 2016 [14]. According to these data, the resistance rates of *S. aureus* isolates stratified by materials have not significantly changed from 2015 to 2018. Generally, the higher resistance rates were observed for penicillin, erythromycin, clindamycin followed by oxacillin and levofloxacin. On the contrary, vancomycin, tigecycline, teicoplanin, linezolid and daptomycin showed the lowest resistance rates in all evaluated materials.

Also, Hao-Yuan Lee et al., in a 2015 study, found a

reduced susceptibility to vancomycin, teicoplanin, daptomycin and linezolid [15].

Despite the absence of non-significant differences, it is quite interesting to highlight the behaviour of the oxacillin resistance rates. Generally, they are higher than 25% in all the materials. In particular, oxacillin resistance rates decreased with time in urines and bronchial aspirates, while they remained constant in blood, pus and sputum specimens. On the other hand, the oxacillin resistance rates have significantly ranged according to the clinical specimens. In fact, the higher resistance rates were detected in clinical isolates obtained from pus (43.12%) and urine (37.18%), followed by blood (30.30%) and sputum (27.78%). Isolates obtained from bronchial aspirates exhibited oxacillin resistance less than 25% (23.81%). However, it is necessary to highlight that the overall effect size estimation may be considered small (Cramer's V=0.15).

Resistance to clindamycin and erythromycin was higher for isolates from urine and sputum (>50%, respectively) while the lowest rates were detected in isolates from blood and bronchial aspirates. The behaviour of the levofloxacin resistance was quite different. In fact, higher resistance was observed in urine isolates followed by pus, blood and sputum, respectively, while the lowest resistance rate was detected in isolates from bronchial aspirates (<25%). Penicillin resistance was around 75%, but the higher values were detected in isolates from pus and sputum. The isolates from the same specimens exhibited the highest resistance rates to gentamicin, while higher resistance to vancomycin was detected in urine isolates.

Empiric antimicrobial therapy is guided by several considerations, including the local resistance pattern of the isolated bacteria. For this reason, cumulative antibiograms may help to avoid both ineffective therapies and excessive prescription of broad-spectrum antibiotics. Kohlmann et al. compared resistance rates of blood culture isolates and other isolates. They reported that the resistance rates of *S. aureus* (oxacillin) and *Pseudomonas aeruginosa* (imipenem) were considerably lower in blood culture isolates (23.0% vs 30.9% and 15.1% vs 21.1%, respectively). On the other hand, the cefotaxime differences were less pronounced for *Escherichia coli* and *Klebsiella pneumoniae* (16.8% vs 15.0% and 13.0% vs 17.3%, respectively) [16]. On the contrary, Daxboeck et al. didn't find any differences comparing MRSA rates of clinical isolates from blood cultures to those from respiratory tract samples or wound swabs or urine, whereas Álvarez-Paredes et al. reported lower resistance in *S. aureus* isolates in blood cultures [17, 18]. However, both Kohlmann et al. and Álvarez-Paredes didn't report any effect size in their studies. Notably, the calculated Cramer's V values for oxacillin resistance rates of *S. aureus* and the imipenem resistance rates of *P. aeruginosa* in Kohlmann et al. study were small (0.05 and 0.02, respectively). Interestedly, the comparison of MRSA rates of *S. aureus* clinical isolates from respiratory tract samples, blood cultures/sterile liquids, skin/soft tissues was always statistically significant (calculated Chi squared p value<0.05), but the effect size was small (calculated Cramer's V 0.05, 0.09, 0.06, 0.10, respectively), regardless of the different criteria used to eliminate duplicates.

Our data have highlighted that oxacillin resistance of *S. aureus* isolated from blood culture is intermediate between pus/urine (higher) and sputum/bronchial aspirate (lower). However, the distribution of the resistance rates among the different tested antibiotics showed quite unpredictable deviations. Moreover, the results confirm that the stratification of the data by specimens may significantly impact on the evaluation of the resistance rates of *S. aureus* isolates. However, the small Cramer's V values raise some issues regarding the clinical relevance of such differences. CLSI guidelines recommend including the first isolate from each patient as a criterion to eliminate duplicates but several studies have questioned this statement showing that when the other criteria are applied, there are statistically significant changes in the evaluation of the resistance rates [16-18]. Finally, the data support the hypothesis on the constancy, through time, of resistance rates of *S. aureus* clinical isolates in hospitalized and ambulatory care patients in South Italy. This result is partially in line with a paper by Facciolà et al. which showed a general 7% decrease of MRSA in South Italy from 2015 to 2017. Moreover, they showed that the highest percentage was found in wards belonging to the surgery area and in clinical isolates obtained from wound swabs [19].

MRSA control interventions have become an important issue widely implemented in health-care facilities worldwide. These containing measures

aim to reduce the MRSA spreading by implementation of antimicrobial stewardship, detection of the asymptomatic carriers and contact precautions to prevent MRSA nosocomial transmission. Because of the remarkable persistence of MRSA in the Italian territory and its impact on the healthcare facilities, a strengthening of such measures to reduce the burden of this health care-associated infection is highly desirable.

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Ethical approval
This study was approved by the Ethical Committee of Azienda Ospedaliero-Universitaria "Consorziale Policlinico", Bari (N. 5947), Italy. The study followed the local ethical guidelines of the Azienda Ospedaliero-Universitaria Policlinico of Bari, Italy.

Informed consent
Not necessary

Conflict of interest
The authors declare that they have no conflict of interest to disclose.

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