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Susceptibility of *Aerococcus urinae* to Fluoroquinolones: Broth Microdilution and Gradient Diffusion Methods

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Background

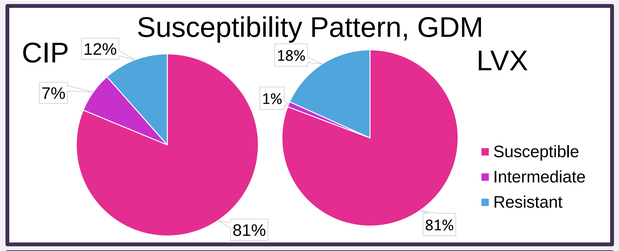
Aerococcus urinae is an emerging urinary pathogen increasingly identified owing to MALDI-TOF[1]. It is generally susceptible to beta-lactams. However, its susceptibility pattern to **fluoroquinolones (FQ)** is variable[2]. The current gold standard for **antimicrobial susceptibility testing (AST)**, **broth microdilution method[3] (BMD)**, is laborious. A less fastidious method is needed. The goals of this study were 1) to evaluate the performance of a **gradient diffusion method (GDM)** and 2) to estimate the resistance rate of *A. urinae* towards FQ in Quebec's hospitals.

Methods

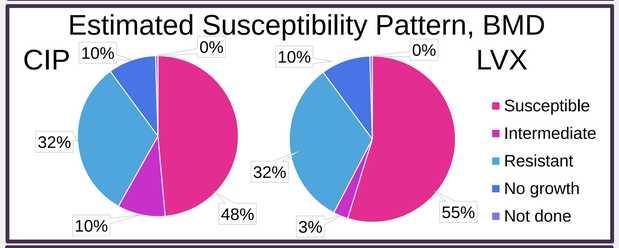
208 consecutive clinical isolates of *A. urinae* from urine specimens were collected in 5 hospitals. Identification was later re-confirmed using Vitek-MS MALDI-TOF +/- RNA 16s gene sequencing in our reference laboratory (**Laboratoire de Santé Publique du Québec, LSPQ**). All isolates were tested by GDM and BMD for **ciprofloxacin (CIP)** and **levofloxacin (LVX)** susceptibilities. EUCAST recommends MH agar with lysed horse blood and 20mg/L β-NAD for AST of *Aerococcus*, but these are not widely available in Canada. The GDM was carried out in duplicates using Etest® strips on MH agar with 5% sheep blood. BMD was done in triplicates for every isolates and followed current CLSI recommendations. Susceptibility rates were calculated for every method. Performance and agreement of the GDM was assessed in comparison to BMD.

Results

208 clinical isolates were tested. We obtained a 81% susceptibility pattern (SP) for both CIP and LVX by GDM. Results were reproducible for GDM.



We couldn't adequately establish the susceptibility pattern using the BMD because of inadequate growth (20%) or trailing (15%).



Therefore, neither categorial or essential agreement between both methods could be calculated. However, performance of the GDM couldn't be determined.

Discussion

A. urinae is now recognized as a frequent urinary pathogen. The current recommended AST method in North America[3], BMD, is fastidious and hard to interpret.

A more efficient and widely available method for AST of *A. urinae* is needed.
AST results obtained by our GDM were similar to those previously published.
Neither errors nor agreement could be calculated due to insufficient growth and reading issued with the BMD. Furthermore, results obtained by BMD don't correlate with current literature. Our SP obtained by BMD with those results would imply excessive very major errors.

% of Susceptibility of *A. urinae* to FQ in Current Literature

	CIP	LVX
Etest[5, 6, 7, 8]	85-88%	87.5-88%
BMD[9]	89.3%	84%
Agar dilution[10]	-	67%

Repetition of the method, increased inoculum and longer incubation couldn't solve the issue.
Some problematic clinical isolates were sent to University of California, Los Angeles (UCLA) and were tested again by BMD. They also observed insufficient growth and trailing issues. Results remain inconclusive. For BMD, considering 80% of growth inhibition as susceptible also gave discordant susceptibility rates (CIP 63% and LVX 70%).
Correspondence with other authors revealed that the problem had already been noticed. It has been previously reported by EUCAST but not by the CLSI.
A third method, such as agar dilution method is needed to arbitrate discrepancy between BMD and GDM.

Conclusions

The method recommended by the CLSI for *A. urinae* susceptibility testing of FQ presented several challenges, including insufficient growth and difficult reading. The GDM appears to be a promising method for susceptibility testing of FQ for urinary tract isolates. It will first require a further comparison with agar dilution methods.

By any method, the rate of FQ non-susceptibility of *A. urinae* exceeded 15% for CIP and LVX. Therefore, they may not be recommended for the empirical treatment of urinary tract infections caused by *A. urinae*.

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Disclosure

Biomerieux provided the Etest® and refunded the cost of the BMD