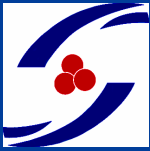


Direct Sensitivity Testing of Urine Samples Based on the Microscopic Quantitation and Morphology of Organisms



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Introduction

The usefulness of direct sensitivity testing of urine samples containing coliform bacteria (determined microscopically) has been established previously¹. Implementation of this procedure, as described, frequently resulted in an unacceptably light inoculum, necessitating the retesting of isolates using the CDS method² and thus delaying reporting by 24 hours. Direct sensitivity testing of enterococcal/streptococcal isolates has not been previously evaluated against the CDS method.

Aim

- To devise a CDS compliant direct sensitivity testing method. The inoculum was varied, based upon the microscopic quantitation of bacteria, to obtain a bacterial suspension equivalent to the standard CDS suspension² (10⁷cfu/ml). The resulting bacterial lawn was evaluated against that expected of a standard CDS suspension.
- To evaluate CDS compliant direct sensitivity testing for enterococci and streptococci.

Method

Microscopy: Each sample was loaded into one chamber on KOVA[®] slide and examined at 400x under phase contrast illumination.

Bacterial Quantitation: The bacterial concentration in each sample was determined by estimating the number of bacteria per small square on the KOVA[®] slide as shown in figure 1 and table 1.

Culture: When bacteria were detected microscopically the sample was cultured on a full MacConkey agar plate and one half of a BioMerieux CPSIII chromogenic agar plate to assist in 24-hour identification of isolates. When cocci were detected microscopically the sample was also cultured onto a full horse blood agar plate.

Bacterial Suspension: Bacterial suspensions for direct sensitivity testing were prepared by adding 25µl or 250µl (40µl or 400µl for cocci) of the urine sample, as determined by the bacterial concentration (see figure 1 and table 1), to 2.5ml saline.

Sensitivity Testing: Sensitest Agar (Oxoid CM409) for coliform sensitivity testing. Sensitest Agar (Oxoid CM409) supplemented with 5% Horse blood for enterococcal/ streptococcal sensitivity testing. All plates were incubated in air for 18 hours at 35°C. After 18-hour incubation sensitivities were repeated on all enterococcal and streptococcal isolates using the CDS method.

Inoculum Evaluation:

Coliforms: After 18-hour incubation the density of the bacterial lawn was evaluated in conjunction with the annular radii around the antibiotic discs to grade the inoculum as: too light, acceptable or too heavy.

Enterococci/Streptococci: After 18-hour incubation the annular radii were recorded for correlation with the CDS method.

Figure 1: Quantitation of bacteria and direct sensitivity inoculum

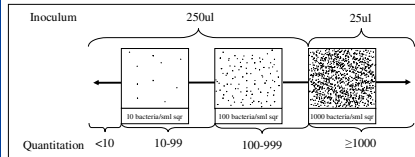


Table 1: Direct sensitivity inoculum (bacilli)

Bacteria per small square	Concentration in urine	Inoculum in 2.5ml saline [§]	Final conc/ml [*]
< 10	< 9 x 10 ⁶ /L	250µl	< 10 ⁶
10-99	9 x 10 ⁶ /L – 9 x 10 ⁹	250µl	10 ⁵ - 10 ⁸
100-999	9 x 10 ⁹ /L – 9 x 10 ¹⁰	250µl	10 ⁶ - 10 ⁷
≥1000	≥ 9 x 10 ¹⁰ /L	25µl	≥ 10 ⁶

[§] Where enterococci/streptococci were seen microscopically 400µl and 40µl inoculums were used in place of the 250µl and 25µl inoculums respectively.

^{*} Final concentrations are rounded to the nearest power of ten. Actual concentration are 9 x 10^{p-1}, where p is the exponent given in the above table.

Results

Of seventy-eight coliforms evaluated only 4 (5.1%) required repeat sensitivity testing using the CDS method. In each case this was caused by too light an inoculum (see table 2).

Direct and CDS sensitivity testing was performed on 50 samples with enterococci/streptococci isolates. Table 3 lists the mean and standard deviation of the difference between the zone sizes (Direct – CDS) for each antibiotic tested. For each antibiotic, results are reported as either Sensitive or Resistant and in this context 100% correlation was achieved between the two methods (table 3).

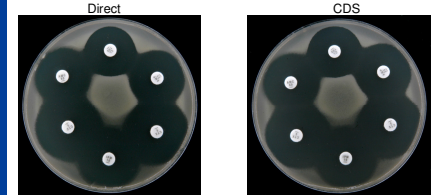
Table 2: Evaluation of inoculum based on microscopic quantitation of coliform bacteria.

Bacteria per small square	Urine inoculum	Bacterial Lawn (Sample Tally)		
		Light	Acceptable	Heavy
< 10	250µl	2	1	0
10 – 99	250µl	2	12	0
100 – 999	250µl	0	36	0
≥ 1000	25µl	0	25	0

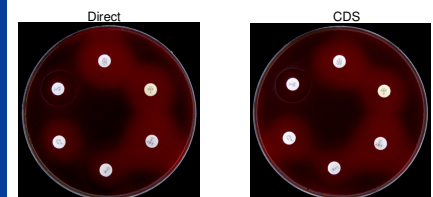
Table 3: Mean and standard deviation of differences between the annular radii using direct and CDS sensitivity testing methods for enterococci and streptococci.

Statistic	Antibiotic Disc			
	Ampicillin 5 µg	Penicillin 0.5 µg	Nitrofurantoin 200 µg	Vancomycin 5 µg
Samples Evaluated	42	8	50	50
Sum of Differences	5.5	11.0	29.0	17.0
Mean of Differences	0.1	1.4	0.6	0.3
Standard Deviation	0.911	0.834	1.14	0.86
Concordant Result	42	8	50	50
Discordant Results	0	0	0	0

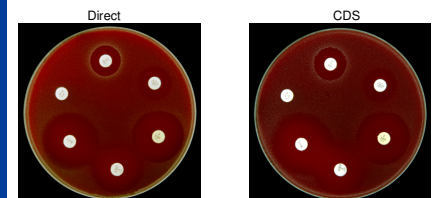
Figure 2: Comparison of Direct and CDS sensitivities



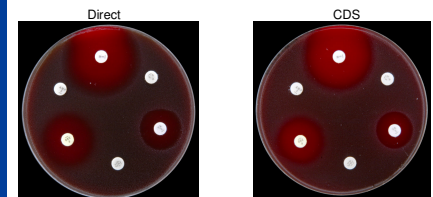
Escherichia coli



Group B Streptococci



Enterococcus faecalis



Enterococcus faecium

Conclusion

Adjusting the inoculum based on the microscopic quantitation of bacteria to obtain a suspension approximating the standard CDS suspension has decreased the number of coliform sensitivities that need to be repeated.

Direct sensitivity testing of samples containing enterococci or streptococci produces zone sizes comparable to those obtained using the CDS method and shows 100% concordance in reported sensitivities.

Coupled with the use of chromogenic agar media these procedures have resulted in improved turnaround times for many samples.

References

- C. Mukerjee and E. Reiss-Levy. Evaluation of Direct Disc Diffusion Susceptibility Testing for Bacteriuria Using Diluted Urine Against Standard CDS Method. Pathology (1994), 26, pp. 201-207.
- S. M. Bell, B. J. Gatus, J. N. Pham & D. L. Rafferty Antibiotic Susceptibility testing By The CDS Method. A Manual For Medical And veterinary Laboratories 2004.