Gemifloxacin and Ciprofloxacin Susceptibility Testing: Comparison of NCCLS Broth MIC Method With Five MIC Methods

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Abstract

Variations in susceptibility methodology were assessed for gemifloxacin (SB-265805) and ciprofloxacin. NCCLS broth MIC results were compared with results obtained with E test and with methods used in France (Société Française de Microbiologie (SFM)), in Germany (Deutsches Institut für Normung (DIN)), in the United Kingdom (British Society for Antimicrobial Chemotherapy (BSAC)) and in Sweden (Swedish Reference Group for Antibiotics (SRGA)). Susceptibility testing was performed in triplicate on 100 stock isolate: 26 Gram positive isolates (*Streptococcus pneumoniae*, staphylococci and enterococci) and 74 Gram negative isolates (*Haemophilus influenzae*, *Enterobacteriaceae* and *Pseudomonas aeruginosa*). Essential/category agreement rates for gemifloxacin were: E test 100/83.0, DIN 91.1/93.0, SRGA 86.6/86.0, SFM 74.7/87.0 and BSAC 86.2/87.0. Essential/category agreement rates for ciprofloxacin were: E test 95.2/99.0, DIN 86.8/94.0, SRGA 86.1/63.0, SFM 80.2/94.0 and BSAC 73.3/90.2. Ciprofloxacin and gemifloxacin essential agreement rates were <90% with SFM, SRGA and BSAC methods, due to larger variations in MICs at concentrations <0.06 µg/ml. Differences in interpretive criteria also contributed to category discrepancies, as was most evident with ciprofloxacin SRGA results. Overall, the error rates were very low for gemifloxacin (1% major error (SRGA) and 1% very major error (SRGA and BSAC) and for ciprofloxacin (2% major error (SFM and SRGA), 1.1% major error (BSAC) and 1% very major error (E test)). At concentrations of >0.06 µg/ml, results for all methods were comparable.

Introduction

- Variations in susceptibility methodology and interpretive breakpoints may impact on the integration of MIC data from multi-national studies.
- In this study, gemifloxacin (SB-265805) and ciprofloxacin data from MIC methods used in Europe and the USA were compared to determine if there were any significant differences.

Methods

Isolates

One hundred isolates with previously characterized gemifloxacin susceptibilities (as determined by NCCLS methodology) were tested. Strains were selected in order to test a range of concentrations, especially at or near the interpretive breakpoint concentrations. Table 1 shows the number of each species tested.

Methods and Testing Laboratories

- The testing was performed by five laboratories in France, Germany, Sweden, the United Kingdom and the USA.
- Each isolate was tested in triplicate by all MIC methods.
- Gemifloxacin and ciprofloxacin interpretive criteria used for the category and error analysis are summarized in Table 2.
- The appropriate quality control strains for each method were tested for every day of testing.

Data Analysis

The results were analyzed according to four different methods. As statistical analysis indicated no difference between replicates, the first replicate for each method was used for the analyses.

1 **Doubling dilution difference** (Figure 1): The number of dilutions difference for each method compared with the NCCLS broth method was determined.

2 **% Cumulative MICs** (Figure 2): This analysis shows the similarities and differences between all methods at each MIC or zone.

3 **Statistical analysis**: MIC data were log transformed prior to analysis to account for the doubling nature. Analysis of variance (ANOVA) was used to determine whether results for any of the methods were considered statistically different. Tukey post hoc test was carried out when the analysis was significant to determine where differences occurred.

4 **Essential agreement, category agreement and error analysis**: The essential agreement rate was the percentage of results that were within +/- one dilution of results of the NCCLS broth method. The category agreement rate was the percentage of results that were similar in interpretive category (susceptible, intermediate and resistant) compared with results of the NCCLS broth method. Minor error was when the comparative method and the NCCLS broth method results differed by one category (i.e. susceptible *versus* intermediate, resistant *versus* intermediate). Major error was when the comparative method result was resistant and the NCCLS broth method was susceptible. Very major error was when the comparative method was susceptible and the NCCLS broth method was resistant.

Results

Results based on the three methods of analysis are shown in Figures 1 and 2 and Tables 2–4.

Figure 1. Comparison With NCCLS Broth MIC Results: Doubling Dilution Differences

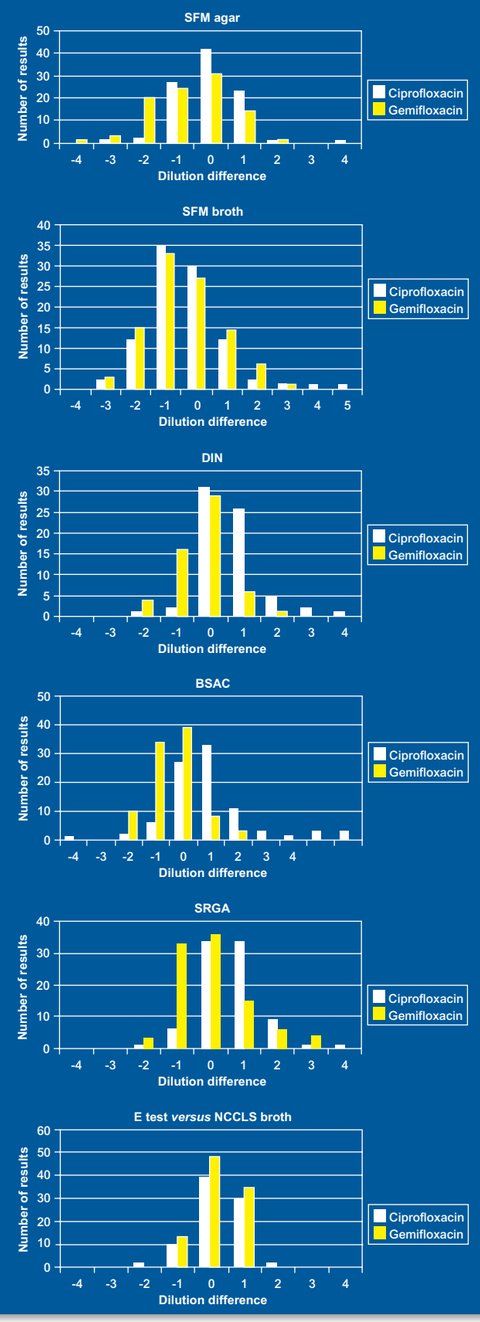


Table 1. Number of Microrganisms Tested

Microrganism	n
S. pneumoniae	10
S. aureus	5
S. epidermidis	2
S. saprophyticus	1
E. faecalis	8
H. influenzae	10
E. coli	13
E. aerogenes	4
E. cloacae	5
P. mirabilis	4
P. vulgaris	3
P. aeruginosa	12
S. marcescens	4
K. pneumoniae	19

Table 2. Gemifloxacin[a] and Ciprofloxacin Interpretive Criteria

Method	Microrganism	MIC (µg/ml) Susceptible	Intermediate	Resistant
Gemifloxacin				
All methods[a]	*Enterobacteriaceae*	≤0.25	0.5	≥1
	P. aeruginosa	≤0.25	0.5	≥1
	Enterococcus spp.	≤0.25	0.5	≥1
	Staphylococcus spp.	≤0.25	0.5	≥1
	S. pneumoniae	≤0.25	0.5	≥1
	H. influenzae	≤0.25	0.5	≥1
Ciprofloxacin				
BSAC	*Enterobacteriaceae*	≤1	–	≥2
	P. aeruginosa	≤1	2–4	≥8
	Enterococcus[b]	–	–	–
	Staphylococcus	≤1	–	≥2
	S. pneumoniae	–	≤2	≥4
	H. influenzae	≤1	–	≥2
DIN	All	≤1	2	≥4
NCCLS and E test	*Enterobacteriaceae*	≤1	2	>4
	P. aeruginosa	≤1	2	≥4
	Enterococcus spp.	≤1	2	≥4
	Staphylococcus spp.	≤1	2	≥4
	S. pneumoniae	≤1[c]	2[c]	>4[c]
	H. influenzae	≤1	–	–
SFM	All	≤1	–	–
SRGA	*Enterobacteriaceae*	≤0.12	0.25–1	≥2
	P. aeruginosa	≤1	–	≥2
	Enterococcus spp.	≤0.12	0.25–2	≥4
	Staphylococcus spp.	≤0.06	0.12–2	≥4
	S. pneumoniae	≤0.12	0.25–2	≥4
	H. influenzae	≤0.12	0.25	≥0.5

[a]Gemifloxacin breakpoints have not yet been established. The breakpoints that are used in this study are for comparative purposes only and represent the most conservative values. Approved breakpoint values may vary and may differ by organism group and by method (as is the case with ciprofloxacin)
[b]No BSAC breakpoints exist for *Enterococcus* spp., therefore results for eight enterococci were excluded in calculation of essential/category agreement rates
[c]No NCCLS breakpoints exist for *S. pneumoniae*. For comparative purposes the NCCLS 'non-*S. pneumoniae*' breakpoints were used

Table 3. Comparison of Mean MICs

Method	Gemifloxacin mean (µg/ml)	Ciprofloxacin mean (µg/ml)
BSAC	0.1224	0.5047
DIN	0.2054	0.5217
NCCLS (USA)	0.1717	0.2726
SFM broth	0.1295	0.2247
SFM agar	**0.0984**[a]	0.2579
SRGA	0.1619	0.3347
E test	0.2000	0.3710

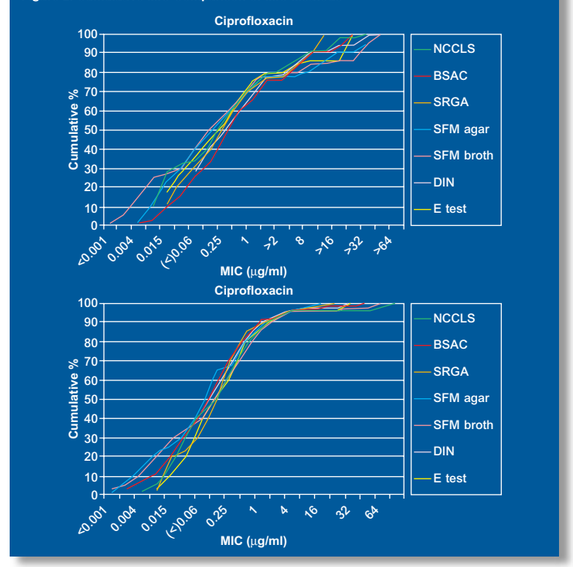
[a]Mean in bold print is statistically different compared with NCCLS mean

Table 4. Essential/Category Agreement and Error Rates (Ciprofloxacin/Gemifloxacin) Compared with NCCLS Broth

	BSAC	DIN	E test	SFM (agar)	SFM (broth)	SRGA
Number on scale[a]	90/94	68/56	83/96	97/93	96/99	86/97
Agreement						
Essential (n)	66/81	59/51	79/96	92/69	77/74	74/84
% Essential[b]	73.3/86.2	86.8/91.1	95.2/100	94.8/74.2	80.2/74.7	86.1/86.6
% Category	90.2/87.0	94.0/93.0	99.0/83.0	94.0/83.0	94.0/87.0	63.0/86.0
Errors (%)						
Minor (+/- 1 dilution)[c]	4.3/11.0	5/5	3/17	5/13	3/11	34/11
Minor (≥2 dilution)	1.1/1.0	1/2	0/0	0/3	1 / 2	1/1
Major	1.1/0	0/0	0/0	1/0	2/0	2/1
Very major	1.1/0	0/0	1/0	0/1	0/0	0/1

[a]On scale results are those results that are not below the lowest dilution tested or above the highest dilution tested
[b]Essential agreement is based on Essential (n)/Number on scale
[c]Even though results are within +/- 1 dilution, a category difference occurs

Figure 2. Cumulative MIC Comparison of MIC Methods



Conclusions

- Specific organism observations (in comparison to NCCLS broth) were:
  - *H. influenzae*: lower BSAC, DIN and SFM; higher SRGA
  - *Enterococcus faecalis*: higher BSAC
  - *Enterobacteriaceae*: higher BSAC
  - *Staphylococcus* spp.: lower SFM
  - *P. aeruginosa*: lower SRGA.
- The lower category agreement rate (63.0%) for ciprofloxacin SRGA agar compared with NCCLS broth was due to variation in interpretive category between the two methods. As a result, many organisms with MICs in the susceptible range by NCCLS were in the intermediate range by SRGA.
- Although the DIN and NCCLS (Germany) broth methods did not include testing below 0.06 µg/ml, the category agreement rates were not affected as the breakpoint values are well above this concentration. However, as many strains were extremely susceptible, essential agreement rates were impacted.
- In this preselected group of isolates, there was a higher percentage of isolates with MICs at the breakpoint dilutions compared with normal organism populations. Therefore, based on the results of this study, category agreement rates would be expected to be higher if a typical group of organisms was tested.
- The majority of minor errors were a result of MICs that differed by only +/- 1 dilution, but differed in category (i.e. susceptible *versus* intermediate, intermediate *versus* resistant).
- Most variation occurred at very low dilutions. At concentrations of >0.06 µg/ml, all MIC methods were comparable.