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Stability of Three Oral Vancomycin (Va) Preparations Stored at 2–5ºC and Ambient Temperatures for Up to 60 Days

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Introduction

Oral vancomycin (VA) is used to treat *Clostridium difficile* infection (CDI). Several different oral preparations are available including pharmacy-prepared IV solutions and vancomycin capsules (USP, Rockville, MD) as well as FIRST®-Vancomycin grape-flavored liquid compounding kits, (CutisPharma, Inc., Wilmington, MA).

The refrigerated shelf-life of the solutions after reconstitution varies between 7–14 days for pharmacy-prepared IV vancomycin and a standard 30 days for FIRST®-Vancomycin. This study examined various storage conditions and durations for all three preparations and compared the results to a vancomycin control solution to determine the impact on VA potency.

Methods

Vancomycin IV solution. Vancomycin hydrochloride for injection 4 g was reconstituted in 160 ml sterile water for a final concentration of 25 mg/ml.

Vancomycin Hydrochloride Capsules. Vancomycin hydrochloride capsules (equivalent to 3.75 g vancomycin) was reconstituted in 150 ml sterile water for a final concentration of 25 mg/ml.

FIRST®-Vancomycin. FIRST®-Vancomycin 25 Compounding Kit (FIRST) consists of a bottle containing premeasured vancomycin hydrochloride powder (equivalent to 3.75 g vancomycin) and a bottle of white grape-flavored diluent (150 ml). The diluent was added to the bottle containing the vancomycin hydrochloride powder and mixed by shaking for 10 seconds; for a final concentration of 25 mg/ml.

Sigma Vancomycin Control. Sigma laboratory standard vancomycin powder was used as the control. Powder was reconstituted according to the manufactures' instructions, and stored at −70ºC. On the day of testing, a new tube of stock solution was thawed and diluted according to the instructions in CLSI M11-A8 document [1].

The impact of storage on the potency of VA was determined by testing clinical isolates of *C. difficile* and *S. aureus* as well as *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Bifidobacterium adolescentis* and *Bacteroides fragilis* against the three test solutions and the Sigma vancomycin control solution in 3 different methods and comparing the MICs.

- Reconstituting the FIRST kit and then testing.
- Reconstituting all four preparations, storing at room temperature or refrigerated, and then testing the solutions at T_o, and after 14, 30 and 60d.
- Storing FIRST kits at RT for 17 and 21 months, reconstituting and then testing.

Bacterial strains were recovered from recent clinical samples and stored at −70°C in 20% skim milk. Strains were taken from the freezer and subcultured at least twice. Colonies were taken from pure culture plates and suspended in Brucella broth for anaerobes or cation-adjusted Mueller Hinton broth (CAMHB) for aerobes to a turbidity of 0.5 McFarland standard.

On the day of testing, the VA solutions were diluted according to the instructions in the CLSI M7-A10 and M11-A8 documents [1, 2]. Anaerobic testing was performed using CLSI standard methods for agar dilution [1]. The strains were applied to the plates using a Steers multipronged inoculator for a final concentration of approximately CFU 10^5 CFU/spot. After 44h incubation at 36°C in the anaerobic chamber incubator, the plates were examined for growth, and the MICs interpreted. Aerobic testing was performed using standard CLSI methods for microbroth dilution [2]. MIC trays were prepared in-house using the Quick-Spense apparatus (Sandy Spring Instrument Co. Inc., Germantown, MD) using CAMHB, and stored at −70°C until used. Each inoculate well had a final concentration of ~5 x 10^5 cfu/ml. Trays were incubated for 20h at 35°C before reading, and the MICs were interpreted.

Quality control strains were included each test day and were comprised of *Clostridium difficile* ATCC 700057 and *Bacteroides fragilis* ATCC 25285 for anaerobic testing and *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 for aerobic testing.

The MIC was defined as the concentration of antimicrobial agent that completely inhibited growth or resulted in a marked decrease in growth compared to the growth control as indicated by CLSI guidelines [1, 2]. The susceptibility of the test isolates to VA was determined per CLSI interpretive breakpoints [3]. All testing was done in triplicate.

Results

Table 1. Geometric mean MICs (µg/ml) for *C. difficile* tested against three oral vancomycin preparations under different storage conditions.

Test No.	Storage Time, T_x	No Strains	Manufacturer: Cutis RT[a]	Cutis 2–5°C	IV solution RT	IV solution 2–5°C	Capsule RT	Capsule 2–5°C	Sigma Control −70°C
Reconstituted (no storage) and tested at T_o									
1-1	0	50	1.87						1.87
1-2	0	50	1.18						1.16
Reconstituted, stored for T_x and then tested									
1-3	0	25	1.96		2.12		2.08		1.48
1-4	14 d	25	1.44	1.44	1.52	1.52	1.48	1.40	1.48
1-5	30 d	25	2.20	1.84	2.28	2.12	2.12	1.92	1.38
1-6	60 d	25	1.45	1.34	1.58	1.46	1.12	1.06	1.38
Stored for T_x, reconstituted and then tested									
1-7	17 mo	25	1.30						1.27
1-8	21 mo	25	1.27						1.28

[a] Storage temperature; RT, room temperature

Table 2. Geometric mean MICs (µg/ml) for *S. aureus* tested against three oral vancomycin preparations under different storage conditions.

Test No.	Storage Time, T_x	No Strains	Manufacturer: Cutis RT*	Cutis 2–5°C	IV solution RT	IV solution 2–5°C	Capsule RT	Capsule 2–5°C	Sigma Control −70°C
Reconstituted (no storage) and tested at T_o									
2-1	0	50	—						0.79
2-2	0	27	0.69						0.68
Reconstituted, stored for T_x and then tested									
2-3	60 days	25	0.67	0.53	0.70	0.53	0.69	0.53	0.53
Stored for T_x, reconstituted and then tested									
2-4	17 months	27	0.64						0.63
2-5	21 month	27	0.70						0.73

* Storage temperature; RT, room temperature

Table 3. VA range for miscellaneous clinical isolates (all manufacturers and storage conditions)

	No. strains	Range
Enterococcus faecalis	5	0.5–2
Escherichia coli	5	>16
Klebsiella pneumoniae	5	>16
Bifidobacterium adolescentis	5	0.5–1
Bacteroides fragilis	5	>16

Results

The triplicate MICs (µg/ml) were averaged and the geometric mean calculated for all preparations, storage conditions and time periods for *C. difficile* and *S. aureus* and are shown in Tables 1–2.

In the preliminary study (tests 1-1,2 and 2-1,2), the geometric mean MICs for FIRST and the Sigma vancomycin control against 50 strains of *C. difficile* and *S. aureus* were very similar.

The geometric mean MICs for each preparation and organism were less than one dilution different for all of the values and were thus considered insignificant regardless of storage temperature or duration (tests 1-3,4,5,6 and 2-3). When stored at RT for 60 days, FIRST and IV prep showed no growth; however, the IV and capsule preparations had a ground-glass, cloudy appearance, and the capsule preparation grew a mold at a concentration of 4 x 10^5 CFU/ml.

The FIRST kits that were stored for 17 and 21 months, reconstituted and tested had MICs that were comparable to the Sigma vancomycin control (tests 1-5,6 and 2-4,5).

The FIRST diluent did not show any inhibition of any of the test strains and passed as a negative control.

The vancomycin MIC ranges for *E. faecalis, E. coli, K. pneumoniae, B. adolescentis* and *B. fragilis* are listed in Table 3.

QC values were within CLSI specified ranges [3] throughout testing.

Conclusions

- All VA preparations that were reconstituted and tested from T_o to 60 days showed remarkably similar MICs compared to the Sigma vancomycin control regardless of storage temperature for both *C. difficile* and *S. aureus*.
- Storage 2–5°C maintained a clear appearance and full potency for all of the preparations.
- The capsule preparation grew a mold after 60d at RT. But unlike FIRST, which retained a clear appearance, the IV and capsule preps showed evidence of crystallization.
- All FIRST kits that were stored at RT for 17 or 21 months and then reconstituted and tested had similar MICs compared to the Sigma control for both *C. difficile* and *S. aureus*.
- FIRST®-Vancomycin provides a convenient and more palatable way to administer vancomycin to patients with CDI.

References

[1] Clinical Laboratory Standards Institute. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard-8th edition. CLSI document M11-A8. Wayne, Pa.: CLSI; 2015.
[2] Clinical Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-10th edition. Clinical and Laboratory Standards Institute document M7-A10. Wayne, Pa.: CLSI; 2015.
[3] Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 26th informational supplement. CLSI document M100-S26. Wayne, Pa.: CLSI; 2016.

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