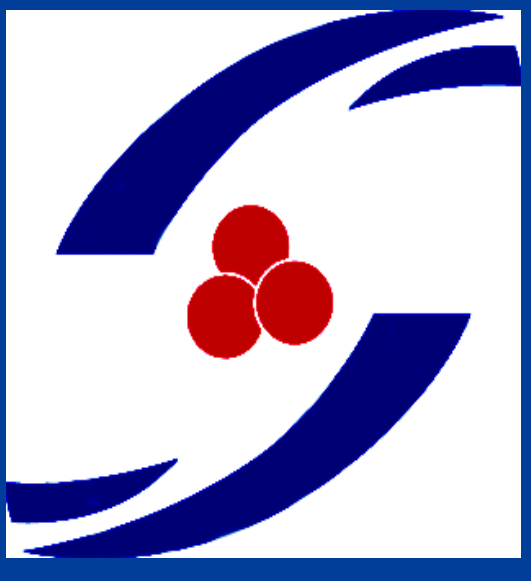


Performance of a Disposable Plastic Inoculating Needle in the Preparation of the CDS Inoculum



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Introduction

The CDS method of antimicrobial susceptibility testing stipulates that a Nichrome wire with a diameter of 0.56mm be used to prepare the inoculum for flooding the susceptibility test plate. Open flames and other heat sources for sterilisation of metal inoculating needles and loops are not always available in microbiology laboratories. Gas may not be available in small laboratories, and open flames may be a fire hazard or have consequences for occupational health and safety. Other methods of sterilisation have similar disadvantages. For these reasons some microbiology laboratories use only disposable plastic inoculating loops and needles for tasks in the laboratory. The same laboratories therefore are unable to conform with the specified requirements for preparing a CDS inoculum with Nichrome wire.

Aim

Prompted by requests from laboratories who were in the predicament where they were unable to use Nichrome wire, we undertook this study to determine if substituting plastic needles for Nichrome wire compromised the validity of the CDS test.

Method

We evaluated the performance of a sterile, disposable plastic inoculating needle (COPAN innovation™) for the preparation of the CDS suspension. First, we correlated transmittance of the suspension with the subsequent radius of inhibition around antimicrobial discs, using plastic and wire inoculating needles. We analysed a historical set of quality control measurements of zone sizes, collected using the same plastic needles applied to the CDS reference strains. We compared these data with the quality control ranges stated in the CDS manual. We prospectively investigated issues arising from this historical data using reference strains, as well as isolates with known borderline susceptibility using the CDS method.

Results

Correlation of transmittance with zone size

The transmittance of a bacterial suspension and the resulting CDS zone size each appeared to follow an approximately normal distribution. A best-fit linear equation derived from transmittance could account for one third of the variance in zone size ($R^2=0.35$, data not shown). Suspensions created with the plastic needle had a lower transmittance ($P=0.0004$ by t-test), implying heavier inocula.

Figure 1 compares the appearance of CDS plates prepared using plastic and wire inoculating needles.

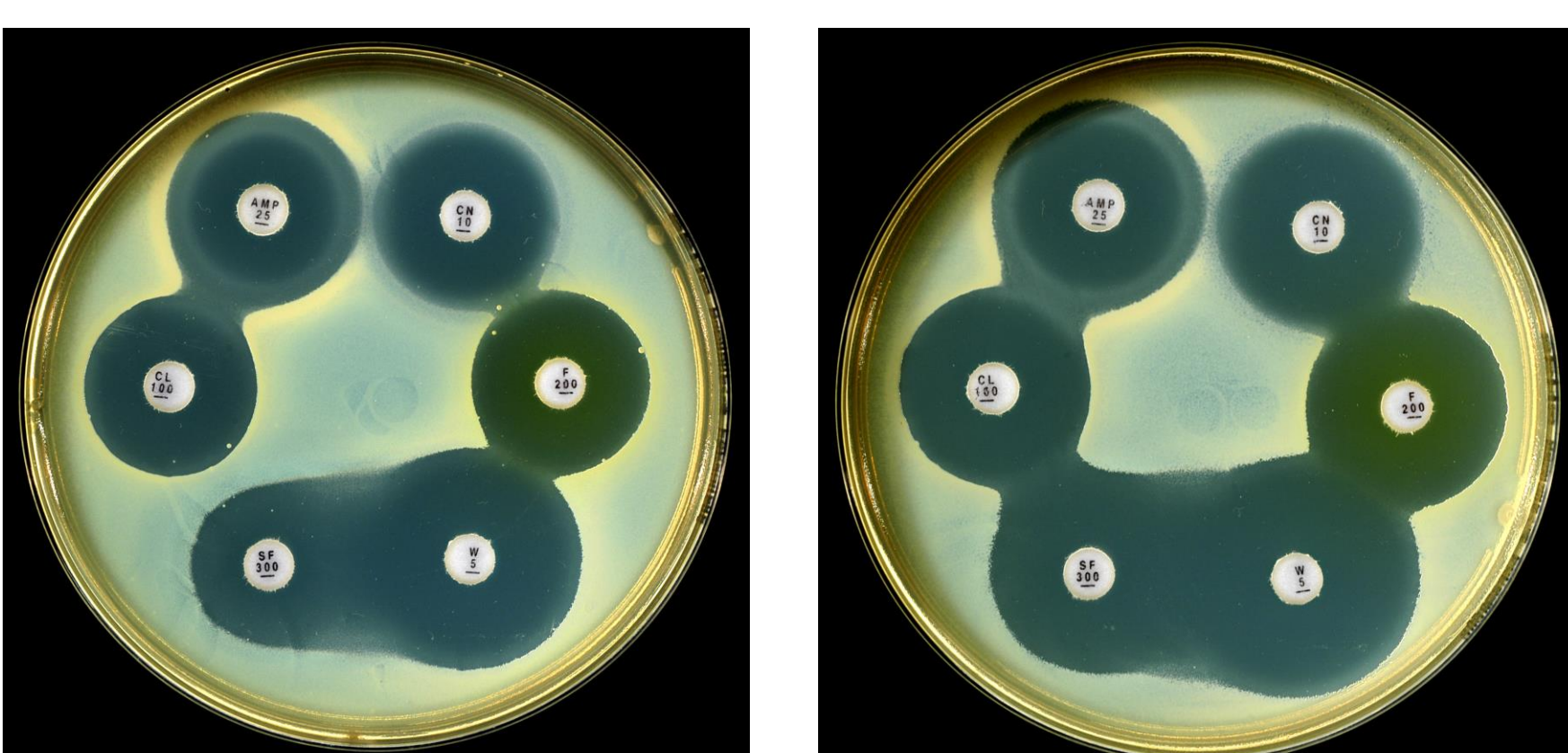


Figure 1: CDS susceptibility test performed on *E. coli* (ACM 5185) using the plastic inoculating needle (left) and the standard Nichrome wire (right).

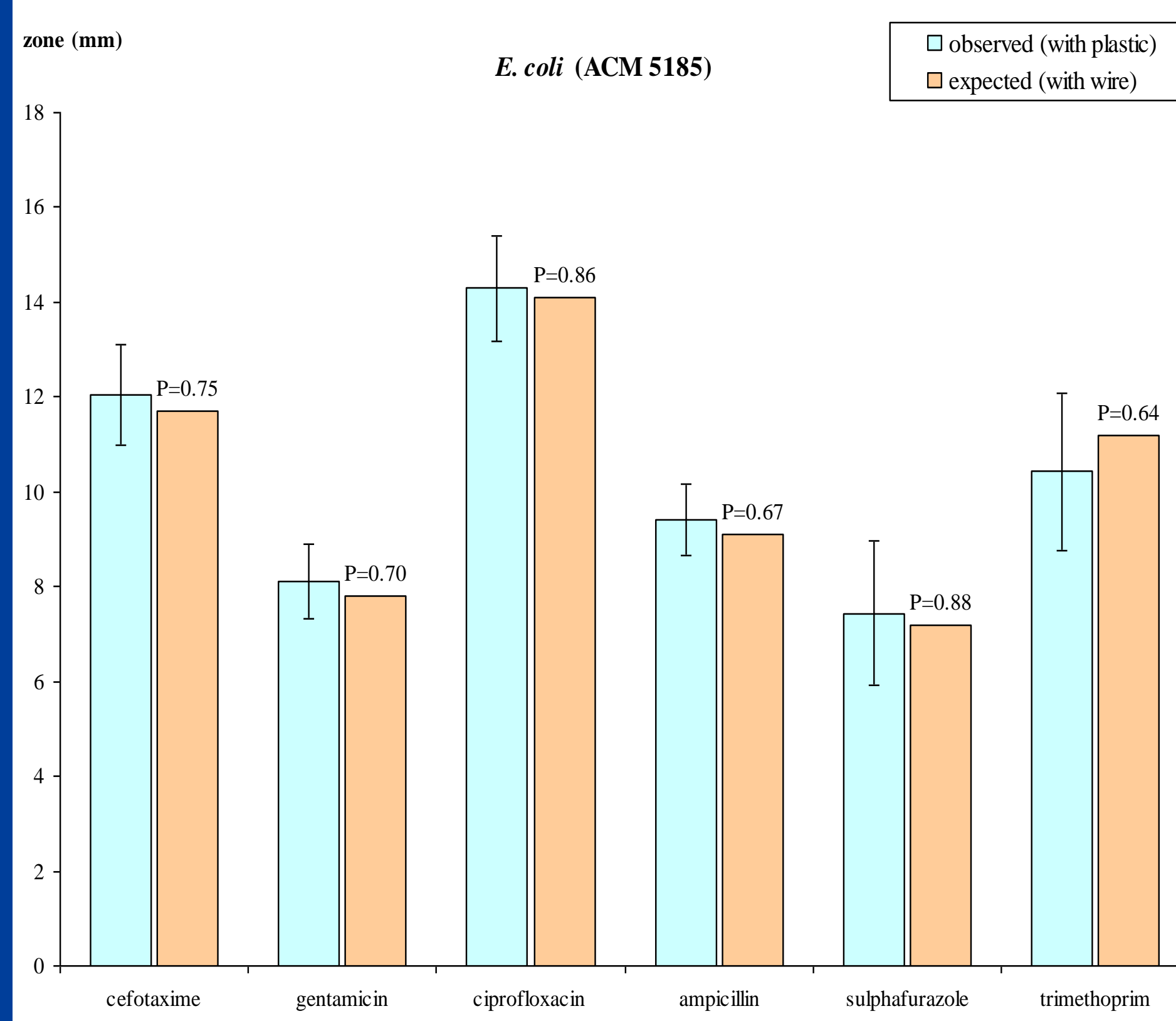
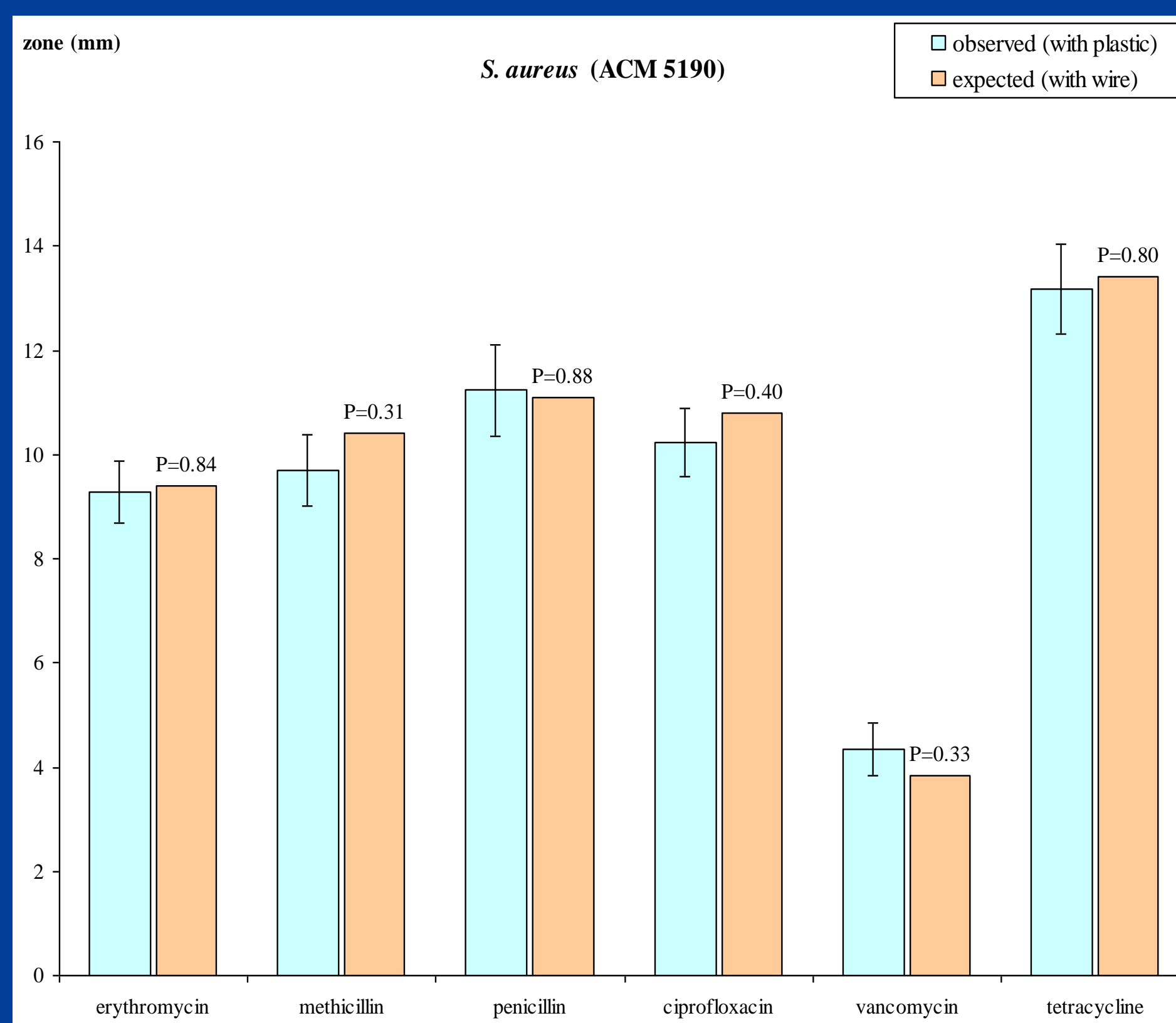


Figure 2: Historical QC data showing the mean and standard deviation of the zone size produced using the plastic needle in comparison with the reference mean zone size (using wire). P values were generated using Student's T-test.

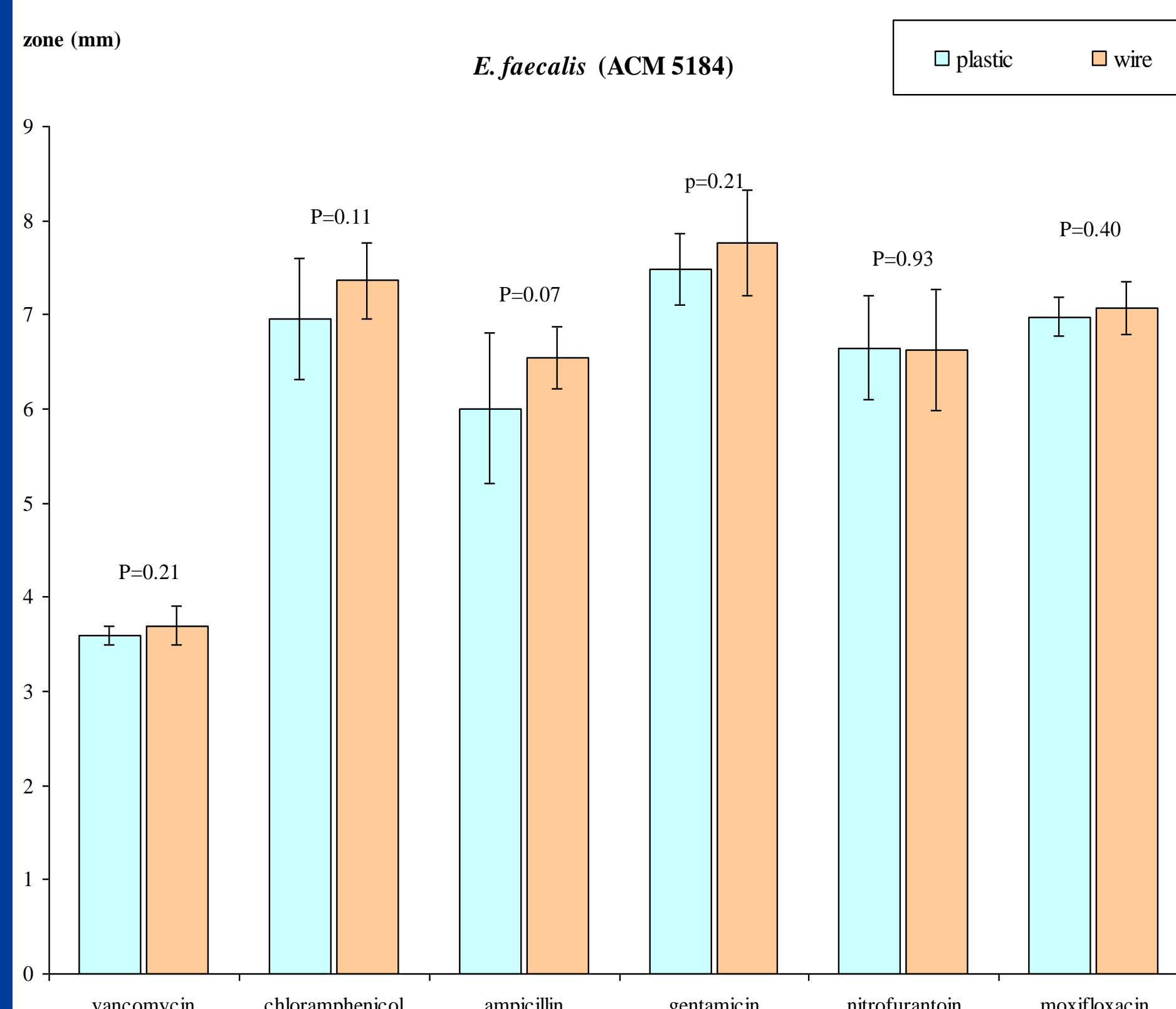
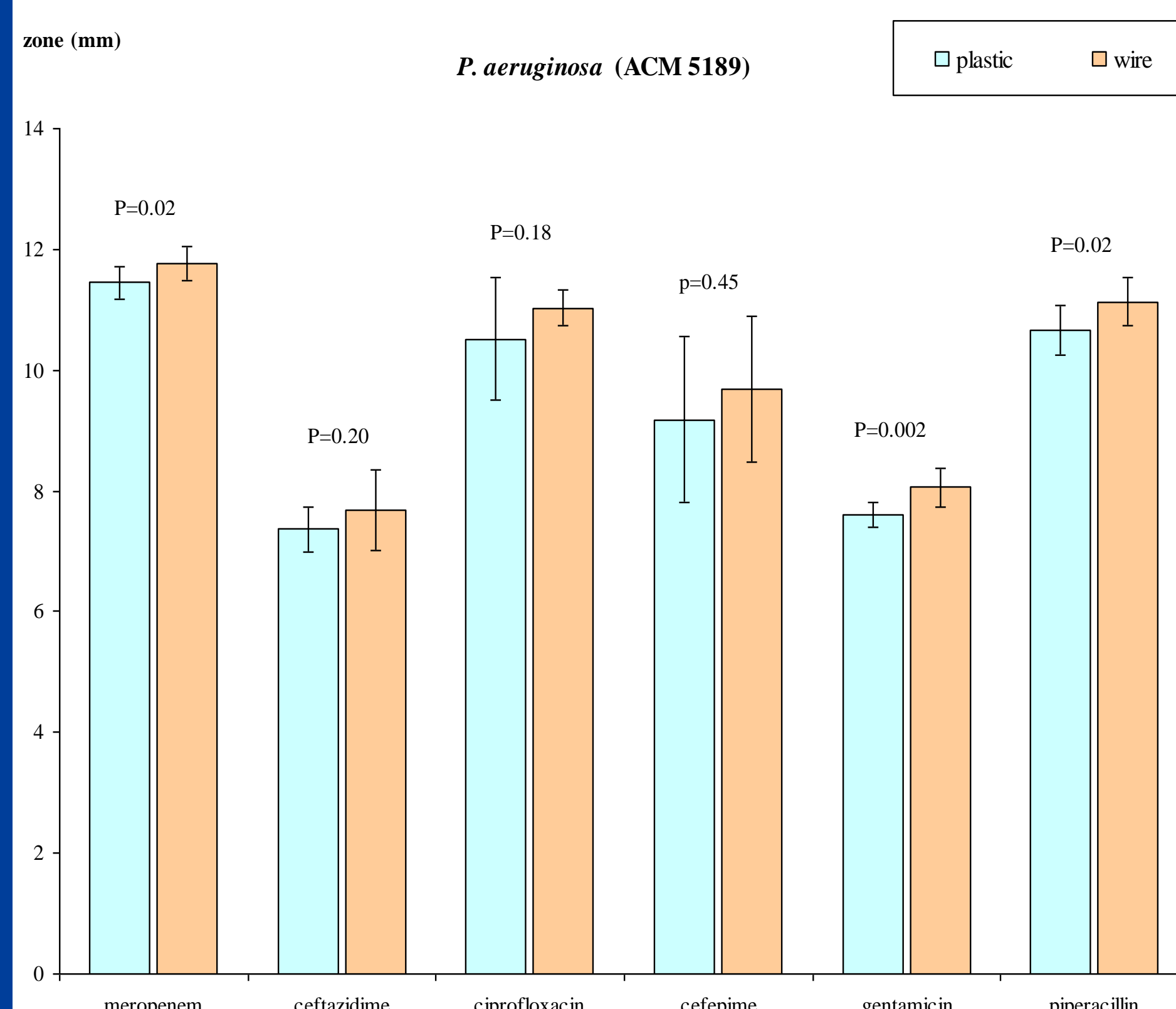


Figure 3: Mean and standard deviation of zone sizes generated prospectively with plastic and wire inoculating needles for the same reference strains. P-values were calculated using Student's T-test.

Results (continued)

Historical data

Zone size data were extracted from a historical collection of CDS susceptibility results performed by several operators using the plastic needle and five reference strains (*S. aureus*, *E. coli*, *H. influenzae*, *S. pneumoniae* and *P. aeruginosa*).

Figure 2 shows the results for two of these organisms. No significant difference was found for *S. aureus*, *E. coli*, or *H. influenzae*. Zone sizes were larger than expected for *S. pneumoniae* (implying a lighter inoculum), and were smaller than expected for *P. aeruginosa* (implying a heavier inoculum).

Prospective evaluation of zone size

Contrary to the historical data, in our experiments the zone sizes for *S. pneumoniae* prepared with the plastic needle were no larger than those prepared with wire. Inocula of this organism prepared using the plastic needle tended to be heavier than those prepared with wire (data not shown). This was in keeping with our earlier findings regarding transmittance.

One possible explanation for lighter than expected inocula in the historical data is autolysis within the reference strain. There are special procedures within the CDS manual to address this issue with *S. pneumoniae*. It is possible that these procedures were not followed consistently during collection of the historical data.

Figure 3 shows our findings regarding *P. aeruginosa*. There was a statistically significant difference in zone sizes, but for all agents this was less than or equal to 0.5 mm.

Figure 3 also shows our findings for another important clinical isolate, *E. faecalis*. There was no statistically significant difference in zone sizes.

"Borderline" susceptibility

Figure 4 shows our findings for a strain of *S. aureus* with so-called "borderline" susceptibility to methicillin tested against cefoxitin.

The suspensions were prepared by three operators with both plastic and wire. The difference between the two sets of zones approaches statistical significance, but remains less than 0.3mm.

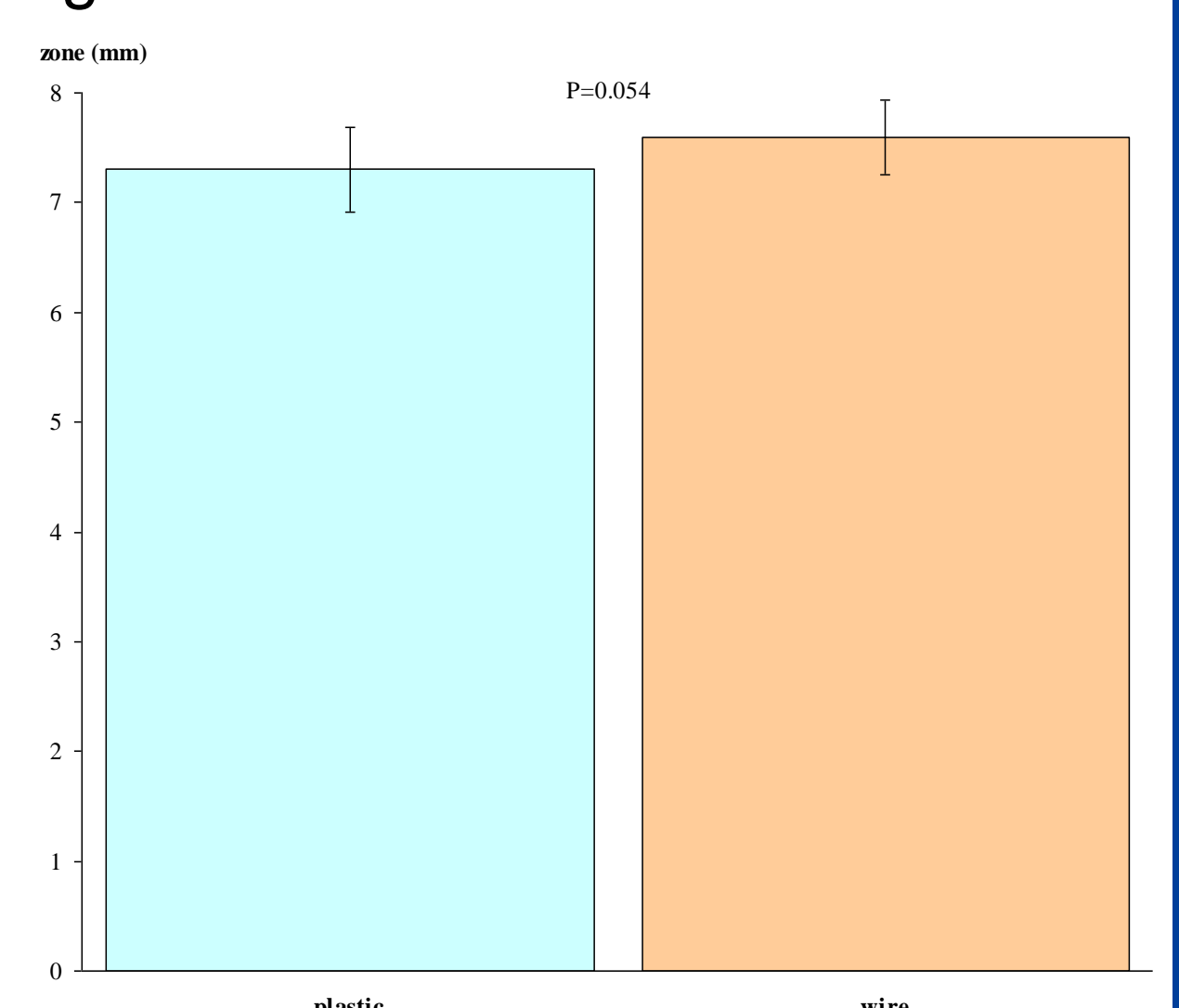


Figure 4: Comparison of cefoxitin zone sizes for a clinical isolate of *S. aureus* with "borderline" susceptibility, showing mean, standard deviation and P-value.

Conclusion

Although the plastic needle does produce a heavier inoculum, the CDS method is sufficiently robust to accommodate this. At most, zones shrunk by 0.5mm. Potential clinical consequences are minor – rarely, an isolate within 0.5mm of the cut-off for susceptibility may be reported incorrectly as resistant. There was no indication that the plastic needle would produce inocula that were too light, leading to falsely susceptible results.

The plastic needle we have evaluated can be substituted for Nichrome wire in laboratories where the wire cannot be used.