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Quality Control Parameters for Broth Microdilution Susceptibility Testing of Amoxicillin and Amoxicillin-Clavulanic Acid

Two control strains of *Escherichia coli* are currently used for monitoring broth microdilution susceptibility tests of amoxicillin-clavulanic acid (1). The standard control strain (ATCC 25922) is susceptible to amoxicillin with or without clavulanic acid. A beta-lactamase-producing strain (ATCC 35218) is also used because it is susceptible to amoxicillin only in the presence of clavulanic acid or other beta-lactamase inhibitors. Both strains should be tested in order to control the two components in the amoxicillin-clavulanic acid combinations (2). At this time, there are no control limits for tests with amoxicillin since ampicillin is normally tested and the two are often assumed to be equivalent (data to be published).

Since amoxicillin-clavulanic acid might be used for treating infections due to some gram-positive microorganisms, in vitro susceptibility tests are required periodically. Microdilution panels that have been designed for testing gram-positive microorganisms are usually monitored by testing gram-positive control strains, but MIC control limits for such strains have not yet been developed. For that reason, we carried out a collaborative study that established MIC control limits for testing amoxicillin and amoxicillin-clavulanic acid (3). The two standard *Escherichia coli* control strains, *Enterococcus faecalis* (ATCC 29212) and two *Staphylococcus aureus* strains (ATCC 29213 and NCTC 11561) were all evaluated. The latter *Staphylococcus aureus* strain was selected because it produces beta-lactamase enzymes that are neutralized by clavulanic acid. Consequently, if the inhibitor is degraded during storage, the MIC for the latter control strain will increase but the beta-lactamase-negative control strains should not be affected.

All five of our laboratories prepared broth microdilution trays with dilutions of amoxicillin and amoxicillin-clavulanic acid (2:1 combination). Each participant used a different lot of cation adjusted Mueller-Hinton broth purchased from Difco Laboratories (USA). A sixth lot of broth was used to prepare control trays that were distributed to all participants for inclusion in the testing process. The procedures outlined by the National Committee for Clinical Laboratory Standards were followed as precisely as possible (1). The inocula were adjusted to give 3 to 5×10^5 cfu/ml in each well. Each participant tested the five control strains on 30 different occa-

Table 1: Results of replicated broth microdilution susceptibility tests of amoxicillin and amoxicillin plus clavulanic acid (CA) with five control strains in five independent laboratories. Proposed MIC limits are enclosed within brackets representing a four-dilution range when there is marked skewing away from the mode.

Antimicrobial Agent	No. of times each amoxicillin MIC[a] (μg/ml) was reported (n = 150)											
	0.06	0.12	0.25	0.5	1.0	2.0	4.0	8.0	16	32	64	>64
Staphylococcus aureus ATCC 29213												
Amoxicillin				[5	79	54	12]					
Amoxicillin + CA	3	[0	84	64	1]							
Staphylococcus aureus NCTC 11561												
Amoxicillin[b]							2	22[b]	6	0	0	[120]
Amoxicillin + CA				[36	105	9]						
Enterococcus faecalis ATCC 29212												
Amoxicillin			[1	123	26]							
Amoxicillin + CA			[1	145	4]							
Escherichia coli ATCC 25922												
Amoxicillin					1	0	[58	90	1]			
Amoxicillin +CA				1	4	[10	101	34]				
Escherichia coli ATCC 35218												
Amoxicillin												[150]
Amoxicillin +CA						4	[0	146	0]			

[a] Dilutions of amoxicillin or a 2:1 combination of amoxicillin plus clavulanic acid were tested and in both cases MICs were recorded as the concentration of amoxicillin that inhibited growth.
[b] One laboratory reported all 30 MICs to be 4.0 to 16 μg/ml, all others reported MICs > 64 μg/ml. There was no obvious explanation for those aberrant results.

sions, using 20 test lots of trays unique to that laboratory and 10 control lots of trays common to all participants. This exercise generated 150 MIC values for each drug-microorganism combination. Table 1 shows the distribution of those MICs.

With most drug-microorganism combinations, the modal MIC was easily identified and a three-dilution control range was proposed as the mode plus/minus one dilution. When *Staphylococcus aureus* ATCC 29213 is tested against either drug, a four-dilution range may be needed because of marked skewing toward the upper end of the usual three-dilution range. The two strains of *Escherichia coli* gave amoxicillin-clavulanic acid MICs that were within the currently recommended ranges (1). However, for the susceptible strains (ATCC 25922) amoxicillin MICs were one doubling dilution greater than the 2.0 to 8.0 μg/ml range that is described for tests with ampicillin (1). In the same way, *Staphylococcus aureus* ATCC 29213 gave amoxicillin MICs that were one doubling dilution greater than the 0.25 to 1.0 μg/ml limits that are proposed for tests with ampicillin (1). *Enterococcus faecalis*, on the other hand, gave amoxicillin MICs that were one doubling dilution lower than the 0.5 to 2.0 μg/ml that are proposed for ampicillin (1). Clearly, MIC control limits for ampicillin are not necessarily appropriate for tests with amoxicillin.

The two beta-lactamase-producing control strains (*Escherichia coli* ATCC 35218 and *Staphylococcus aureus* NCTC 11561) were fully resistant to amoxicillin (MIC > 64 μg/rml), although one laboratory reported that all 30 of their MICs for strain NCTC 11561 ranged from 4.0 to 16 μg/ml; these aberrant observations could not be explained. Both strains were inhibited by the amoxicillin-clavulanic acid combination, but modal MICs were one doubling dilution greater than those obtained with the susceptible strain of the same species.

The MIC control limits designated within brackets in Table 1 should provide useful guidelines for those who wish to establish a quality control program for broth microdilution susceptibility tests that include dilutions of amoxicillin and/or amoxicillin-clavulanic acid.

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Detection of Human Papilloma Virus Type 6 DNA in an Esophageal Squamous Cell Papilloma

Esophageal squamous cell papilloma (ESCP) has been regarded as a rare tumor (1). The etiology of ESCP had been unknown until 1982, when Syrjanen et al. (2) described an ESCP in which human papilloma virus (HPV) antigens were discovered by immunohistochemistry, implicating possible involvement of HPV in the development of these tumors. These findings were confirmed by other authors, demonstrating HPV-suggestive morphological changes and HPV 11 DNA in few

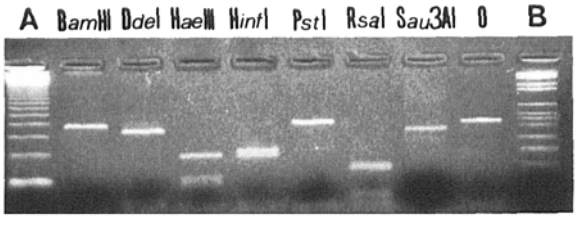

Figure 1: Endonuclease cleavage patterns of t he HPV PCR product from esophageal squamous cell papilloma. Ten µl of the PCR product (450 bp) was mixed with 10 U of each enzyme (*Bam*HI, *Dde*I, *Hae*III, *Hin*fI, *Pst*I, *Rsa*I and *Sau*3AI, all from Gibco BRL, USA) in the optimal buffer to reach a total volume of 15 µl. Reaction was performed in a 37 °C water bath for 1 h and stopped by adding 2 µl of DNA gel loading buffer. Digested bands were separated by electrophoresis in 3 % agarose gel prepared with 0.5 x TBE buffer. DNA ladder 123 bp (Gibco BRL) (lane A) and DNA molecular weight marker no. VI (Boehringer Mannheim) (lane B) were used for the determination of fragment sizes.

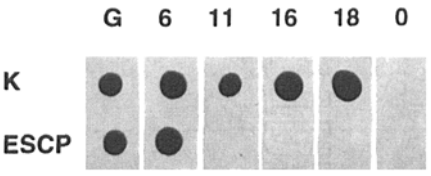

Figure 2: Dot-blot hybridization analyses of the PCR product from esophageal squamous cell papilloma. Three µl of the PCR products from HPV-positive esophageal squamous cell papilloma (lane ESCP) and HPV-positive tissue control specimens (lane K) were applied to each of six replicate filters. Each filter was separately hybridized with a ^{32}P-labelled, HPV L1 type-specific probe (MY12 for HPV6, MY13 for HPV11, MY14 for HPV16, and WD74 for HPV18) and HPV L1 generic probes (GP1 and GP2) (lane G) and autoradiographed for 10 h without an intensifying screen. Lane O, negative control.

ESCPs (3–5). In contrast, Chang et al. (1) were unable to detect the presence of HPV 6, 11, 16 or 18 DNA in 14 ESCPs using in situ hybridization and even the most sensitive polymerase chain reaction (PCR).

In the present study seven formalin-fixed, paraffin-embedded endoscopic biopsy specimens showing histologic characteristics typical for ESCP were screened for HPV infection by in situ hybridization and PCR. In situ hybridization was performed with HPV 6/11, 16/18 and 31/33/51 biotin-labelled probes from the Enzo PathoGene HPV kit (Enzo, USA) according to the manufacturer's directions. After DNA extraction PCRs that target a highly conserved portion (450 bp) of the L1 region of at least 25 types of HPVs were performed using the degenerative consensus primers MY09 and MY11 as described previously (6).

No HPV DNA was found in any of the esophageal tumors or the negative control tissues examined by in situ hybridization. Specific hybridization signals were observed only in our representative positive control tissues. On the other hand, using PCR we detected the presence of HPV DNA in one of the seven ESCPs (14.3 %). To determine the HPV type, the PCR product of the positive tumor was digested using seven restriction endonucleases and analyzed by agarose gel electophoresis (Figure 1). The restriction enzyme digestion patterns were found to be unique for HPV type 6. This finding was additionally confirmed by dot-blot hybridization analyses of the PCR product using ^{32}P-labelled HPV generic and type-specific probes (6); however, only HPV generic and HPV type 6 specific hybridization signals were observed (Figure 2). All other ESCPs were successfully amplified only with internal control human beta-globin primers PC04 and GH20 (Perkin Elmer, USA).