

Introduction

Moraxella catarrhalis is a gram-negative diplococcus and normal flora of the upper respiratory tract with other bacteria such as the pneumococcus and *Haemophilus influenzae*. It commonly causes acute otitis media in children and serious lower respiratory tract infections in elderly and immunocompromised patients (Verduin *et al.*, 2002).

Agar disk diffusion method for antibiotic susceptibility testing (AST) is currently used as the official method of AST in clinical laboratories due to its simple and well-standardized procedure (Jorgensen and Ferraro, 2009). In 2019, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) introduced a new term called the Area of Technical Uncertainty (ATU) in AST (EUCAST, 2019).

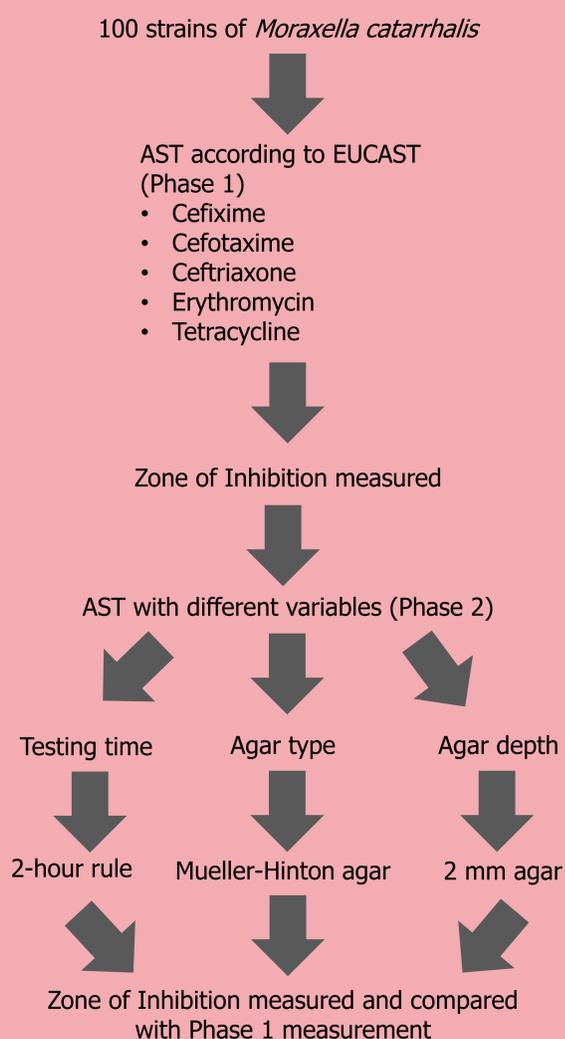
Aim & Objectives

- To determine whether the uncertainty of measurement affects the interpretation of an antimicrobial susceptibility testing measurement of *Moraxella catarrhalis*.

Objectives

- To determine the area of uncertainty of clinical isolates of *Moraxella catarrhalis* against cefixime, cefotaxime, ceftriaxone, erythromycin and tetracycline.
- To assess the EUCAST 15-minute rule of AST by increasing it to 2 hours for each time interval.
- To assess the measurement of antibiotic susceptibility testing of *Moraxella catarrhalis* using different agar types.
- To examine the effects of variation of agar depth on the measurement of antibiotic susceptibility testing of *Moraxella catarrhalis*.

Methodology



Results

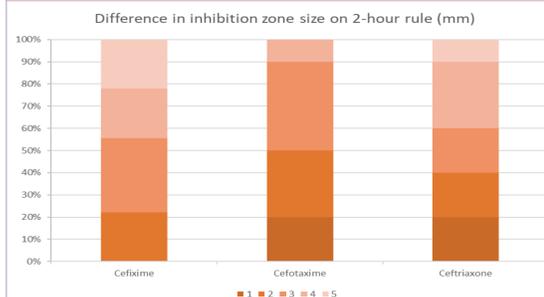


Figure 1: Difference in inhibition zone size when performing AST against three antibiotics (cefixime, cefotaxime, ceftriaxone) using a 2-hour rule. The different colour of boxes represent the difference of inhibition size when compared to the reference. The different size of the boxes represent the amount of strain that produced the same inhibition zone size difference.

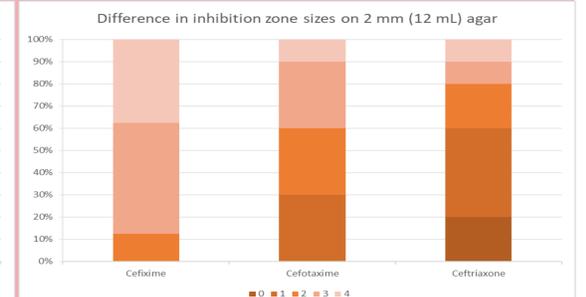


Figure 2: Difference in inhibition zone size when performing AST against three antibiotics (cefixime, cefotaxime, ceftriaxone) using 12ml agar. The different colour of boxes represent the difference of inhibition size when compared to the reference. The different size of the boxes represent the amount of strain that produced the same inhibition zone size difference.

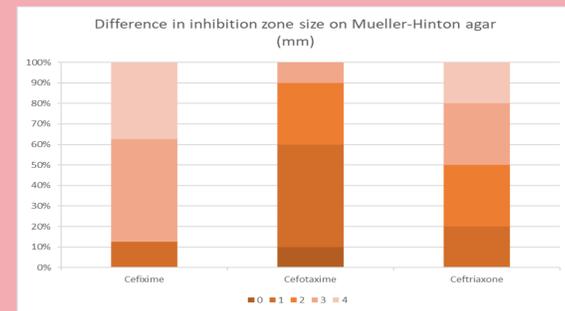


Figure 3: Difference in inhibition zone size when performing AST against three antibiotics (cefixime, cefotaxime, ceftriaxone) using an unsupplemented Mueller-Hinton agar. The different colour of boxes represent the difference of inhibition size when compared to the reference. The different size of the boxes represent the amount of strain that produced the same inhibition zone size difference.

Discussion & Conclusion

The size of inhibition zone increased when changing the EUCAST 15-minute rule to 2 hours (Figure 1). Cefixime and cefotaxime gave 3mm difference while for ceftriaxone, most of the strains produced a 4 mm difference. The antibiotic disk was able to pre-diffuse into the agar when the plates were left at room temperature for 2 hours prior to incubation (ASM, 2009; Matuschek, Brown and Kahlmeter, 2014). This finding is also similar to that by Frimodt-Moller *et al.* (1986).

Based on the results, the size of the inhibition zone for all three of the antibiotics increased when the thickness of the agar reduced from 4 mm to 2 mm. Biggest difference of inhibition zone can be seen in 12ml agar for cefixime with the majority being 3-4 mm. The results were consistent with the findings from previous study by Flanagan and Steck (2017) where *Burkholderia multivorans* produced smaller inhibition zone size for two of the antibiotic used, ceftazidime and meropenem as agar plate depth increases.

The results showed that there is not much difference in inhibition zone for cefotaxime when using unsupplemented Mueller-Hinton (MH) agar with half of the strains having only 1mm difference. This finding was also reported by Niederstebuch and Sixt (2013) that found that the average zone of inhibition difference of *Streptococcus sp.* between blood-supplemented Mueller-Hinton agar and standard nutrient agar is 0.32mm.

In conclusion, changing the standardize protocol set by EUCAST does have an impact on the inhibition zone sizes. It is important to follow the EUCAST protocols for AST. The uncertainty of measurement in AST will always exist due to technical variability that cannot be eliminated. In this study, the ATU does contribute to the measurement of AST but does not change the reporting and susceptibility interpretation of it. As ATU is a fairly new term and has not been investigated as much. Therefore, further studies need to be done on the implementation of ATU in AST.

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