D-47

Inter-Method Susceptibility Comparisons Produced by Several National Methods: Report of Garenoxacin Results Produced by Methods from France, Germany, Spain, Sweden and United Kingdom versus NCCLS

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AMENDED ABSTRACT

Background: Laboratories worldwide perform MIC/disk diffusion (DD) tests using various national methods, often published by local organizations. Potentially different results may complicate drug development/registration between the US methods (NCCLS) and those used in EU. This study uses a new des F(6)quinolone, garenoxacin (GRN; formerly BMS284756), to compare in vitro results among the most utilized methods.

Methods: Investigators in France (F), Germany (G), Spain (Sp), Sweden (Sw) and United Kingdom (UK) tested 2 bacterial sets designated, challenge (CS; n=330) with quinolone (Q) resistances (R); national (NS; n=540) that were recent isolates (2000-2001). Results were compared to NCCLS data performed by the US coordinating site. Discords (>4-fold) were repeated (x3). Results were analyzed for variation from the NCCLS MIC results (± 1 or 2 dilutions, > 3 or 6 mm) and by regression statistics. Ciprofloxacin (CIP) was the control.

Results: CS compliance to testing all strains was 97.3 - 98.8% and correlation (r) of the national method NCCLS/MIC was: F (0.98), G (0.95), Sp (0.98), Sw (0.96) and UK (0.95). CS produced MICs the same as NCCLS (Sp) to 0.5 x $\log_2$ greater (G), but % strains within ± 2 dil versus NCCLS was 98 (G) - 100% (Sw, UK). Similar patterns were observed for the NS (r=0.93 - 0.98) and all nations had results > 99% within ± 2 dil (± 6 mm) of NCCLS and each nation's MIC was slightly elevated versus NCCLS (ave. 0.2 x $\log_2$). Control CIP MIC and disk results showed similar patterns.

Conclusions: Intermethod GRN susceptibility results indicate that MIC and DD endpoints derived from 5 EU methods compare favorably to the NCCLS and generally were identical or a fraction of a $\log_2$ dilution higher. In contrast, zone diameters for GRN and CIP were usually larger (3 - 4 mm) by EU methods. This unique "bridging" experiment allows regulatory agencies a greater quantitative understanding of in vitro test results produced in the EU or where the NCCLS methods predominate.

INTRODUCTION

The vast majority of antimicrobial susceptibility testing methods that have been developed by national organizations or professional societies have been based on the comprehensive studies summarized by Ericcsson and Sherris in 1971. In that 90 page document, results from numerous investigations by a multitude of scientists were summarized leading to recommendations for the technical aspects of three methods (broth and agar dilution, disk diffusion). However, the evolving problems of inter-method discords in technical details and interpretation of results among the various national methods has compromised international antimicrobial development.

Since 1972, the National Committee for Clinical Laboratory Standards (NCCLS) in the United States (US) has had an active standardization program, initially focused on the disk diffusion method. Later committee activities expanded the consensus standards process to include: agar dilution, broth dilution, broth microdilution and reference methods for testing anaerobic bacteria (agar dilution and broth microdilution). Concurrently, similar organizations in several developed nations formalized the standardization processes, mostly found in Europe and Japan. With the birth of the European Union and the goal of common regulatory standards for developing new antimicrobials, the need for harmonization of in vitro antimicrobial susceptibility testing methods has assumed a high level of importance. Also via the collaboration of the European Society for Clinical Microbiology and Infectious Disease and the EU regulatory agencies, a newer pan-EU initiative called EUCAST (European Committee for Antimicrobial Susceptibility Testing) was organized to develop common methods, quality assurance and interpretive standards.

Statistics from some European programs illustrate the complex problem encountered in this geographic region when trying to combat emerging antimicrobial resistances. The European Antimicrobial Surveillance System (EARSS) in their resistance monitoring projects, indicated that a wide variety of methods and interpretive standards were utilized. Bruinsma et al. (2002) noted that among 24 European nations participating in 2001, the primary guidelines for testing were from diverse sources such as the NCCLS, British Society for Antimicrobial Chemotherapy (BSAC), Deutsche Industrie Norm-Medizinische Mikrobiologie (DIN), la Société Francaise de Microbiologie (SFM), Swedish Reference Group for Antibiotics (SRGA), Commissie Richtlijnen Gevoeligheidsbepalingen, and non-national methods (Rosco and Stokes). However, overall in Europe the NCCLS guidelines were used by 73% of EARSS participants. Other guidelines only predominated within a single nation for Germany (DIN), Denmark (SRGA), France (SFM), Sweden (SRGA) and United Kingdom (BSAC). Clearly the field of medical microbiology needs to achieve greater international standardization of antimicrobial susceptibility test methods.

As new and novel antimicrobial agents are developed for clinical use, worldwide development standards for susceptibility testing and their relationship to pre-registration clinical trials will streamline the regulatory process, and hopefully reduce the confusion and high costs of current practices in Europe, North America and elsewhere. To this end, an investigation was designed to use a novel des(6)fluoroquinolone, garenoxacin, to study the inter-relationships of six national standards for antimicrobial susceptibility testing by dilution (MICs) and disk diffusion (zone diameters) methods. Dominant methods used in the EU (BSAC, DIN, Mese Espanole de Normalizacion de la Susceptibilitad y Resistencia a los Antimicrobianos [MENSURA], SFM and SRGA) would be compared to the results of the most used tests worldwide and in the EU, the NCCLS M2-A7 and M7-A5 (2000) methods.

MATERIALS AND METHODS

Investigators and Methods: The BSAC (Professor French), DIN (Professor Rodloff), MENSURA (Professor Canton), SFM (Professor Soussy), and SRGA (Professor Kahlmeter) methods were represented. The work done in the laboratories of these investigators was compared in a blinded manner to that produced by the NCCLS method.

Organisms: The strains were selected by collaboration of the participants, sponsor and the study monitor. The project was performed in two major phases related to organism processing.

- A "Challenge Collection" of organisms (330 strains) was selected to provide a wide variety of pathogens and organisms that had unusual resistance patterns in some nations monitored. These organisms included samples of penicillin- and ciprofloxacin-(MIC, ≥ 4 µg/ml) resistant *S. pneumoniae*; oxacillin-resistant staphylococci, vancomycin-resistant (*van A* and *van B* patterns) enterococci, ß-lactamase-producing *H. influenzae* and *H. parainfluenzae*, fluoroquinolone-resistant enteric species and ciprofloxacin-resistant non-fermentative Gram-negative bacilli.

  The number of results received for evaluation (compliance) included: France (321; 97.3%), Germany (324; 98.2%), Spain (323; 97.9%), Sweden (325; 98.8%) and United Kingdom (325; 98.5%). The overall compliance in Phase I was 98.4%.

- A second group of strains called the "National Collection" was selected by each national investigator from those isolates referred by SENTRY Program participants for the 2001 study year. These strains were selected based on target numbers by species, and would total 540, if fully compliant. All strains were to be representative of the susceptibility patterns (by organism) that were endemic within that nation.

  Compliance in Phase II was as follows: France (463; 85.7%) Germany (456; 84.4%), Spain (501; 92.8%), Sweden (494; 91.5%), and United Kingdom (458; 84.8%). The all-nation compliance for the "National Collection", unique to each country, was 86.4%. The compliance to protocol numbers of organisms processed was 90.9% for the entire study.

- Quality control strains were also circulated and all results were within NCCLS or sponsor recommended limits.

Other Study Procedures: Results compared to NCCLS values and discords of ≥ three $\log_2$ dilution steps or > 6 mm were repeated by the national and NCCLS investigation. Repeated values (triplicate) became the result utilized in the study record. Each result by nation was compared to the NCCLS value by regression analysis and by error-rate techniques. Target correlations that would be considered acceptable would be r-values of ≥ 0.90, ≥ 90% of MICs within ± one $\log_2$ dilution step (≥ 95% with ± two $\log_2$ dilution steps, ≥ 90% categorical agreement between method results using the same quantitative MIC breakpoints, all as applied to garenoxacin and ciprofloxacin. All of these goals were achieved.

Table 1. Variations of garenoxacin MIC results produced by five national methods against two collections/sets of organisms when compared to the MIC values obtained with the NCCLS M7-A5 broth microdilution method.

Organism collection/national method (no. tested)	% variations from the NCCLS MIC in $\log_2$ dilutions: >-2	-2	-1	Same	+1	+2	>+2
Challenge							
France (321)	0.3	0.3	13.7	51.1	32.4	2.2	0.0
Germany (330)	0.6	2.1	8.2	37.9	40.3	9.4	1.5
Spain (323)	0.0	2.2	17.0	59.8	20.4	0.6	0.0
Sweden (325)	0.0	0.3	8.9	43.4	38.2	9.2	0.0
United Kingdom (324)	0.0	5.9	24.1[a]	33.0[a]	22.5[a]	14.5	0.0
All methods (1,623)	0.2	2.2	14.4	45.0	30.8	7.2	0.3
National							
France (474)	0.2	2.1	17.1	57.2	19.4	3.4	0.6
Germany (442)	0.2	0.7	7.5	38.5	45.0	7.9	0.2
Spain (460)	0.0	0.7	15.0	58.9	23.0	2.2	0.2
Sweden (479)	0.0	0.4	12.9	57.3	26.1	3.3	0.0
United Kingdom (478)	0.0	10.0	20.1[a]	28.9[a]	27.6[a]	13.2	0.2
All methods (2,333)	<0.1	2.8	14.6	48.2	28.0	6.0	0.3
All collections and methods (5,956)	0.1	2.6[b]	14.5[b]	46.9[b]	29.2[b]	6.5[b]	0.3

a. Lowest rates of agreement at ± one $\log_2$ dilution = 76.6 - 79.6%.
b. Overall rates of agreement were 90.6 and 99.6% for ± one $\log_2$ dilutions and ± two $\log_2$ dilution steps, respectively.

Table 2. Trends in the garenoxacin MIC values for five national methods in Europe compared to the NCCLS broth microdilution method (M7-A5, 2000).

National method	Trend by organism set ($\log_2$ dilutions): Challenge	National	Overall
France	+ 0.2	Same	+ 0.1
Germany	+ 0.5	+ 0.6	+ 0.6
Spain	Same	+ 0.1	+ 0.1
Sweden	+ 0.4	+ 0.2	+ 0.3
United Kingdom	+ 0.1	+ 0.3	+ 0.2
All methods	+ 0.2	+ 0.2	+ 0.2

RESULTS

- MIC results by the five tested European methods (BSAC, DIN, MENSURA, SFM and SRGA) compare favorably with the MICs generated by the NCCLS broth microdilution method. Correlation coefficients averaged well over 0.90, intermethod serious categorical errors were at acceptable levels (≤ 1.5%; actual 0.3%) and a modest trend toward higher MICs was observed for the European methods. The latter trend was greatest for the DIN and SRGA methods, but averaged only + 0.2 x $\log_2$ dilution, overall.

- Garenoxacin zone diameters around the 5-µg disk were routinely larger by 3 - 4 mm using the European disk diffusion methods when compared to the NCCLS M2-A7 method. However, correlation between methods (zone versus zone) remained high at > 0.90 (average). Also correlate zones could be selected for each method that would achieve excellent categorical agreement with the NCCLS results.

- Garenoxacin tests using the six national antimicrobial susceptibility testing methods for MIC and disk diffusion tests yielded comparable results, if the interpretive criteria were identical for the MIC breakpoints and when correlate zone diameters for susceptibility and resistance were adjusted for medium and/or inoculum differences.

Table 3. Overall inter-method discord rates when five national method results for garenoxacin by susceptibility category were compared to the results of the NCCLS MIC method.[a]

National method (total no. tested)	No. serious errors by set (%):[b] Challenge	National	No. minor errors by set (%):[b] Challenge	National	Total % errors
France (795)	0 (0.0)	2 (0.4)	20 (6.2)	15 (3.2)	4.7%
Germany (772)	4 (1.2)	3 (0.7)	16 (4.8)	47 (10.6)	9.1%
Spain (783)	0 (0.0)	0 (0.0)	10 (3.1)	17 (3.7)	3.4%
Sweden (804)	0 (0.0)	0 (0.0)	21 (6.5)	14 (2.9)	4.4%
United Kingdom (802)	2 (0.6)	0 (0.0)	21 (6.5)	26 (5.4)	6.1%
Total (3,956)	6 (0.4)	5 (0.2)	88 (5.4)	119 (5.1)	5.5%[c]

a. Susceptible defined as ≤ 2 µg/ml and resistance at ≥ 8 µg/ml for all tested strains for this analysis.
b. Serious errors defined as a resistant-to-susceptible or visa versa discord between method categories. Minor error indicates an intermediate category for one of the methods and resistant or susceptible for the other.
c. 94.5% absolute categorical agreement.

Table 4. Organisms producing serious categorical errors (11; 0.3%) between methods using the challenge and national collections of organisms.

National method (no./% serious errors)	Organism (no. tested) associated with the most serious errors
France (2/0.2%)	*P. aeruginosa* (2)
Germany (7/0.9%)	*A. baumannii* (1) Coagulase-negative staphylococci (1) *E. faecium* 2) *H. influenzae* (1) *Serratia* spp. (2)
Spain (0/0.0%)	None
Sweden (0/0.0%)	None
United Kingdom (2/0.2%)	*E. faecalis* (1) *S. maltophilia* (1)

Table 5. Median 5-µg garenoxacin zone diameter for each national method for strains having zones of 25 and 30 mm by the NCCLS disk diffusion method for each organism sent.

National method	Median zone in mm: Challenge Collection 25 mm	Challenge Collection 30 mm	National Collection 25 mm	National Collection 30 mm
France	28	36	28	36
Germany	28	30	30	34
Spain	27.5	30	29	33
Sweden	31	35	31	35
United Kingdom	30	33	30	34
Average variation (mm)	+ 3.9	+ 2.8	+ 4.6	+ 4.4

Figure 1. Scattergram demonstrating excellent correlation between SFM and NCCLS method MICs for garenoxacin (Challenge Collection of organisms; 321 strains). Solid lines indicate ± one $\log_2$ dilution limits and broken lines the ± two $\log_2$ dilution range.

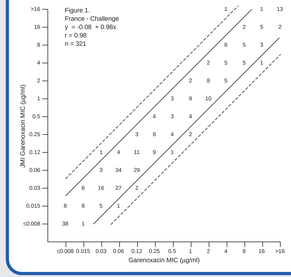
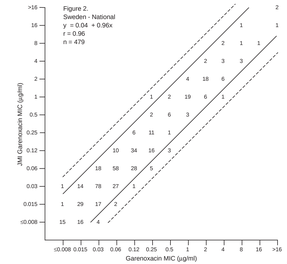


Figure 2. Scattergram demonstrating excellent correlation between SRGA and NCCLS method MICs for garenoxacin (National Collection of organisms; 479 strains). Solid lines indicate ± one $\log_2$ dilution limits and broken lines the ± two $\log_2$ dilution range.



CONCLUSIONS

- Garenoxacin clinical trial susceptibility testing results from these assessed national systems appear to be closely related and compatible for the merging of data in preparation for new drug registrations in the US (FDA) and in the EU.

- These unique collaborative study results should also lead to greater harmonization of susceptibility test methods within the EU nations, and between the laboratories using the NCCLS methods in Europe and elsewhere.

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