Methicillin-Resistant \textit{Staphylococcus aureus} (MRSA): A major concern in the Northern Cameroon

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Abstract

\textit{Staphylococcus aureus} remains the most virulent pathogenic staphylococcal species known to human beings. Oxacillin is still the best antibiotic used for treating infections with \textit{S. aureus}. However, cases of resistance are increasingly recorded and cause a serious public health concern. The objective of this study was to evaluate the resistance of \textit{S. aureus} to oxacillin. The study was carried out in the North region of Cameroon on strains isolated at Centre Pasteur in Cameroon. Three hundred (300) strains of \textit{Staphylococcus} were recorded, among them 200 (66.67%) were identified as \textit{Staphylococcus aureus}, mainly coming from six (06) different types of samples. The susceptibility test to oxacillin was carried out using two methods: the diffusion method using discs (oxacillin, cefoxitin) and the determination of Minimum Inhibition Concentration (MIC) by E-test. Overall 84 (42%) resistant strains were detected by both methods. Cervico-vaginal specimen harbored the highest number of \textit{S. aureus} strains (41%), compared to other specimen types. However, pus recorded the highest number of resistant cases (79%), followed by semen culture (50%). Among all resistant strains recorded, 84.5% were tested positive for beta-lactamase production.

Keywords: \textit{Staphylococcus aureus}; Methicillin resistance; Oxacillin resistance; Resistance rate; Beta-lactamase production

1. Introduction

\textit{Staphylococcus aureus} (\textit{S. aureus}) has been recognized as an important public health problem. Penicillin antibiotic families are used for those health conditions. The prevalence rate of Methicillin-resistant \textit{Staphylococcus aureus} in the world is very heterogeneous [1-6]. In World Health Organization (WHO) regions, a report on Methicillin resistance call Oxacillin resistance in \textit{Staphylococcus aureus} documented to be ranged from 20% to above 80% in some regions [7]. In Asian countries such as Shanghai, high prevalence rates are noted. Some of them could reach 64 % [8]. In North America the rates varied according to studies from 36 to 62.6% [9-11]. The same variability is observed in south European countries, where the oxacillin resistance rate ranged from 20 to 50% [11-15]. The result of 9 African countries reported by their National data Institute showed a high prevalence rate, between 12% to 80%, with some countries exceeding 82% [16-17]. In east African countries, for example, a high rate prevalence was recorded in Uganda from 31.5% to 42%, among patients and health workers [18, 19]. Oxacillin resistance rate was 31% to 82% in Rwanda [20,21]. While in Cameroon it was around 34.6% [22]. In Cameroon, Penicillin antibiotic group is still the first choice in the treatment of...
infections related to *Staphylococcus aureus* [22]. Unfortunately, high antibiotic treatment failure cases are increasingly recorded in patients. As observed in many studies on animals and animal products in different areas [23-26]. Previous investigations in North Cameroon region showed: an important antibiotics misuse in human and livestock for *Staphylococci* infections [27] and a high frequency of multiresistant *S. aureus* (53,2%) were reported [28]. Besides, few studies in Cameroon have been conducted to assess the problem of *S. aureus* resistance to penicillin which is the first line prescribe antibiotic when *S. aureus* infection is diagnosed [29]. Our main objective was to assess the resistance of *Staphylococcus aureus* species isolated in this region to oxacillin, which is the antibiotic the most used when multidrug resistance is noted.

2. Material and methods

A prospective study were conducted in the North region of Cameroon at Centre Pasteur in Cameroon (CPC). Our sample was constituted mainly of *staphylococcus* strains from five sources: stools, urines, vaginal discharge, urethral swaps and semen culture.

Samples were subculture into Mannitol salt Agar (Chapman) following the technical standard platform [30,31]. The inoculated plates were incubated at 37°C for 24 h. Primary isolation of bacteria was done based on their colonies' morphology and Gram staining. Then biochemical tests: Catalase, Coagulase, and Slide x staph were used for specy identification.

Antimicrobial drug discs were placed using a disc dispenser into the Muller Hinton agar and incubated at 37°C for 18–24 h. At the end of the incubation period, the diameter area of growth inhibition was measured by using a digital caliper. The growth inhibition zone was interpreted as susceptible or resistant following the guidelines of the CASFM 2020 [31]. Two antibiotics were tested: Oxacillin (disc of 5µg) and Cefoxitin (disc of 30µg) (Bio-Rad, France). Strips E-test oxacillin was carried out for confirmation of observed resistance using disc diffusion technic. For the Cefoxitin disc all diameters less than 25 mm, were assigned resistant and any diameter greater than or equal to 27 mm was referred to as susceptible. For oxacillin disc when the diameter was less than 20 mm the strain was considered resistant and if the diameter was larger or equal to 20 mm, the strain was considered sensitive to Oxacillin.

The beta-lactamase production test was performed on the resistant strains derived from the E-test using a sterile slide on a cefinase disc. One or two colonies were collected from the media and applied on top of the disc and a reaction is observed: a positive reaction is characterized by the appearance of red color on the disc. The reaction is considered negative if no coloration appeared after an hour. This interpretation was done according to the manufacturer's recommendations (Biomerieux, France).

Data were entered into Excel and exported to Epi info 2.0 for analysis. Descriptive statistics like mean, frequency, and percentage were performed on different variables. The variables with P-value < 0.05 were considered statistically significant.

3. Results

Three hundred (300) strains of bacteria described as Gram positive cocci were identified. After running various identification tests (Gram staining, Catalase, Coagulase, and Slide x Staph), 200 out of 300 (66,67%) strains were *Staphylococcus aureus*. In our study, the vaginal collection was the most represented samples (82/200), followed by urine (46/200) (table1). Among 200 strains of *Staphylococcus aureus*, 104 strains (52%) were resistant to oxacillin and 84 (42%) were resistant to cefoxitin (Figure 1). However, the E-test oxacillin was applied on the 200 strains recorded as *Staphylococcus aureus* to determine the Minimum Inhibition Concentration (MIC). Our results showed that 84 strains (42%) displayed a MIC of oxacillin greater than 2 mg/L interpreted as resistant strains. The same value (84 out of 200) was observed for the cefoxitin disc with P < 0.05. We focused on those 84 resistant strains to both oxacillin (E-test) and cefoxitin. *S. aureus* resistance rates were considered in each sample independently one from another. Pus specimen harbored 79% of resistant strains despite it wasn't the most represented one in our study, followed by semen culture (50%) and stool (40%) (P < 0.05) (Figure 2). The ages of the patients varied from 7 months to 67 years with a mean age of 29 years. Women were more represented in this study (58 %, P < 0.05). Each female above 60 years of age harbored a resistant strain (table2). Of the total of 84 resistant strains recorded, 71 (84.5%) produced beta-lactamase (Figure 3).
### Table 1 Antimicrobial (oxacillin and cefoxitin) susceptibility according to specimen types and, patient’s age and gender

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Variables</th>
<th>Resistance</th>
<th>Sensitivity</th>
<th>Records</th>
<th>P-value</th>
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<tr>
<td></td>
<td>N %</td>
<td>N %</td>
<td>N %</td>
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<tr>
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<td>84 42</td>
<td>116 58</td>
<td>200 100</td>
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<td>09 60</td>
<td>15 7</td>
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<tr>
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<td>30 65</td>
<td>46 23</td>
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<td>18 67</td>
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<td></td>
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<tr>
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<td>05 21</td>
<td>24 12</td>
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<td>03 50</td>
<td>06 3</td>
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<td>166 200</td>
<td>100</td>
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<td>27 14</td>
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<td>52 63</td>
<td>83 41</td>
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<tr>
<td>Records</td>
<td>84 116</td>
<td>166 200</td>
<td>100</td>
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</table>

**Figure 1** S. aureus sensitivity and resistance rates to both oxacillin and cefoxitin.

For cefoxitin disk (30µg, Bio-Rad France), diameters < 25 mm, S. aureus are assigned resistant and the strain with diameters ≥ 27 mm are susceptible. For oxacillin disc (5µg, Bio-Rad France), when diameters < 20 mm, we have resistant strain and when it is ≥ 20 mm, the stain is considered susceptible. About Strips E-test oxacillin (Biomerieux, France), when a MIC > 2 mg/L, S. aureus is resistant, but if the MIC ≤ 2 mg/L the strain is sensitive to oxacillin.
Figure 2 Sensitivity and resistance rates of *S. aureus* to both oxacillin and cefoxitin by sample types. Procedure in figure 1.

Figure 3 Beta-lactamase production of oxacillin resistant strains. The Beta-lactamase production was performed on the resistant strains derived from the E-test using a cefinase disc (Biomerieux, France). A positive reaction is characterized by the appearance of a red color on the disc. No coloration after an hour suggest a negative result.

4. Discussion

Despite the extent of damage caused by this *Staphylococcus aureus* bacteria, little data about antimicrobial resistance topics are available in North Cameroon [32]. Our research showed 42% methicillin-resistant *S. aureus* strains. This result is not too far from those obtained by Gonsu et al in 2013 (34.6%) in Yaounde-Cameroon[22]. However, Omuse et al in Kenya and Wangai et al in East Africa have obtained more prevalence respectively 53.4% and 58.2% [6, 33], when others showed 90.2% MRSA [34]. These studies were conducted in hospital settings, which strengthens the evidence that *S. aureus* is the first cocci Gram-positive implicated in nosocomial infections [35]. Moreover, in our study, pus recorded the highest cases of resistance, similar to Dilnessa’s findings in Ethiopia (2016) [35] and Wangai in Kenya (2019) [6]. We suggest that the observed resistance of *Staphylococcus aureus* might be due to the selection pressure resulting from years of exposure to antibiotics used to kill germs in routine hospital sanitization which was responsible of high rate of multiresistant *S. aureus* observed in this same area (53.2%)[28]. In our questionnaire, participant
information about antibiotic consumption right before sample collection was noted. And it appears that some of the patients took antibiotics before coming to consult their doctors. This may also explain the high rate of resistance in our study. Other factors explaining this phenomenon might be auto medication and abusive antibiotic prescription by medical practitioners in case of staphylococci infection suspicion, without prior antibiotic susceptibility test. High use of antibiotics in livestock for staphylococci infection may also be pointed out as one of the reason why the resistance rate of *S. aureus* is so elevated in north Cameroon [27]. In Cameroon, Northern regions is breeding area by excellence. In this region, farmers give antibiotics to cattle for either prevention of infectious diseases or treatment of some animal elements [27]. Beta-lactamines are mostly uses in unexpected quantity dues to veterinary recommendation. This can lead to bacterial resistance to antimicrobial and can also explain the high frequency of MRSA in North Cameroon. Besides, immunity weakness in the elderly people was cited by Tebelay [35] as the factor that favors resistant strains’ emergence among this population. In our study, there was a high rate of resistance in the elderly over 60 years probably due to immunity weakness at first, then Secondly antibiotic exposure for many decades supported by antimicrobial overuse by the majority of patients older than 60 years. The production of beta-lactamase is still one of the well-characterized resistance mechanisms in *S. aureus*. It is the most recurrent mechanism as mentioned by several authors [36-38]. In *S. aureus*, the genetic basis of oxacillin used in the replacement of methicillin since 1960 is associated with the carriage of a mobile cassette of genes known as the staphylococcal cassette chromosome mec (SCCmec) [39]. In this cassette, the mecA gene is the one responsible for resistance to β-lactams. The product of mecA is the peptidoglycan synthesis enzyme, penicillin-binding protein (PBP2a) involved in cross-linking of peptidoglycan in the bacterial cell wall [40-41]. Moreover, many studies have shown that the presence of beta-lactamase highly sustained the methicillin resistance transduction [32, 42]. Various clinical tests are used for the detection of beta-lactamase production, the cefinase disc method which we used, has the advantage that the test gives the rapid result with optimal prediction [43]. In our study 84.5% of *S. aureus* strains produced beta-lactamase. It is known that the production of beta-lactamase is a major resistance mechanism that *Staphylococcus aureus* uses to prevent the action of oxacillin [44]. These results allow us to state that most of our patients were already in contact with antibiotics, which confer this resistance gene that stimulates the production of beta-lactamase.

5. Conclusion

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified as one of the major risk pathogens associated with the development of antimicrobial resistance (AMR). The emergence of resistant strains has important social and economic impacts worldwide. Studies conducted in many countries in Africa have shown a high prevalence of Methicillin Resistance *Staphylococcus aureus* (MRSA), Cameroon is involved. This study showed that 42% of *Staphylococcus aureus* isolated were resistant to oxacillin in the northern regions of Cameroon. Pus specimen recorded the highest number of resistant strains (79%) followed by semen culture (50%). Cervico-vaginal specimens registered the greatest number of infections with 82 (41%) confirmed cases compared to other samples. Among resistant strains, 84.5% (71 strains) produced beta-lactamase. We all should be aware of the danger MRSA occurrence and spreading might be for our health. In the long run we might not be able to handle medically *S. aureus* associated health issues due to restricted number of antibiotic efficient against MRSA. At individual scale we should sensitized our surrounding on the danger of abusive antibiotic consumptions. To prevent the emergence and spread of multiresistant bacteria the authorities in charge of Public Health must regulate the importation, sale and usage of antibiotics in Cameroon both in human and veterinary medicines. We also highly recommend to these authorities to implement in the hospitals a committee to fight against antimicrobial resistance and also to provide to hospitals suitable technical equipments. For medical staff, we suggest prescribing a test with an antibiogram before any antibiotic prescription.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.
References


