Development of an Antimicrobial Susceptibility Testing Method Suitable for Performance During Space Flight

James H. Jorgensen,[1*] Joyce A. Skweres,[2] S.K. Mishra,[2] M. Letticia McElmeel,[1] Louise A. Maher,[1] Ross Mulder,[3] Michael V. Lancaster[4#] and Duane L. Pierson[5]

Department of Pathology, the University of Texas Health Science Center, San Antonio Texas 78284, [1] Krug Life Sciences, Houston, Texas, [2] bioMerieux Vitek, Hazelwood, Missouri,[3] Accumed International, Westlake, Ohio,[4] NASA/Johnson Space Center, Houston, Texas, [5]

Running Title: Susceptibility Testing in Microgravity

*Corresponding author:

James H. Jorgensen, Ph.D.
Department of Pathology
University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, Texas 78284-7750
Telephone 210-567-4088
Fax 210-567-2367

#Current Address:

Hospital Infections Program
National Center for Infectious Diseases
Centers for Disease Control and Prevention
Atlanta, Georgia 30333

ABSTRACT

Very little is known regarding the affects of the microgravity environment of space flight upon the action of antimicrobial agents on bacterial pathogens. This study was undertaken to develop a simple method for conducting antibacterial susceptibility tests during a Space Shuttle mission. Specially prepared susceptibility test research cards (bioMerieux Vitek, Hazelwood, MO) were designed to include 6-11 serial two-fold dilutions of 14 antimicrobial agents, including penicillins, cephalosporins, a ß-lactamase inhibitor, vancomycin, erythromycin, tetracycline, gentamicin, ciprofloxacin, and trimethoprim/sulfamethoxazole. Minimal inhibitory concentrations (MICs) of the drugs were determined by visual reading of color endpoints in the Vitek research cards made possible by incorporation of a colorimetric growth indicator (alamarBlue™, Accumed International, Westlake, OH). This study has demonstrated reproducible susceptibility results when testing isolates of *Staphylococcus aureus,* Group A *Streptococcus, Enterococcus faecalis, Escherichia coli* (beta-lactamase positive and negative strains), *Klebsiella pneumoniae, Enterobacter cloacae,* and *Pseudomonas aeruginosa.* In some instances, the MICs were comparable to those determined using a standard broth microdilution method, while in some cases the unique test media and format yielded slightly different values, that were themselves reproducible. The proposed in-flight experiment will include inoculation of the Vitek cards on the ground prior to launch of the Space Shuttle, storage of inoculated cards at refrigeration temperature aboard the Space Shuttle until experiment initiation, then incubation of the cards for 18-48 h prior to visual interpretation of MICs by the mission's astronauts. Ground-based studies have shown reproducible MICs following storage of inoculated cards for 7 days at 4-8°C to accommodate the mission's time schedule and the astronauts' activities. For comparison, ground-based control (normal gravity) MIC values will be generated by

simultaneous inoculation and incubation of a second set of test cards in a laboratory at the launch site. This procedure can provide a safe and compact experiment that should yield new information on the affects of microgravity on the biological activities of various classes of antibiotics.

INTRODUCTION

Extended space flight missions of greater than 90 days aboard the Russian Space Station Mir are currently underway and even longer duration missions on the International Space Station are planned during this decade. Early studies in the space craft environment showed distinct effects on microorganisms, including apparent increased growth rates and increased cell density (4). Relatively little is known about the effect of microgravity during space flight on the susceptibility of bacteria to antimicrobial agents. Bacteria recovered from astronauts during the Apollo-Soyuz Test Project in 1975 demonstrated somewhat increased antimicrobial resistance in post flight isolates as compared to pre-flight baseline isolates (1,10,13). Limited antimicrobial susceptibility studies were performed by a French-Soviet team involved with the Salyut 7 spacecraft mission in 1982 (3,14). Isolates of *Staphylococcus aureus* and *Escherichia coli* recovered from one of the cosmonauts were tested in vitro against five antibiotics during the flight. Resistance was said to increase in both organisms, as indicated by MICs of oxacillin, chloramphenicol, and erythromycin against *S. aureus* that were found to be approximately twice the ground-based control values, and the MICs for colistin and kanamycin against *E. coli* were more than four-fold higher than in ground-based controls (4,6,14). In a 1985 experiment during the Spacelab D1 mission (European Space Agency Biorack program), increased resistance to a single antibiotic, colistin, was demonstrated a second time (6,14). In this experiment, in-flight cultures of *E. coli* were tested against colistin in a static incubator (microgravity conditions) and in a centrifuge that simulated 1 g. Increased resistance was evident in the in-flight tests as compared to the ground- and flight- controls.

Postflight analyses of microorganisms collected from in-flight experiments have indicated that the space-grown microbes, after subculture several times on the ground, return to normal levels of antibiotic susceptibility (3,13,14). From the results of these

initial experiments, the apparent increase in resistance did not appear to be an acquired characteristic of the organisms, but rather a temporary adaptation to conditions of microgravity. Electron-microscopy of space-grown cultures have shown an increase in cell-wall thickness (3). This may have altered cellular permeability, thus affecting the penetration of antibiotics into the cells. However, increased resistance to antibiotics due to temporary modification of molecular targets or alterations in cell physiology cannot be excluded. Thus, there is some evidence that a microgravity environment adversely affects some drugs or classes of drugs, which could have clinical significance in the eventuality of astronaut illness during prolonged missions.

The constraints associated with space flight do not allow the use of traditional antibiotic susceptibility testing methods. Safety issues preclude transferring liquids containing viable microorganisms to prepare and standardize inocula for susceptibility tests, and open tubes or plates of broth or agar cannot be handled safely without a burdensome biological safety cabinet. The purpose of this study was to design a simple, safe susceptibility testing procedure suitable to be conducted on an upcoming Space Shuttle mission that would evaluate several antimicrobial agents of current therapeutic relevance against common bacterial pathogens. A susceptibility test method has been developed that uses specially prepared antibiotic research test cards (bioMerieux Vitek, Hazelwood, MO) incorporating a growth indicator (alamarBlue™; Accumed, Westlake, OH) for manual susceptibility determinations by astronauts during a Space Shuttle mission.

MATERIALS AND METHODS

Bacterial test strains. A group of commonly occurring bacterial pathogens consisting of both ATCC stock cultures and selected clinical isolates were utilized in these studies. These included *Staphylococcus aureus* ATCC 29213, Group A *Streptococcus, Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and 35218, *Pseudomonas aeruginosa*

ATCC 27853, *Klebsiella pneumoniae,* and *Enterobacter cloacae.* Several of the strains were selected because of known susceptibility or resistance to the study antimicrobial agents.

Antimicrobial agents selected for study. Antimicrobial agents were chosen for inclusion in the study based on their potential clinical utility and because they represented examples of different chemical classes and modes of antimicrobial action. These included the cell wall active agents penicillins, cephalosporins, and vancomycin; the beta-lactamase inhibitor clavulanate (combined with amoxicillin); protein synthesis inhibitors, tetracycline, erythromycin, and gentamicin; a DNA gyrase inhibitor, ciprofloxacin; and the folate metabolism inhibitors trimethoprim/sulfamethoxazole.

Specially prepared Vitek cards. Specially configured Vitek research susceptibility testing cards were kindly provided for this study by bioMerieux Vitek. The cards incorporated $\log_2$ dilutions of the study antibiotics without growth medium in standard 30 well clear plastic Vitek cards. The test concentrations of each antibiotic were selected in order to encompass a range of approximately two dilutions on either side of the expected MICs for each test strain. Cards 1 and 2 used for gram-positive bacteria included penicillin, 0.008-8 μg/ml; oxacillin, 0.03-2 μg/ml; cephalothin 0.008-0.5 μg/ml; vancomycin 0.12-16 μg/ml; ciprofloxacin, 0.06-16 μg/ml; erythromycin, 0.06-16 μg/ml; and trimethoprim/sulfamethoxazole, 0.25-16 μg/ml (based on the trimethoprim component tested in a fixed 1:19 ratio). For gram negative test species, cards 3 and 4 contained ampicillin, 1-32 μg/ml; amoxicillin/clavulanate, 2-64 μg/ml (based on the amoxicillin component tested in a fixed 2:1 ratio); cephalothin, 4-128 μg/ml; cefuroxime, 0.5-8 μg/ml; ceftazidime, 0.06-8 μg/ml; gentamicin, 0.12-32 μg/ml; tetracycline, 1-32 μg/ml; ciprofloxacin, 0.004-0.12 μg/ml, and trimethoprim/ sulfamethoxazole, 0.06-2 μg/ml (based on the trimethoprim component).

Specially formulated test media. Growth media were selected and optimized based on preliminary experiments for each organism or group as indicated below. In order to make manual readings of the Vitek cards possible, a redox growth indicator

(alamarBlue™ U.S. patent no. 5,501,959; kindly provided by Alamar Biosciences [now Accumed, International, Westlake, Ohio]) was incorporated into each test medium. The indicator system allowed growth of a test organism to be visually detected when the indicator turned from blue to bright pink. Preliminary experiments were conducted to define a suitable test medium and optimal indicator concentration for each test organism. For the staphylococci, *Enterobacteriaceae* and *P. aeruginosa*, the test medium consisted of cation adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, MI), 1% yeast extract (Difco) and triple strength alamarBlue™ (equivalent to 0.0045% resazurin). For Group A *Streptococcus* and *E. faecalis*, BBL Sceptor Gram-Positive Broth (Becton-Dickinson Microbiology Systems, Sparks, MD) was chosen for these experiments and double strength alamarBlue™ (equivalent to 0.003% resazurin) was used.

Inoculation of the Vitek cards. The Vitek cards were simultaneously filled with test medium and inoculated by using the test medium as the final diluent for the bacterial inoculum. This was accomplished by first preparing a fresh bacterial suspension in 0.45% saline equivalent in turbidity to a 0.5 McFarland standard. For gram-positive organisms, 100 µl of a 1:10 dilution (in saline) of the 0.5 McFarland suspension was added to 1.8 ml of the special test medium described above. For gram-negatives, 50 µl of a 1:10 dilution of the McFarland suspension was placed in 1.8 ml of test medium. The inoculated test media were then used to fill the Vitek cards using a Vitek vacuum-filling module in the usual manner. The final inoculum density in each well of the filled Vitek cards was approximately 1-3 x 10^5 CFU/ml.

Storage and incubation of the Vitek cards. The inoculated cards were placed in a standard Vitek card holder and then either placed in a standard 35°C bacteriologic incubator or stored at 4-8°C for a defined period prior to incubation. In most experiments, the inoculated cards were stored under refrigeration for approximately seven days prior to incubation. In other experiments, Vitek cards were incubated either at 35°C for 16-20 hours or at room temperature for 48 hours.

Determination of MICs using the special cards. Following incubation of the cards, the individual wells were examined visually for a change in color of the growth indicator. If growth occurred in the presence of an antibiotic, the indicator changed from blue to bright pink, indicating resistance to the antibiotic at that test concentration. Conversely, lack of growth and color change of a well (i.e., remaining blue) indicated susceptibility to that antibiotic concentration. The data were recorded by marking each pink well on a specially designed data sheet which pictorially represented the configuration of that card.

Determination of reference susceptibility results. For the stock culture isolates of clinical origin, MICs of the study antibiotics were determined according to the broth microdilution procedure advocated by the National Committee for Clinical Laboratory Standards (NCCLS) (7). Specifically, cation adjusted Mueller-Hinton broth was used for the non-fastidious species, and 3% lysed horse blood-supplemented cation adjusted Mueller-Hinton broth was used for the Group A *Streptococcus* isolate (7).

Evaluation of susceptibility results. The MIC results determined using manual readings of the special Vitek cards were compared either to the NCCLS expected ranges for the ATCC control strains included in this study (7), or to NCCLS reference broth microdilution susceptibility results determined on the test isolates.

RESULTS

Use of the specially configured Vitek cards and incorporation of alamarBlue™ as a growth indicator allowed MICs to be visually determined without difficulty. Relatively enriched, supplemented test media were used in order to provide rapid growth of the organisms and sharply defined growth end-points with the indicator system. Because of the clarity of the visual indicator system, each Vitek card could be read and the results recorded on the special report sheets in less than 30 seconds per card.

Preliminary studies indicated that the Vitek test cards could be inoculated and stored at 4°C for seven or eight days prior to incubation for 16-18 h at 35°C without adversely affecting the test results (data not depicted). This approach was taken in order to allow cards to be inoculated in a laboratory on the ground prior to launch of the Space Shuttle, stored at refrigerated temperature, then incubated and read at a convenient time during the mission using a special refrigerator-incubator module. This would avoid the difficulties and safety concerns that would ensue from attempting to standardize inoculum suspensions and inoculate the test cards during the flight.

There was reasonably good agreement between MICs determined in the special Vitek cards and those determined by use of NCCLS reference methods using 35°C incubation preceded by storage of the inoculated test cards at 4°C for seven to eight days (see Tables 1-2). However, there were some antimicrobial agent-organism combinations that resulted in MICs outside the NCCLS control range, or that were different than the NCCLS reference procedure MIC values. The trimethoprim/ sulfamethoxazole MICs were generally higher in the experimental cards than when determined by the standard NCCLS methodology, and the gentamicin, tetracycline, and ciprofloxacin MICs likewise tended to be higher when determined using the Vitek cards.

Room temperature incubation of the Vitek cards also provided reproducible susceptibility test results that were generally comparable to the conventional 35°C incubation temperature values (Tables 3 and 4). However, there were a number of off-scale values that prevented a precise comparison of the MICs of some of the agents included in this part of the study. In some instances the Vitek card MICs were lower than the reference values (e.g., vancomycin and erythromycin with *S. aureus* ATCC 29213 and cefuroxime with *E. coli* ATCC 25922). More often, the Vitek card MICs were higher than the reference values under room temperature incubation, as compared with the same agents incubated at 35°C (Tables 1-4).

DISCUSSION

The risk of infectious diseases is increased during space flight because of the crew working and living in crowded conditions, the use of reclaimed/recycled air and water, the absence of infection isolation facilities, as well as human physiological changes in such an environment (1,8,9). In addition, the observed attenuation of the human immune response reported by American, Russian, and other international investigators may be the most obvious reason for an increased risk of infectious diseases during space flight (2,5,12). The dosage of antibiotics for infections that develop in space may be affected by alterations in the absorption rates and the pharmacokinetics and pharmacodynamics of antibiotics that have been demonstrated during space flight (11). Any decrease in microbial susceptibility to antibiotics during space flight could markedly influence decisions on the management of infectious diseases that might develop during long-duration missions. Several previous studies, although limited in scope, suggest that bacteria cultivated in microgravity may show increased resistance to certain antibiotics (3,4,6,10,13,14).

The methodology described in this report would allow simple determinations of the antimicrobial susceptibility of several bacterial species to be made by astronauts during a Space Shuttle mission. The color end point method of defining MICs can provide on-board measurements of susceptibility during the prolonged period of microgravity that occurs during a shuttle flight. The feasibility of incubating the test cards at ambient room temperature was demonstrated in this study in the event that 35°C incubation could not be provided on board the Space Shuttle.

The MICs recorded in this ground-based pilot study were reproducible and sometimes encompassed within the NCCLS reference ranges with four ATCC control strains commonly used in clinical laboratory susceptibility determinations. However, some MIC values were higher when determined in the Vitek cards. Elevated

trimethoprim/sulfamethoxazole MICs likely were the result of excess thymidine contributed by the yeast extract added to the Mueller-Hinton broth to promote rapid, luxuriant growth of the test organisms. The tendency for higher gentamicin, erythromycin, tetracycline, and ciprofloxacin MICs in the special Vitek cards, may have been the result of a decrease in pH of the test media during prolonged storage of the inoculated cards at 4°C prior to the period of incubation. Future experiments will include periodic pH measurements to assure maintenance of the proper medium pH during the pre-incubation period. Despite some deviations from the standard reference values, the MICs determined in the special Vitek cards were quite reproducible, and should allow recognition of any significant increase or decrease in susceptibility that might occur during the microgravity incubation period as compared to the simultaneously inoculated and incubated ground-based control card MICs. In order to increase the statistical confidence of the MIC results determined in the test system by the astronauts and the ground-based laboratory staff, each organism will be tested in triplicate in a blinded fashion.

The goal of this proposed Space Shuttle experiment using the susceptibility procedures described herein is to confirm or refute those earlier reports of increased resistance by examining strains representing a wider spectrum of clinically significant species and to examine antibiotics of every clinically relevant class and mechanism of action. The susceptibility testing methodology described in this report (special Vitek cards and color growth indicator system) has been developed to facilitate simple, fast, and safe handling and reading by the Space Shuttle crew with minimal crew time required for the experiment. The authors are hopeful that the final version of this experiment will be scheduled for a Space Shuttle mission in the near future. The results of that experiment should clarify whether there is any adverse effect of microgravity on the activities of antimicrobial agents of various classes against commonly encountered bacterial pathogens.

REFERENCES

1. **Ferguson, J. K., G.R. Taylor, and B.J. Mieszkuc.** 1975. Microbiological Investigations. In: Johnston, R. S., L.F. Dietlein, C.A. Berry, eds. Biomedical Results of Apollo. Washington, D.C.: National Aeronautics and Space Administration, Scientific and Technical Information Office, p. 83-103.

2. **Konstantinova, I. V., M.P. Rykova, A.T. Lesnyak, and E.A. Antropova.** 1993. Immune changes during long-duration missions. J. Leukocyte Biol. **54**:189-201.

3. **Lapchine, L., N. Moatti, G. Gasset, G. Richoilley, J. Templier, and R. Tixador.** 1986. Antibiotic activity in space. Drug Exp. Clin. Res. **12**:933-938.

4. **Mishra, S. K., and D.L. Pierson.** 1992. Space flight effects on microoganisms. In: Lederberg, J., ed. Encyclopedia of Microbiology. San Diego, CA: Academic Press, **14**: 53-60.

5. **Mishra, S. K., E. Segal, E. Gunter, V.P. Kurup, J. Mishra, P.S. Murali, D.L. Pierson, H. Sandovsky-Losica, and D.A. Stevens.** 1994. Stress, Immunity, and Mycotic Diseases. J. Med. Vet. Mycol. **32**:379-406.

6. **Moatti, N., L. Lapchine, G. Gasset, G. Richoilley, J. Templier, and R. Tixador.** 1986. Preliminary results of the "antibio" experiment. Naturwissenschaften 73: 413-414.

7. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, PA.

8. **Nefedov, Y. G., V.M. Shilov, I.V. Konstantinova, and S.N. Zaloguyev.** 1971. Microbiological and immunological aspects of extended manned space flight. Life Sciences and Space Research. **9**:11-16.

9. **Pierson, D. L., V.J. Bassinger, T.C. Molina, E. Gunter, T.O. Groves, L.A. Cioletti, and S.K. Mishra.** 1993. Preflight and postflight microbiological results from 25 Space Shuttle crews. Society of Aeronautics Engineers (SAE) Publication No. 932139.

10. **Pierson, D. L., M.R. McGinnis, and A.N. Viktorov.** 1994. Microbiological Contamination. In: Sulzman, F. M., and A.M. Genin, eds. Foundations of Space Biology and Medicine, Volume II: Life Support and Habitability. Washington, D.C.: American Institute of Aeronautics and Astronautics.

11. **Putcha, L., and N.M. Cintron.** 1990. Pharmacokinetic consequences of spaceflight. In: Hrushesky, W. J., M. R. Langer, and F. Theeuwes, eds. Annals of the New York Academy of Sciences, Vol. 618. Proceedings of the Temporal Control of Drug Delivery Meeting. New York, NY. February 26-28, 1990.

12. **Taylor, G. R.** 1993. Immune changes during short-duration missions. J. Leukocute Biol. **54**:202-208.

13. **Taylor, G. R.** 1977. Medical microbiological analysis of U.S. crewmembers. In: Nicogossian, A. F., compiler. The Apollo-Soyuz Test Project Medical Report. Washington, D.C.: National Aeronautics and Space Administration, Scientific and Technical Information Office; p. 69-81.

14. **Tixador, R., G. Richoilley, G. Gasset, J. Templeir, J.C. Bes, N. Moatti, L. Lapchine.** 1985. Study of minimum inhibitory concentration of antibiotics on bacteria cultivation in space. Aviat. Space Environment. Med. 56:748-751.

Table 1. MICs determined using special Vitek cards on NCCLS-ATCC control strains following storage for 7-8 days at 4° C, then incubation for 16-18 h at 35° C.

S. aureus ATCC 29213		MIC (µg/ml)			
	(n)	mode	range	NCCLS range	% within NCCLS Range
penicillin	7	1	1	0.25-1	100
oxacillin	7	0.25	0.25-0.5	0.12-0.5	100
cephalothin	7	0.12	0.06-0.25	0.12-0.5	57
vancomycin	7	1	1-2	0.5-2	100
erythromycin	7	0.5	0.5	0.12-0.5	100
trim/sulfa	7	4	1-4	≤0.5	0
ciprofloxacin	7	1	1	0.12-0.5	0

E. faecalis ATCC 29212		MIC (µg/ml)			
	(n)	mode	range	NCCLS range	% within NCCLS Range
penicillin	7	4	4	1-4	100
oxacillin	7	>2	>2	8-32	N.D.
cephalothin	7	>0.5	>0.5	8-32	N.D.
vancomycin	7	2	2	1-4	100
erythromycin	7	4	2-4	1-4	100
trim/sulfa	7	16	16->16	≤0.5	0
ciprofloxacin	7	4	2-4	0.25-2	29

E. coli ATCC 25922		MIC (µg/ml)			
	(n)	mode	range	NCCLS range	% within NCCLS Range
ampicillin	6	8	4-8	2-8	100
amox/clav	6	8	8	2-8	100
cephalothin	6	16	8-32	4-16	83
cefuroxime	6	4	4	2-8	100
ceftazidime	6	0.25	0.12-0.25	0.06-0.5	100
gentamicin	6	-	1-2	0.25-1	50
tetracycline	6	4	4	1-4	100
trim/sulfa	6	0.25	0.25	≤0.5	100
ciprofloxacin	6	0.03	0.03	0.004-0.015	0

N.D. = Could not be determined based on concentrations tested.

E. coli ATCC 35218

	(n)	mode	range	MIC (µg/ml) broth microdilution	% within ± 1 dil
ampicillin	7	>32	>32	> 64	N.D.
amox/clav	7	16	8-16	4-16[a]	100[b]
cephalothin	7	16	16-32	16	100
cefuroxime	7	2	2	4	100
ceftazidime	7	0.12	0.12-0.25	0.25	100
gentamicin	7	1	1-2	0.5	43
tetracycline	7	8	4-8	2	43
trim/sulfa	7	1	0.5-1	0.5	100
ciprofloxacin	7	0.03	0.03	≤ 0.25	N.D.

P. aeruginosa ATCC 27853

	(n)	mode	range	MIC (µg/ml) NCCLS range	% within NCCLS range
ampicillin	7	>32	>32		N.D.
amox/clav	7	>64	>64		N.D.
cephalothin	7	>128	>128		N.D.
cefuroxime	7	>8	>8		N.D.
ceftazidime	7	2	2	1-4	100
gentamicin	7	4	4	0.5-2	0
tetracycline	7	>32	>32	8-32	0
trim/sulfa	7	>2	>2	8-32	N.D.
ciprofloxacin	7	>0.12	>0.12	0.25-1	N.D.

N.D. = Could not be determined based on concentrations tested.

[a] NCCLS approved range

[b] % within NCCLS approved range

Table 2. MICs determined using special Vitek cards on strains of clinical origin following storage for 7-8 days at 4° C, then incubated for 16-18 h at 35° C.

Group A *Streptococcus* 2726		MIC (µg/ml)			
	(n)	mode	range	broth microdilution	% within ± 1 dil
penicillin	7	0.016	0.016	≤0.015	100
oxacillin	7	0.06	≤0.03-0.06	≤0.125	100
cephalothin	7	0.03	0.03	≤0.25	100
vancomycin	7	0.5	0.5	≤0.25	100
erythromycin	7	≤0.06	≤0.06	≤0.06	100
trim/sulfa	7	≤0.25	≤0.25-2	>2	43
ciprofloxacin	7	1	1	≤0.25	0

K. pneumoniae 2588		MIC (µg/ml)			
	(n)	mode	range	broth microdilution	% within ± 1 dil
ampicillin	6	>32	>32	32	100
amox/clav	6	64	64	N.T.	N.D.
cephalothin	6	>128	>128	>64	100
cefuroxime	6	2,4	2-4	8	50
ceftazidime	6	0.5	0.5	1	100
gentamicin	6	1	1-4	0.5	83
tetracycline	6	4,8	4-8	≤1	0
trim/sulfa	6	0.25	0.25	≤0.25	100
ciprofloxacin	6	0.03	0.03	≤0.25	N.D.

E. cloacae 2596		MIC (µg/ml)			
	(n)	mode	range	broth microdilution	% within ± 1 dil
ampicillin	6	>32	>32	16	0
amox/clav	6	64	64->64	N.T.	N.D.
cephalothin	6	>128	>128	>64	N.D.
cefuroxime	6	4	4-8	32	0
ceftazidime	6	0.25	0.25	N.T.	N.D.
gentamicin	6	1	1-4	0.5	83
tetracycline	6	8	8	4	100

trim/sulfa	6	0.25	0.25	N.T.	N.D.
ciprofloxacin	6	0.03	0.03-0.06	≤0.25	N.D.

N.D. = Could not be determined based on concentrations tested.

N.T. = Not tested.

Table 3. MICs determined using special Vitek cards on NCCLS ATCC control strains following storage for 7-8 days at 4° C, then incubated for 48 h at room temperature.

S. aureus ATCC 29213		MIC (µg/ml)			
	(n)	mode	range	NCCLS range	% within NCCLS Range
penicillin	6	0.5	0.25-1	0.25-1	100
oxacillin	6	0.25,0.5	0.25-0.5	0.12-0.5	100
cephalothin	6	0.06	0.015-0.06	0.12-0.5	0
vancomycin	6	0.25	0.25-0.5	0.5-2	50
erythromycin	6	0.06,0.25	0.06-0.25	0.12-0.5	50
trim/sulfa	6	0.25,1	0.25-1	≤0.5	50
ciprofloxacin	6	1	0.25-1	0.12-0.5	50

E. faecalis ATCC 29212		MIC (µg/ml)			
	(n)	mode	range	NCCLS range	% within NCCLS Range
penicillin	12	4	4	1-4	100
oxacillin	12	>2	>2	8-32	N.D.
cephalothin	12	>0.5	>0.5	8-32	N.D.
vancomycin	12	2	2	1-4	100
erythromycin	12	>16	16->16	1-4	0
trim/sulfa	12	16	8->16	≤0.5	0
ciprofloxacin	12	4	4	0.25-2	0

E. coli ATCC 25922		MIC (µg/ml)			
	(n)	mode	range	NCCLS range	% within NCCLS Range
ampicillin	9	4	4-8	2-8	100
amox/clav	9	8	4-8	2-8	100
cephalothin	9	≤4	≤4-8	4-16	100
cefuroxime	9	2	1-2	2-8	67
ceftazidime	9	0.12	0.12-0.25	0.06-0.5	100
gentamicin	9	4	4	0.25-1	0
tetracycline	9	4	4-8	1-4	78
trim/sulfa	9	0.5	0.25-0.5	≤0.5	100
ciprofloxacin	9	0.008	0.008	0.004-0.015	100

N.D. = Could not be determined based on concentrations tested.

E. coli ATCC 35218

	(n)	mode	range	MIC (µg/ml) broth microdilution	% within ± 1 dil
ampicillin	9	>32	>32	> 64	N.D.
amox/clav	9	8	8-16	4-16[a]	100[b]
cephalothin	9	8	≤4-32	16	89
cefuroxime	9	1	≤0.5-2	4	33
ceftazidime	9	0.12	≤0.06-0.12	0.25	78
gentamicin	9	2,4	1-4	0.5	22
tetracycline	9	8	≤1-8	2	11
trim/sulfa	9	1	≤0.06-1	0.5	78
ciprofloxacin	9	0.016	≤0.004-0.016	≤ 0.25	N.D.

P. aeruginosa ATCC 27853

	(n)	mode	range	MIC (µg/ml) NCCLS range	% within NCCLS Range
ampicillin	9	>32	>32	-	N.D.
amox/clav	9	>64	>64	-	N.D.
cephalothin	9	>128	>128	-	N.D.
cefuroxime	9	>8	>8	-	N.D.
ceftazidime	9	2	2	1-4	100
gentamicin	9	4	4	0.5-2	0
tetracycline	9	>32	>32	8-32	0
trim/sulfa	9	>2	>2	8-32	N.D.
ciprofloxacin	9	>0.12	>0.12	0.25-1	N.D.

N.D. = Could not be determined based on concentrations tested.

[a] NCCLS approved range

[b] % within NCCLS approved range

Table 4. MICs determined using special Vitek cards on strains of clinical origin following storage for 7-8 days at 4° C, then incubated for 48 h at room temperature.

Group A *Streptococcus* 2726		MIC (μg/ml)			
	(n)	mode	range	broth microdilution	% within ± 1 dil
penicillin	12	0.008	≤0.008-0.016	≤0.015	100
oxacillin	12	≤0.03	≤0.03-0.06	≤0.12	N.D
cephalothin	12	0.015	≤0.008-0.015	≤0.25	N.D.
vancomycin	12	0.25	≤0.12-0.25	≤0.25	100
erythromycin	12	≤0.06	≤0.06	≤0.06	100
trim/sulfa	12	≤0.25	≤0.25-0.5	>2	0
ciprofloxacin	12	1	0.5-1	≤0.25	58

K. pneumoniae 2588		MIC (μg/ml)			
	(n)	mode	range	broth microdilution	% within ± 1 dil
ampicillin	12	>32	>32	32	100
amox/clav	12	>64	>64	N.T.	N.D.
cephalothin	12	>128	>128	>64	100
cefuroxime	12	4	4-8	8	100
ceftazidime	12	0.5	0.5-2	1	100
gentamicin	12	2	2-4	0.5,1	67
tetracycline	12	8	4->32	≤1	0
trim/sulfa	12	0.5	0.25-0.5	≤0.25	100
ciprofloxacin	12	0.03	0.03-0.06	≤0.25	N.D.

E. cloacae 2596		MIC (μg/ml)			
	(n)	mode	range	broth microdilution	% within ± 1 dil
ampicillin	12	>32	>32	16	0
amox/clav	12	>64	>64	N.T.	N.D.
cephalothin	12	>128	>128	>64	100
cefuroxime	12	8	8->8	32	0
ceftazidime	12	0.05	0.05	N.T.	N.D.
gentamicin	12	2	2-4	0.5	0
tetracycline	12	8	8->32	4	50

trim/sulfa	12	0.5	0.12-0.5	N.T.	N.D.
ciprofloxacin	12	0.03	0.016-0.06	≤0.25	N.D.

N.D. = Could not be determined based on concentrations tested.

N.T. = Not tested