

Local Production of Low Cost Quality Antibiotic Susceptibility Disks for the Philippines*

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The success of treatment of infections depends on the use of appropriate antimicrobial agents directed against the pathogen. In the absence of laboratory facilities for the isolation, identification and susceptibility testing of the microbial agents, the prevailing antibiotic susceptibility patterns in the regions usually serve as the clinical guide in the choice of the appropriate antimicrobial. Many hospitals and clinics have facilities for antimicrobial susceptibility testing but various constraints result in the use of inappropriate antibiotic sensitivity disks. As a consequence, antibiograms frequently include only a list of many heavily promoted drugs, the disks of which are given free, but exclude the older less expensive drugs whose patents have lapsed. The disks of these drugs, which are no longer being promoted, have to be purchased at a cost ranging from eighty centavos to one peso and fifty centavos per piece. The exclusion of disks which have to be purchased generates data biased towards the use of the newer antimicrobial drugs that are being heavily promoted, and which are generally more expensive than the older drugs like penicillin, erythromycin, tetracycline and chloramphenicol. The economic implications are clearly disadvantageous for the cost of medical care. Community acquired infections, with some exceptions, continue to remain susceptible to common antimicrobial drugs¹ and it is for these infections that inclusion of the older less expensive antimicrobial drugs in the antibiograms offers the most benefits.

Local production of antibiotic susceptibility disks is one possible solution to this problem of inappropriate antibiograms. The immediate benefits expected are continuous local supply of disks for useful and less expensive antibiotics for testing common pathogens in the laboratories; significant reduction in demand and use of newer expensive antimicrobials and more importantly patterns can be utilized as a comprehensive guide for the choice of appropriate antimicrobials for treatment of infections. The choice of less expensive antibiotics in the treatment of infections among Filipino patients should be expected to significantly cut the cost of medical care. The feasibility of producing antibiotic susceptibility disks was explored by this group of researchers with the following objectives:

1. To produce quality antimicrobial susceptibility disks and to determine the various factors which influence the quality of disks.
2. To determine the most cost efficient method of disk production;
3. To provide adequate supply of antibiotic susceptibility disks to the Microbiology Laboratory and other hospitals in the country; and
4. To help reduce demand and use of expensive antibiotics in the community.

MATERIALS AND METHODS

Materials

Paper

Four types of paper, namely blotting paper made from imported raw materials but is locally manufactured, Whatman Filter paper #3, Whatman Chromatographic #2 and #3, and Neigele chromatographic paper which are all imported were selected for preparing the disks. The selections were based on its ability to uniformly absorb sufficient volumes of antibiotic solution. These materials including imported blank disks were initially tested for presence of inhibitory activity. Commercially available antibiotic disks (BBL) served as the control.

Standard Antibiotic Powders

Ampicillin, oxacillin and tetracycline were obtained from Bristol Laboratories while chloramphenicol, methicillin and nalidixic acid were bought from Sigma Chemicals (USA).

Tests organisms for quality control

Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 were used as organisms for quality control of antibiotic disks.

Culture media

Mueller Hinton Agar (Difco) and Tryptic Soy Broth (Difco) were used.

Petri dishes (150 x 20 mm)

Micropipette (Oxford)

Silica gel Screw capped vials

Hole puncher

Production Methods

Labeling of paper disks

To facilitate identification of disks, code names of antibiotics and their corresponding potency were printed on the sheets of paper before holes were punched from the four types of paper.

Preparation of paper disks

Using an ordinary office two-hole puncher, paper disks with approximate diameter of 6.3 mm. were punched out one by one from a sheet of paper, Precautions were taken to avoid overlapping of holes, Since the paper disks had a tendency to curl after punching, these were flattened by spreading them in a single-layer on a clean smooth surface then pressed by rolling a bottle repeatedly. The disks were placed in vials then autoclaved for 15 minutes at 15 lbs. pressure and allowed to cool.

Preparation of antibiotic solutions

The concentrations of the antibiotic solution expressed in mcg/ml were based on the potency per disk prescribed by WHO² and the National Committee on Clinical Laboratory Standards³ (NCCLS) of the United States. It was assumed that a paper disk could absorb 0.02 ml of solutions. In the preparation of ampicillin with a potency of 10 mcg, the antibiotic solution

must contain 500 mcg of ampicillin per ml of solution. The following formula was used in determining the amount of antibiotic powder to be used:⁴

$$\text{Vol. (ml)} \times \text{desired conc. (ug/ml)} \text{ Weight (mg)} = \text{Potency (ug/mg)}$$

Example:

$$5 \text{ ml} \times 500 \text{ ug/ml} \text{ Weight (mg)} = 860 \text{ ug/ml} = 2.91 \text{ mg}$$

The antibiotic powders were dissolved in their appropriate solvents and further diluted in distilled water, Appendix I.

Impregnation of disk

Two methods of impregnating antibiotics to disks were employed. In the immersion method, blank disks were soaked in known concentration of antibiotics and then allowed to dry for 2 hours. In the pipette delivery method, the sterile disks were placed in petri dishes (150 x 20 mm) approximately 5mm apart. Using a mechanical pipettor with a fixed volume delivery of 0.02 ml, the disks were loaded with antibiotic solutions one by one, taking precautions that the tip was in light contact with the disk.

Drying and storage

Without covering the petri dishes, the disks were allowed to dry in a clean incubator at 35°C for 2-3 hours.⁵ After drying, 50 to 100 disks were placed in small sterile air-tight labelled containers with a dessicant at the bottom. A layer of sterile cotton or foam was placed over the dessicant to avoid contact with the disks. The disks were stored in a freezer at -14°C. Unopened containers were removed from the freezer 1 or 2 hours before use to equilibrate to room temperature before this is opened to minimize the amount of condensation that may occur when warm room air reaches the cold containers.

Performance Test

The first batch of locally produced disks were tested after preparation to determine if the zone of inhibition produced fell within the limits set by WHO or NCCLS for the control organisms *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Initial testing was done five times, then two times every month and five times on the sixth month after preparation. A selected batch of commercially prepared disks (BBL) and blank disks were used simultaneously. The procedures applied in doing the sensitivity tests were in accordance with those prescribed by WHO⁶ for the modified Kirby-Bauer technique. With the use of a sliding caliper the zones of inhibition were measured after 16-18 hours of incubation and recorded.

RESULTS

Evaluation of performance

The performance of different types of paper is seen in Table 3. The performance of the prepared antibiotic disks was evaluated using *S. aureus* ATCC 25923 as control organism. The mean zone diameters (in mm) of the control (A), blank (B), blotting paper (C) and filter paper (D) disks for ampicillin were 27.28, 24.48, 27.22 and 28.00 mm respectively, and were within the limits of the expected zone diameters of 27.35 mm, Table 1.

The mean zone diameters produced by penicillin, erythromycin, chloramphenicol, nalidixic acid and trimethoprim/sulfa disks were also within the limits of the expected .zone diameter regardless

of the materials used. Table 3 further shows that 90% confidence intervals of the mean zone diameters were within the said limits.

Table 1. Quality Control Susceptibility of Control Strains*

Antibiotic Disk	Expected Range Zone Diameters of Inhibition (mm)		
	Potency	S. aureus (ATCC 25923)	E. coli (ATCC 25922)
Ampicillin	10 mcg	24 - 35	15 - 20
Chloramphenicol	30 mcg	19 - 26	21 - 27
Erythromycin	15 mcg	22 - 30	-
Methicillin	5 mcg	17 - 22	-
Nalidixic Acid	30 mcg	-	23 - 28
Oxacillin	1 mcg	18 - 24	-
Penicillin G	10 u	26- 37	-
Tetracycline	30 mcg	19 - 28	18 - 25
Trimethoprim/Sulfa	1.25/23.75 mcg	24- 32	24 - 32

*From WHO Guidelines for Antimicrobial Susceptibility Testing •-.

Table 2. Criteria for Interpretation Based on Kirby-Bauer Method* (Disk diameter 6.3 mm)

Antibiotic or Chemotherapeutic Agent	Disk Potency	Diameter of Zone Inhibition (mm)		
		Resistant	Intermediate	Susceptible
Ampicillin vs	10 mcg			
Gram (-) enteric organisms		11 or less	12 - 13	14 or more
Staphylococci		20 or less	21 - 28	29 or more
Penicillin susceptible organisms		19 or less		20 or more
Hemophilus sp.				
Penicillin G	10 U			
vs Staphylococci		20 or less	21 - 28	29 or more
vs other organisms		11 or less	12 - 21	32 or more
Chloramphenicol	30 mcg	12 or less	13 - 17	18 or more
Erythromycin	15 mcg	13 or less	14 - 17	18 or more
Methicillin	5 mcg	9 or less	10 - 13	14 or more
Nalidixic acid	30 mcg	13 or less	14 - 18	19 or more
Oxacillin	1 mcg	10 or less	11 - 12	13 or more
Tetracycline	30 mcg	14 or less	15 - 18	19 or more
Trimethoprim/ Sulfa	1.25/23.75	10 or less	11 - 15	16 or more

*From WHO Guidelines for Antimicrobial Susceptibility Testing

Comparison of two methods of impregnating antibiotics to paper disks

Thirty mcg tetracycline disks impregnated with antibiotic by pipette delivery produced mean zone diameters of 21.78 and 21.44 mm for Disks D and E, respectively, while Disks D and E impregnated by immersion method had mean zone diameters of 20.50 and 20.30 mm, respectively, Table 4. Although analysis of variance showed that a statistically significant difference was demonstrated between the two methods, all the mean zone sizes and their 90% confidence interval were within the limits of the expected range for 30 mcg Tetracycline disk.

Performance against common bacteria

Only disks prepared from filter and chromatographic paper were chosen for testing against common bacteria. Blotting paper was temporarily excluded. Although initial evaluation proved its comparability with the other materials, blotting paper was temporarily excluded because rigid production quality-control procedures were not performed as routinely applied to Whatman filter and chromatographic paper. Table 5A, 5B and 5C show the mean zone diameters produced by control (A), filter paper (D) and chromatographic paper disks (E) tested against

common bacteria. Results showed that the mean zone diameters and the 90% confidence intervals produced, by ampicillin, penicillin, chloramphenicol, erythromycin, methicillin, trimethoprim/sulfa and nalidixic acid were within the limits of the expected range, Table 2.

Table 3. Performance of Disks Made from Different Types of Papers

Antibiotic (No. of Tests)	Mean Zone Diameter (mm)				
	Control (A)	Blank (B)	Blot (C)	Filter (D)	Chroma (E)
	Mean (90% CI)	Mean (90% CI)	Mean (90% CI)	Mean (90% CI)	Mean (90% CI)
Ampicillin 10 mcg (9) (ER* = 27-35 mm)	27.28 (26.74-27.2)	27.48 (27.03-27.93)	27.22 (26.86-27.86)	28.00 (27.41-28.59)	29.33 (29.05-29.61)
Penicillin 10 units (10) (ER = 26-37 mm)	30.42 (29.96-30.88)	31.41 (31.15-31.67)	31.09 (30.61-31.57)	31.17 (30.89-31.45)	30.76 (30.60-30.92)
Oxacillin 1 mg (10) (ER = 18.24 mm)	19.07 (18.71-19.47)	-	-	21.89 (20.75-23.03)	21.00 (20.19-21.81)
Methicillin 5 mcg (10) (ER = 17.22 mm)	18.95 (18.2-19.08)	-	-	21.25 (20.89-21.61)	20.65 (20.40-20.90)
Erythromycin 15 mcg (10) (ER = 23.30 mm)	21.57 (21.26-21.88)	22.32 (22.06-22.58)	22.76 (22.53-22.99)	23.49 (23.09-23.89)	23.28 (22.98-23.58)
Chloramphenicol 30 mcg (10) (ER = 21.27 mm)	23.06 (22.83-23.29)	24.50 (23.94-25.06)	25.45 (24.86-26.04)	24.99 (24.25-25.53)	23.21 (23.04-23.38)
Tetracycline 30 mcg (10) (ER= 18.25 mm)	21.24 (20.98-21.50)	20.77 (20.52-21.02)	20.20 (20.02-20.78)	22.34 (22.18-22.50)	21.29 (21.11-21.47)
Trimethoprim/sulfa 1.25/23.75 mcg (10) (ER = 23-30 mm)	26.72 (26.46-26.98)	28.64 (28.28-29.00)	29.08 (28.77-29.39)	28.91 (28.56-29.26)	27.99 (27.71-28.27)
Nalidixic Acid 30 mcg (10) (ER = 22.28 mm)	22.37 (22.12-22.62)	24.56 (24.21-24.91)	24.53 (24.28-24.78)	24.97 (24.74-25.20)	24.01 (23.66-24.36)

*ER = Expected Range

Table 4. Comparison of Two Methods of Impregnation of Antibiotic to Paper Disks

Antibiotic Concentration	Mean Zone Diameter (mm)			
	Pipette Delivered		Immersion	
	Filter (D) n=5	Chroma (E) n=5	Filter (D) n=5	Chroma (E) n=5
(Tetracycline 30 mcg) 1500 mcg/ml				
30mcg/0.02ml 90% C.I.	21.78 21.38-22.18	21.44 21.23-21.65	20.50 20.22-20.78	20.30 20.10-20.50
3000 mcg/ml 30 mcg/0.1 ml 90% C.I.	22.26 22.08-22.44	22.08 21.88-22.28	21.70 21.34-22.06	21.64 21.51-21.77

Zone diameter limits for Tetracycline= 18-25mm (WHO and NCCLS)

Mean zone diameter of control disk = 18.58 mm

Evaluation of Stability:

The stability of the first batch of antibiotic disks was determined.

a) Beta-lactam antibiotics (ampicillin, penicillin, oxacillin, methicillin). The mean zone diameter produced by beta-lactam antibiotic disks after 4-6 months of storage were within the acceptable zone limits for all the beta-lactam antibiotics and the 90% confidence interval of the mean is also within the limits, Table 6A.

b) Erythromycin, chloramphenicol, tetracycline, trimethoprim/sulfa and nalidixic acid. The mean zone diameter produced by erythromycin, chloramphenicol, tetracycline, trimethoprim/sulfamethoxazole, and nalidixic acid 4-6 months after storage did not differ significantly from the initial mean zone diameters and was all within the expected range. The results of microbiologic assays^{7,8} of content of control and test disks are not included in this report.

Table 5A. Performance of Locally Produced Chloramphenicol and Ampicillin Disks Compared with Control Disks Tested Against Common Gram-Negative Bacteria

Antibiotic	No. of Tests	Mean Zone Diameter (mm)		
		Control (A)	Filter (D)	Chroma (E)
		Mean (90% CI)	Mean 90% (CI)	Mean (90% CI)
Ampicillin 10 mcg				
E. coli	25	20.34 (19.48-21.20)	20.22 (19.28-21.16)	20.46 (19.55-21.37)
Salmonella sp.	25	22.90 (22.42-23.38)	22.82 (22.32-23.32)	22.70 (22.14-23.26)
Proteus sp.	20	22.38 (22.25-22.51)	21.97 (21.81-22.10)	21.75 (21.60-21.90)
Acinetobacter	20	16.76 (16.55-16.97)	15.92 (15.62-16.22)	16.07 (15.76-16.38)
H. influenzae	25	28.94 (27.57-30.31)	28.97 (27.50-30.44)	28.98 (27.58-30.38)
Chloramphenicol 30 mcg				
E. coli	25	21.76 (21.28-22.24)	23.32 (22.84-23.80)	23.31 (22.90-23.72)
Klebsiella sp.	20	19.90 (19.60-20.20)	21.80 (21.30-22.30)	21.86 (21.50-22.22)
Enterobacter sp.	25	18.86 (18.58-19.14)	20.45 (20.17-20.73)	20.21 (19.86-20.56)
Salmonella sp.	15	20.73 (20.60-20.86)	22.61 (22.43-22.79)	22.43 (22.25-22.61)
Proteus sp.	25	19.64 (19.36-19.92)	20.84 (20.46-21.22)	20.82 (20.46-21.18)
Acinetobacter	20	19.92 (19.10-20.74)	21.79 (21.05-22.53)	21.66 (20.93-22.39)
H. influenzae	25	34.38 (33.49-35.27)	35.59 (34.70-36.48)	35.40 (34.54-36.26)

Table 5B. Performance of Locally Produced Nalidixic Acid, Tetracycline and Trimethoprim/Sulfa Disks Compared with Control Disks Tested Against Common Bacteria

Antibiotic	No. of Tests	Mean Zone Diameter (mm)		
		Control (A)	Filter (D)	Chroma (E)
		Mean (90% CI)	Mean 90% (CI)	Mean (90% CI)
Nalidixic Acid 30 mcg				
E. coli	25	23.65 (23.22-24.08)	25.61 (25.16-26.06)	25.12 (24.69-25.55)
Klebsiella sp.	25	21.42 (20.76-22.08)	23.48 (22.82-24.14)	22.90 (22.26-23.54)
Enterobacter sp.	25	21.91 (21.56-22.26)	24.03 (23.70-24.36)	23.46 (23.13-23.79)
Salmonella sp.	25	21.65 (21.27-22.03)	23.84 (23.46-24.22)	23.34 (22.91-23.77)
Acinetobacter	25	19.37 (19.06-19.68)	21.58 (21.28-22.88)	20.97 (20.62-21.32)
Tetracycline 30 mcg				
E. coli	25	19.02 (18.86-19.18)	20.39 (20.16-20.62)	20.56 (20.35-20.77)
Klebsiella sp.	25	19.96 (19.66-20.26)	21.97 (21.69-22.25)	21.90 (21.60-22.20)
Enterobacter sp.	25	18.96 (18.61-19.31)	21.12 (20.69-21.55)	21.08 (20.62-21.54)
Salmonella sp.	15	19.38 (19.10-19.66)	20.96 (20.65-21.27)	20.94 (20.58-21.30)
Proteus sp.	15	19.83 (19.48-20.18)	21.53 (20.95-21.11)	21.69 (21.11-22.27)
Acinetobacter	25	19.20 (19.02-19.38)	21.55 (21.41-21.86)	21.54 (21.24-21.84)
Trimethoprim/sulfa 1.25/23.75 mcg				
E. coli	25	24.95 (24.32-25.58)	25.41 (24.77-26.05)	25.44 (24.85-26.03)
Klebsiella sp.	20	22.78 (21.48-24.08)	23.42 (22.00-24.84)	23.57 (22.33-24.81)
Enterobacter sp.	25	21.35 (20.49-22.21)	22.47 (21.56-23.38)	22.32 (21.35-23.29)
Salmonella sp.	25	24.43 (23.56-25.30)	25.72 (24.93-26.51)	25.88 (25.10-26.66)
Proteus sp.	25	25.66 (25.36-25.96)	26.89 (26.63-27.15)	26.85 (26.58-27.15)
Acinetobacter	25	17.97 (17.71-18.23)	18.68 (18.23-19.13)	18.64 (18.23-19.05)

DISCUSSION

The results of this study prove that quality antibiotic disks can be produced locally, provided production instructions and quality control requirements are strictly observed. The performance of the locally produced disks: penicillin 10 units, ampicillin 10 mcg, oxacillin 1 mcg, methicillin 5 mcg, erythromycin 15 mcg, chloramphenicol 30 mcg, tetracycline 30 mcg, nalidixic acid 30 mcg and trimethoprim/sulfa 1.25/23.75mcg is comparable to the commercially prepared antibiotic disks as shown by the results of tests against common bacteria isolated in the laboratory. Stability testing revealed that the antibiotic disks maintained their potency even after

storage for 4-6 months. Further testing in the year ahead may substantially lengthen the stability period and improve the flexibility of production schedules.

Table 5C. Performance of Locally Produced Penicillin, Erythromycin & Oxacillin Disks Compared with Control Disks Tested Against Common Bacteria

Antibiotic	No. of Tests	Mean Zone Diameter (mm)		
		Control (A)	Filter (D)	Chroma (E)
		Mean (90% CI)	Mean 90% (CI)	Mean (90% CI)
Erythromycin 15 mcg				
S. aureus	15	22.24 (21.74-22.74)	24.02 (23.52-24.52)	24.15 (23.62-24.68)
S. pneumonia	15	31.07 (30.81-31.33)	32.17 (31.91-32.43)	32.15 (31.85-32.45)
H. influenzae	25	21.88 (20.96-22.80)	22.90 (22.08-23.72)	23.08 (22.17-23.99)
Penicillin G 10 units				
S. aureus	25	29.48 (29.18-29.78)	30.96 (30.63-31.29)	31.13 (30.83-31.43)
S. pneumoniae	15	38.35 (38.09-38.61)	38.85 (38.52-39.18)	39.28 (38.95-39.61)
Oxacillin 1 mcg				
S. aureus	25	23.75* (23.34-24.16)	18.59 (18.16-19.02)	18.29 (17.9-18.65)

*A 5 mcg control disk was used due to unavailability of a m 1 mcg disk

Table 6A. Stability of Locally Produced Antibiotic Disks Compared With Control Disks

Antibiotic (No. of Tests)		Mean Zone Diameter (mm)				
		Control (A)	Blank (B)	Blot (C)	Filter (D)	Chroma (E)
		Mean (90% CI)	Mean (90% CI)	Mean (90% CI)	Mean (90% CI)	Mean (90% CI)
Ampicillin 10 mcg (ER* = 27-35 mm)						
Initial	(5)	27.45 (27.00-27.90)	27.50 (26.86-28.14)	27.50 (26.61-28.39)	-	-
	10	27.28 (26.74-27.82)	27.48 (27.03-27.93)	27.22 (26.86-27.58)	28.00 (27.41-28.59)	29.33 (29.05-29.61)
6 months	5	28.56 (28.35-28.77)	28.50 (28.24-28.76)	28.30 (27.94-28.66)	29.04 (28.43-29.65)	28.56 (28.31-28.81)
Penicillin 10 units (ER = 26-37 mm)						
Initial	5	30.72 (30.13-31.31)	31.50 (31.22-31.78)	31.56 (31.18-31.94)	31.08 (30.60-31.56)	- -
	10	30.42 (29.96-30.88)	31.41 (31.15-31.67)	31.09 (30.61-31.57)	31.17 (30.89-31.45)	30.76 (30.60-30.92)
6 months	5	28.82 (28.32-29.32)	29.36 (28.82-29.90)	29.84 (29.26-30.42)	30.80 (30.37-31.23)	30.09 (29.74-30.44)
Oxacillin 1 mg (ER = 18.24 mm)						
Initial	10	19.07 (18.71-19.23)	-	-	21.89 (21.53-22.25)	21.00 (21.19-21.81)
4 months	10	17.98 (17.88-18.08)	-	-	20.43 (19.98-20.88)	20.20 (19.56-20.84)
Methicillin 5 mcg (ER = 17-22 mm)						
Initial	10	18.95 (18.82-19.08)	-	-	21.25 (20.89-21.61)	20.65 (20.40-20.90)
3 months	10	18.80 (18.50-19.10)	-	-	20.13 (19.72-20.54)	19.88 (19.35-20.41)

The technology developed in this study can now be applied to selected microbiology laboratories. If better facilities can be provided, the production method can further be improved so that a bigger volume of antibiotic disks can be produced in the future. The inclusion of useful but less expensive antibiotics in susceptibility testing of common organisms encountered will not

only guide our medical practitioners in their choice of appropriate antimicrobial agents but will also facilitate cost efficiency and appropriate antimicrobial treatment. More importantly the availability of affordable quality disks is indispensable in the surveillance of the antimicrobial susceptibility of common community acquired infectious organisms. The economic viability of this project for large hospitals depends on volume production. It is estimated that at the current daily consumption of 25.30 disks each for the 6 antimicrobial disks developed in this project, the cost per disk would be 25% less than the price of imported disks. A four-fold increase in production would cut the cost to one-fourth the price of imported disks before packaging costs. Centralized production is preferred over small scale hospital based production units.

Table 6B. Stability of Locally Produced Antibiotic Disks Compared with Control Disks

Antibiotic (No. of Tests)		Mean Zone Diameter (mm)				
		Control (A)	Blank (B)	Blot (C)	Filter (D)	Chroma (E)
		Mean (90% CI)	Mean (90% CI)	Mean (90% CI)	Mean (90% CI)	Mean (90% CI)
Chloramphenicol 30mcg (ER = 21-27 mm)						
Initial	5	23.22 (22.89-23.55)	25.12 (24.38-25.86)	26.40 (26.07-26.73)	25.72 (25.19-26.25)	- -
	10	23.06 (22.83-23.29)	24.50 (23.94-25.06)	25.45 (24.86-26.04)	24.99 (24.45-25.53)	23.21 (23.01-23.41)
6 months	5	21.46 (21.23-21.69)	23.38 (22.92-23.84)	23.46 (23.11-23.81)	23.18 (22.67-23.69)	22.78 (22.62-22.94)
Tetracycline 30 mcg (ER = 18-25mm)						
Initial	5	21.16 (20.81-21.51)	20.54 (20.28-20.80)	20.32 (20.07-20.57)	22.30 (22.14-22.46)	- -
	10	21.24 (20.98-21.50)	20.77 (20.52-21.02)	20.40 (30.20-20.78)	22.34 (22.18-22.50)	21.29 (22.11-21.47)
6 months	5	19.46 (19.31-19.61)	19.56 (19.18-19.94)	18.88 (18.43-19.33)	21.76 (21.40-22.12)	20.93 (20.72-21.14)
Trimethoprim/sulfa 1.25/ 23.75 mcg (ER = 23-30mm)						
Initial	5	26.66 (26.30-27.02)	28.86 (28.45-29.27)	29.16 (28.14-30.18)	28.84 (28.41-29.27)	- -
	10	26.72 (26.46-26.98)	28.64 (28.28-29.00)	29.08 (28.77-29.39)	28.91 (28.56-29.26)	27.99 (27.71-28.27)
6 months	5	26.88 (26.6%29.09)	28.98 (28.85-29.11)	29.56 (29.28-29.84)	29.98 (29.88-30.08)	27.76 (27.55-27.97)
Nalidixic Acid 30 mcg (ER = 22-28mm)						
Initial	5	22.66 (22.40-22.92)	25.06 (24.60-25.52)	24.48 (24.13-24.83)	25.16 (24.75-25.57)	- -
	10	22.37 (22.12-22.62)	24.56 (24.21-24.91)	24.53 (24.28-24.78)	24.97 (24.74-25.20)	24.01 (23.66-24.36)
6 months	5	22.92 (22.72-23.12)	24.98 (24.85-25.11)	25.10 (24.79-25.41)	25.68 (25.58-25.78)	23.89 (23.68-24.10)
Erythromycin 15mcg (ER = 22-30mm)						
Initial	5	21.26 (21.00-21.52)	21.98 (21.72-22.24)	22.52 (22.16-22.88)	23.88 (23.48-24.28)	24.12 (23.64-24.60)
	10	21.57 (21.26-21.88)	22.32 (22.06-22.58)	22.76 (22.53-22.99)	23.49 (23.09-23.89)	23.28 (22.98-23.58)
6 months	5	22.28 (21.95-22.61)	22.96 (22.46-23.46)	23.88 (23.48-24.28)	23.62 (23.36-23.88)	24.12 (23.64-24.60)

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Appendix I

Antimicrobial	Solvent	Diluent
Ampicillin	Phosphate buffer pH 8.0	Distilled water
Chloramphenicol	Methanol	Distilled water
Erythromycin	Methanol	Distilled water
Methicillin	Distilled water	Distilled water
Nalidixic acid	NaOH, 0.1m	Distilled water
Oxacillin	Distilled water	Distilled water
Penicillin G	Distilled water	Distilled water
Sulfamethoxazole	0.85% NSS, 10% NaOH	Distilled water
Trimethoprim	Distilled water	Distilled water
Tetracycline	Methanol	Distilled water