Antimicrobial susceptibility testing: Evaluation of the conformity of 3 medical bacteriology laboratories of Togo according to EUCAST/CA-SFM guidelines.

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ABSTRACT

Objective: Faced with the emergence of antibiotic resistance, the quest for reliable susceptibility test results is becoming a necessity in medical bacteriology laboratories. The aim of this study was to evaluate the conformity of the antimicrobial susceptibility testing of three (03) medical bacteriology laboratories in Togo.

Methodology and results: The conformity of the antimicrobial susceptibility testing was evaluated according to the EUCAST/CA-SFM V1.0 March 2017 guidelines. In addition, the turbidity of prepared inocula was assessed using 0.5 McFarland standard. Compliance rates recorded ranged from 27.78% to 41.05% with an average of 32.61%. At the pre-analytical phase, average compliance was low (16.67%). However, it was higher in the analytical phase (72.84%). As for the compliance rates for the quality control performance, it was very low (8.33%), ranging from 0% to 25%. The concentration of 30 inocula prepared in 2 laboratories were high compared to the threshold recommended by EUCAST (0.5 Mc Farland), 0.83 Mc Farland and 0.86 Mc Farland respectively.

Conclusion and application of results: The data generally showed a low compliance rate with the requirements of the EUCAST/CA-SFM and particularly high inocula concentrations. This may have a negative impact on the sensitivity profile of bacteria. Great efforts must be made by the

laboratories, notably in terms of equipment, staff training on the reference system and technological and documentary monitoring, in order to increase the quality level of these laboratories.

Keywords: Antimicrobial Susceptibility Testing, Conformity, EUCAST/CA-SFM, Togo.

INTRODUCTION

The discovery of antibiotics has been at the origin of a real demographic and social advance, by drastically reducing mortality linked to infectious diseases (Elhani 2011) . However, the emergence and rapid spread of the phenomenon resistance has darkened this brilliant picture (Canton and Morosini 2011). Indeed, the re-emerging diseases, which were thought to be under control and eradicated (plague and cholera) whose incidence in humans has increased over the past two decades, are due, among other things, to the acquisition of resistance to anti-infectious drugs (antibiotics, antivirals, anti-parasites, anti-fungal drugs) (Aubry and Gauzere 2018). The selection pressure exerted by the extensive use of antibiotics and the epidemic spread of resistant strains are the two main factors conditioning the spread of resistant strains (Bourdon 2011, Ebongue, Dongmo Tsiazok et al. 2015). According to the World Health Organisation (WHO): "Antibiotic resistance is one of the most serious threats to global health, food security and development today"; it leads to an increase in medical expenditure, longer medical stays and higher mortality (OMS 2018). WHO has established antimicrobial resistance surveillance programs, in which the laboratory plays a strategic role, in order to monitor the antibiotic resistance of bacteria using reliable susceptibility testing methods and yielding comparable results (OMS 2016). However, laboratory manipulations that are carried out may not give identical results, as there is an uncertainty due to the random nature of the variations observed when a measurement is repeated, particularly in bacteriology where one of the actors is a living microorganism (SFM 2014). Faced with this problem, laboratories are encouraged to obtain known standards such as the Clinical Laboratory Standard Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing of the Comité de l'Antibiogramme de la Société Française de Microbiologie (EUCAST / CA-SFM). These standards ensure that the procedures used follow recommended practices and that the results are reliable; erroneous antimicrobial susceptibility testing results can lead to the prescription of inappropriate antibiotics and induce situations of bacterial resistance to antibiotic therapy. In the absence of a national frame for antimicrobial susceptibility testing, this study was initiated in order to evaluate the conformity of the antimicrobial susceptibility testing according to EUCAST / CA-SFM recommendations in the medical bacteriology laboratories of the hôpital du district II (HD II) of Kpalimé, the Centre Hospitalier Régional (CHR) of Atakpamé and the Institut National d'Hygiène (INH) of Lomé. The bacteriology laboratory of the INH is a central level and reference laboratory for diseases with epidemic potential and is a national reference for medical biology analyses. The laboratories of HD II Kpalimé and CHR Atakpamé are intermediate level laboratories and constitute reference laboratories at the regional level. The aim of this study was to evaluate of the conformity of the antimicrobial susceptibility testing performance of three medical bacteriology laboratories of Togo according to EUCAST/CA-SFM guidelines.

MATERIALS AND METHODS

Framework of the study: This study was conducted from October to November 2017, in 3 medical bacteriology analysis laboratories in Togo. For confidentiality of laboratory data, they are coded laboratories A, B and C. The choice of laboratories was guided by the architecture of the Togolese health system. Indeed, it is a pyramidal system with three levels of care to which correspond equivalent administrative levels: the peripheral level, the intermediate level and the central level. Each level is represented by a laboratory in this study. It was an audit conducted according to the different phases recommended by the ISO 19011:2011 standard, which specifies the guidelines for the audit of management systems. A checklist was developed and the reference guidelines used for interpretation of data were EUCAST V.1.0 March 2017 and the ISO 15189:2012 standard. The evaluation concerned the antimicrobial susceptibility testing only. Preanalytical and analytical phases were evaluated and the compliance rates calculated.

Evaluation of the conformity of the preanalytical phase according to the EUCAST / CA-SFM guideline

Agar preparation: Since the preparation of the agar must be done according to the manufacturer's instructions, at this stage, the laboratories were checked whether they comply with recommendation 1.1.1.4 which requires that the Mueller-Hinton agar for antibiotic susceptibility testing be spread in agar plate to a thickness of 4±0.5mm.

Agar storage: The 3 laboratories were questioned on the short and long-term storage temperature of the agar plates, the storage environment, especially the packaging. Verification of temperature records was performed.

Evaluation of the conformity of the analytical phase according to the EUCAST / CA-SFM repository

Inoculum preparation: The conformity of the laboratories to the recommendations concerning the preparation of the inoculum was evaluated: the use or not of the 0.5 McFarland standards and of a spectrophotometer, the solution used to prepare the inoculum.

Agar plating/inoculation: The parameters evaluated were the incubation time of the inoculum and the method of inoculation (plating) of the agar.

Application of antibiotic-impregnated discs: The technique of disc application, the number of discs used per petri dish and the loads of antibiotic discs according to the bacteria were studied.

Incubation conditions of the petri dishes: The incubation temperature according to the bacteria, the incubation atmosphere (presence of O_2 or CO_2) and the incubation time according to the bacteria was evaluated.

Read out of petri dishes after incubation: The reading technique (naked eye, magnifying glass, transmitted light or automatic) was evaluated according to EUCAST recommendations.

Measurement of inhibition zones and clinical categorization: We have evaluated in these 3 laboratories the technique of measuring the diameters of the zones of inhibition and their interpretation (by correspondence of the zones of inhibition and the critical concentrations).

Performing quality controls: The parameters that are part of the quality control of the antibiogram: quality control of the culture medium (pH, thickness and fertility control of the agar), control of the global performance of the test (verification of the diameters of the inhibition zones within the limits of the controls) were evaluated.

Evaluation of inocula preparation in bacteriology laboratories: This section concerned laboratories that did not use a densitometer or a 0.5 Mc Farland standard. Thus, the inocula of 2 of the 3 selected laboratories were examined. The turbidity of 30 inocula intended for the antimicrobial

susceptibility testing was measured. The densitometer (DENSICHECK from BIOMERIEUX-France) was used for this measurement. To ensure accuracy of the values delivered by the device, we measured at the beginning and at the end the turbidity of a 0.5 Mc Farland standard with the said densitometer.

Calculation of the compliance rate: The compliance rate (CR) of each phase was calculated according to the formula:

$$CR = \frac{\text{Total number of affirmative responses}}{\text{Number of recommendations concerned}} \text{ X } 100$$

Statistical analysis: The evaluation questionnaires (checklist) were developed on Epi-info 3.5.1 software, while the data analysis was done on R Studio 3.3.2 software. The statistical significance level was set at p-value <0.05.

RESULTS

Evaluation of the compliance rate in the pre-analytical phase: In the pre-analytical phase, the requirements for the preparation of the culture media (procedure for the preparation of Mueller-Hinton agar supplemented with 5% horse blood and 20 mg/L of β-Nicotinamide adenine dinucleotide (β-NAD), the measurement of the thickness of the prepared agars) and the storage of the prepared agars (verification of storage temperatures and storage conditions) were evaluated. At the end of this evaluation, the average compliance rates for Laboratories A, B and C were low (16.67%) in the pre-analytical phase. The results were identical for all 3 laboratories. Agar preparation requirements were not complied with at all in the 3 surveyed laboratories (0%), while 25% of the agar storage requirements were complied with. The data are reported in Table 1.

Table 1: Pre-analytical phase compliance rates.

Recommendations / Requirements	Compliance rate		
	Lab A	Lab B	Lab C
Agar preparation	0% (0/2)	0% (0/2)	0% (0/2)
Agar storage	25% (1/4)	25% (1/4)	25% (1/4)
Rate of compliance according to the pre-analytical phase recommendations	16,67% (1/6)	16,67% (1/6)	16,67% (1/6)

Assessment of the compliance rate in the analytical phase: Evaluation of the compliance rate in the analytical phase revealed average rates of 81.48%, 70.37% and 66.67% for laboratories C, A and B respectively (Table 2).

Table 2: Compliance rate for the analytical phase.

Recommendations / Requirements	Compliance rate		
	Lab A	Lab B	Lab C
Preparation of the inoculum	60% (3/5)	40% (2/5)	66,67% (4/5)
Agar inoculation	50% (2/4)	50% (2/4)	75% (3/4)
Disposal antibiotic impregnated discs	83,33% (5/6)	83,33% (5/6)	83,33% (5/6)
Agar incubation	80% (4/5)	80% (4/5)	80% (4/5)

Reading of plates after incubation	33,33% (1/3)	33,33% (1/3)	66,67% (2/3)
Measurement of inhibition zone diameters and clinical categorization	100% (4/4)	100% (4/4)	100% (4/4)
Compliance rate according to recommendations of the analytical phase	70, 37% (19/27)	66,67% (18/27)	81,48% (22/27)

Laboratory C showed the highest compliance rates for inoculum preparation (66.67%), agar inoculation (75%) and plate reading after incubation (66.67%). Laboratories A and B did not assess the concentration of the inoculum prior to agar inoculation. On the other hand, compliance rates were equal for all 3 laboratories at the stages of antibiotics impregnated discs depositing (83.33%), agar incubation (80%) and measurement of inhibition zone diameters and clinical categorization (100%).

Compliance rate of the performance of the quality control of the antimicrobial susceptibility testing: For quality control of the antimicrobial susceptibility testing, laboratories A and B each achieved an average compliance rate of 0%, while laboratory C achieved a rate of 25% (Table 3).

Table 3: Compliance rate for performing quality control of the Antimicrobial Susceptibility Testing.

Requirements /Recommendations	Compliance rate		
	Lab A	**Lab B**	**Lab C**
Quality control of culture medium preparation	0% (0/2)	0% (0/2)	0% (0/2)
Control of the fertility of the culture medium	0% (0/1)	0% (0/1)	100%(1/1)
Assessment of the overall performance of the test	0% (0/1)	0% (0/1)	0% (0/1)
Rate of compliance according to EUCAST quality control recommendations	0% (0/4)	0% (0/4)	25% (1/4)

All 3 laboratories did not comply with the requirements for quality control of culture media preparation (0%) and assessment of overall performance of the test (0%), but only laboratory C applied the requirements for control of the culture media fertility (100%).
**Rate of laboratories compliance according to EUCAST recommendations:** In total, laboratory C had the highest average compliance rate (41.05%) compared to laboratories A (29.01%) and B (27.78%) in relation to the EUCAST criteria. The assessment of the average compliance rates of laboratories A, B and C, all together, compared to EUCAST recommendations revealed in the pre-analytical phase 16.67%, in the analytical phase 72.84% and in the internal quality control 8.33% (Figure 1).
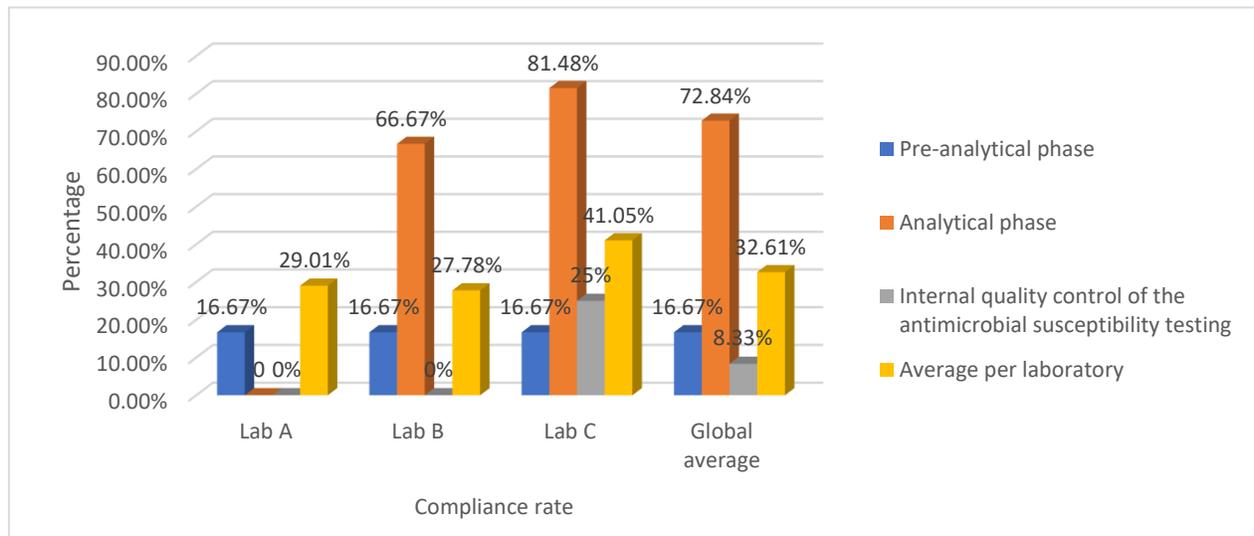
Figure 1: Overall compliance rates according to EUCAST recommendations. Bars indicate the estimated percentage (%) of total compliance versus total number of requirements for each section: pre-analytical phase, analytical phase and internal quality control and then the overall average for each laboratory

Turbidity of inocula: The measurements of the inocula concentration from laboratories A and B revealed an average of 0.83 ± 0.31 Mc Farland with extremes ranging from 0.37 to 1.59 Mc Farland for A and 0.86 ± 0.29 Mc Farland with extremes ranging from 0.46 to 1.87 Mc Farland for B (Figure 2) was observed over the 30 inoculates. The difference in concentration between the inocula from laboratories A and B was significantly greater than that recommended (0.5 Mc Farland, p $_{Labo\ A}$-value = $1.98*10^{-6}$ and p $_{Labo\ B}$-value = $2.69*10^{-7}$).
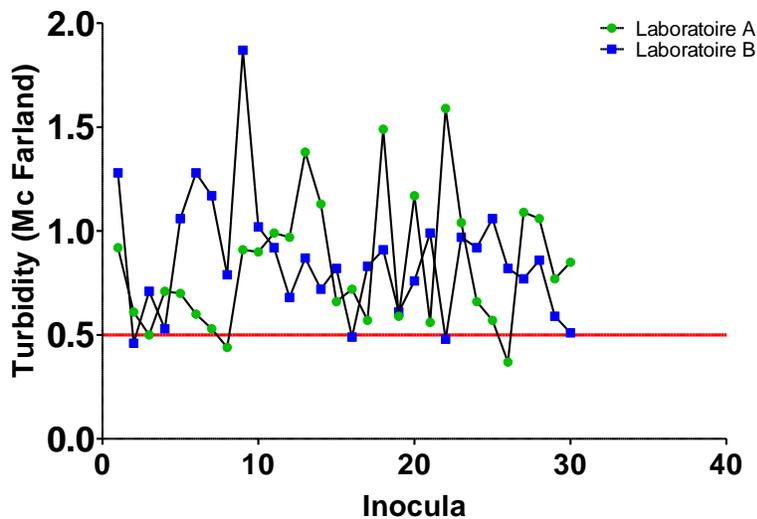
Figure 2: Turbidity of inocula measured in laboratories A and B. The plots show the concentrations (Mc Farland) of 30 inocula measured in laboratories A and B. The red horizontal line shows the concentration (Mc Farland 0.5) recommended for inocula by EUCAST/CA-SFM.

DISCUSSION

The objective of this study was to evaluate the conformity of the performance of the antimicrobial susceptibility testing according to EUCAST/CA-SFM recommendations in 3 medical bacteriology laboratories in Togo. The EUCAST is a reference document whose access is free and which sets the rules for the realization of the antimicrobial susceptibility testing (Kahlmeter, Brown et al. 2006, Leclercq, Canton et al. 2013, Brown, Wootton et al. 2016). Several studies have been carried out using EUCAST recommendations to compare the performance of practices and new methods or techniques (Leclercq, Canton et al. 2013, Skov, Matuschek et al. 2015)**.** Investigations carried out in this study revealed shortcomings in the 3 laboratories. Those shortcomings confirm the interest of using standard operating procedures for performing the antimicrobial susceptibility testing. The absence of an operating procedure does not guarantee the harmonization of the operators' practices. A low compliance rate in the pre-analytical phase was obtained in the 3 laboratories (16.67%). These results indicate insufficiencies of the technical platform of these laboratories. We noted the absence of an automatic media culture dispenser, Mac Farland comparators, pH meter and the lack of an adequate storage packaging for agar plates prepared according to the shelf life or even a refrigerator with adjustable temperature (EUCAST 2017). The very low rate of compliance obtained in the quality control of the antimicrobial susceptibility testing (8.33%) probably reflects not only the lack of material resources, in this case the lack of reference strains, of freezers at -70 °C, but also the lack of technological and documentary monitoring in the laboratories. The evaluation of the analytical phase, which yielded relatively high compliance rates, is the positive point that has been noted during our study; an average compliance rate of 72.84% was obtained in the analytical phase. This is the result of the standardization of the procedures for performing the antimicrobial susceptibility testing in the laboratories due to the availability in the laboratories of the manual for the standardization of the antimicrobial susceptibility testing in Togo by the Ministry of Health and Social Protection through the laboratories division. Since 2015, the laboratories division in Togo through the RESAOLAB+ project has undertaken the training of laboratory staff on antimicrobial susceptibility testing module. This study data are comparable to those obtained by some authors. At the pre-analytical phase, Plebani *et al.,* in 1997 had found errors in the order of 68.2% or a compliance rate of 32.8% (Plebani and Carraro 1997). Carraro et al. obtained in 2007, a 61.9% difference, or a compliance rate of 38.1% (Carraro and Plebani 2007). Katawa *et al.,* obtained 31.1% overall compliance rate in the pre-analytical phase (Katawa, Kpotsra et al. 2011). El Moussaoui et al. found in 2012 that 60 to 85% of laboratory errors are produced during the pre-analytical phase, i.e. a compliance rate ranging from 15 to 40% during this phase (El Moussaoui 2014). For example, a poor conservation of culture media or antibiotic discs will influence the results of the antimicrobial susceptibility testing. In the analytical phase, Plebani *et al.,* in 1997 found errors in the order of 13.3% or a compliance rate of 87.7% (Plebani and Carraro 1997). Carraro et al. obtained in 2007, a 15% difference or a compliance rate of 85% (Carraro and Plebani 2007). El Moussaoui et al. found in 2012 that 4% of errors in the laboratory were produced in the analytical phase or a compliance rate of 96% during this phase (El Moussaoui 2014). However, this study data differ from those obtained by Katawa et al. who obtained a 27.1% compliance rate during the analytical phase (Katawa, Kpotsra et al. 2011). This difference can be explained by the fact that these authors used the requirements of ISO 15189, which is much more organizational. The average concentration of 30 inocula performed by

laboratories A and B are greater than 0.5 McFarland. The concentrations of the microbial suspensions used for performing the antimicrobial susceptibility testing affect the quality of the results. Indeed, when the inoculum is less concentrated, the inhibition diameters are extended and relatively resistant strains can be considered sensitive.

Conversely, a suspension that is too concentrated gives low inhibition diameters. In this case, laboratories A and B may declare resistant susceptible strains. Our results confirm the importance of using a densitometer or 0.5 Mc Farland standard to assess the concentrations of the prepared inocula.

CONCLUSION AND APPLICATION OF RESULTS

In general, the compliance rates of the three laboratories studied are far from satisfactory. The lowest rates are obtained at the quality control level of the antimicrobial susceptibility testing, while the highest rates are obtained in the analytical phase. These observed deficiencies may negatively influence the quality of the antimicrobial susceptibility testing results. Great efforts must be made by the laboratories, notably in terms of equipment, staff training on the reference system and technological and documentary monitoring, in order to increase the quality level of these laboratories

Conflicts of interest
The authors do not declare any conflict of interest.

What is known about this subject?
• The emergence of multi-drug resistant strains is a real public health problem.
• The EUCAST/CA-SFM standard defines the recommendations for the performance of the antimicrobial susceptibility testing.
• The influence of the inoculum concentration on the quality of the antimicrobial susceptibility testing.

What is new in this study?
• First evaluation of antimicrobial susceptibility testing practices according to EUCAST recommendations in Togo.
• The inocula used in bacteriology laboratories that do not have a Mac Farland standard or a densitometer have an average concentration greater than 0.5 Mc Farland.
• Absence of reference strains in laboratories for internal quality control of the antimicrobial susceptibility testing.

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