

## Comparison of Trends of Resistance Rates over 3 Years Calculated from Results for All Isolates and for the First Isolate of a Given Species from a Patient

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Received 29 April 2004/Returned for modification 11 June 2004/Accepted 5 July 2004

**We compared trends of annual resistance rates calculated from results for all isolates and for the first isolate of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* per patient over a 3-year period from 2001 through 2003. Antimicrobial susceptibility results of inpatients were extracted from a computerized database. Annual resistance rates of a species were calculated by two methods: (i) from results for all isolates, even those from patients with multiple isolates in a given year and (ii) from results for the first isolate from a patient in a given year, regardless of susceptibility profile or specimen type. Rates of methicillin-resistant *S. aureus* (MRSA) did not differ among all isolates (79.9, 78.8 and 79.6%;  $P = 0.86$ ), but decreased for the first isolate per patient (70.2, 65.7, and 64.1%;  $P = 0.006$ ) over time. Annual duplication rates of methicillin-susceptible *S. aureus* (MSSA) decreased (39.6, 37.6, and 31.7%;  $P = 0.01$ ), but those of MRSA increased significantly (64.3, 67.8, and 68.9%;  $P = 0.004$ ). Rates of cefotaxime-resistant *K. pneumoniae* did not differ over time by either method, and rates of imipenem-resistant *A. baumannii* decreased over time by both methods. Duplication rates did not differ for either susceptible or resistant isolates of *K. pneumoniae* and *A. baumannii*. The trends in MRSA rate differed by the two methods because of the different proportion of duplicate isolates per year. MRSA rates might be increasingly overestimated for all isolates. These results suggest that the method of calculating results for the first isolate per patient may remove the effect of duplication, allowing the simple and unambiguous analysis of cumulative susceptibility rates.**

Antibiograms are crucial to both the monitoring of susceptibility trends and the empirical selection of antimicrobial therapy. Generally, an antibiogram is a cumulative profile of antimicrobial susceptibility results for a given time period (2, 3). Since it may be desirable to remove duplicate isolates prior to constructing the antibiogram, several methods for defining duplicate isolates have been developed. Studies assessing the impact of duplicate isolates on antibiograms have evaluated time periods of 3 to 365 days for their removal (8, 10) or the use of automated laboratory management software (10).

To date, no consensus has been reached regarding the most appropriate and simplest method for removing duplicate isolates in clinical practice. Recently, the National Committee for Clinical Laboratory Standards (NCCLS) proposed and approved guidelines for analyzing and presenting cumulative antimicrobial susceptibility test data (2, 3). These guidelines recommend that results from the first isolate of a species from a patient per analysis period be used in calculating the percentage of susceptibility, regardless of the body site, antimicrobial susceptibility profile, or other phenotypic characteristics of the isolate. Although some investigators have supported the validity of these NCCLS guidelines because of their simplicity and lack of ambiguity (9), it is not known if application of these guidelines has any effect on trends in annual resistance rates.

We therefore compared trends in annual resistance rates over a 3-year period from 1 January 2001 through 31 December 2003, with and without the removal of duplicate isolates. These comparisons were applied to methicillin-resistant *Staphylococcus aureus* (MRSA), cefotaxime-resistant *Klebsiella pneumoniae* (CRKP), and imipenem-resistant *Acinetobacter baumannii* (IRAB).

### MATERIALS AND METHODS

The Gil Medical Center is a 1,200-bed tertiary-care teaching hospital which includes intensive care units containing 111 beds in Incheon, Korea. The average yearly admission rate is 46,000 patients. Susceptibility testing for clinical isolates was performed by disk diffusion or broth microdilution test with the Vitek (bioMérieux-Vitek, Hazelwood, Mo.) system. All results were classified according to NCCLS guidelines (4, 5).

Antimicrobial susceptibility results for samples from inpatients from 1 January 2001 through 31 December 2003 were extracted from the software package of the Gil Medical Center using the Healthcare Infection Control and Antibiotic Management (HICAM) system (MediCyberCare Co., Ltd., Incheon, Korea). The susceptibility data for *S. aureus*, *K. pneumoniae*, and *A. baumannii* isolation over this 3-year period were transferred to Excel 2000 (Microsoft Corp., Redmond, Wash.) without including the results from surveillance specimens. Isolates with intermediate resistance were classified as resistant. Isolates of *S. aureus* were classified as methicillin-susceptible *S. aureus* (MSSA) or MRSA as determined by the oxacillin disk diffusion or broth microdilution test. Oxacillin screening plates or molecular detections of the *mecA* gene were not performed with clinical isolates of *S. aureus*. *K. pneumoniae* isolates were classified as cefotaxime susceptible (CSKP) or CRKP as determined by disk diffusion or broth microdilution, regardless of extended-spectrum  $\beta$ -lactamase production. The initial screening and phenotypic confirmatory tests recommended by NCCLS were used for the production of extended-spectrum  $\beta$ -lactamase (4, 5). *A. baumannii* isolates were classified as imipenem-susceptible *A. baumannii* (ISAB) or IRAB.

The susceptibility data were sorted in chronological order by date of suscep-

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TABLE 1. Trends of resistance rates calculated from results for all isolates and for the first isolate of a given species per patient from 2001 to 2003

Organism	Year and no. of isolates <sup>a</sup>			P value
	2001	2002	2003	
<b>MRSA</b>				
All isolates	1,661/2,078 (79.9)	2,083/2,644 (78.8)	2,062/2,589 (79.6)	0.86
First isolate per patient <sup>b</sup>	593/845 (70.2)	670/1,020 (65.7)	642/1,002 (64.1)	0.006
<b>CRKP</b>				
All isolates	379/795 (47.7)	1,083/1,676 (64.6)	528/1,090 (48.4)	0.46
First isolate per patient	145/452 (32.1)	300/660 (45.5)	176/603 (29.2)	0.13
<b>IRAB</b>				
All isolates <sup>b</sup>	425/1,375 (30.9)	154/1,389 (11.1)	70/1,314 (5.3)	<0.0001
First isolate per patient <sup>b</sup>	107/538 (19.9)	51/629 (8.1)	22/580 (3.8)	<0.0001

<sup>a</sup> Data represent the number of resistant isolates relative to all isolates or patients. Values in parentheses represent percentages.

<sup>b</sup> Trends over 3 years for which *P* values were <0.05 by the chi-square test for trends.

tibility testing, patient identification number, and specimen type to facilitate subsequent analyses. Annual resistance rates of a given species were calculated by two methods. The first method used the results for all isolates, even for those from patients with multiple isolates in a given year. The second method used the results for the first isolate per patient in a given year, regardless of susceptibility profile, body source, or specimen type.

The annual rates of MRSA, CRKP, and IRAB as calculated by the two methods over this 3-year period were compared by the chi-square test for trends. The annual duplication rate of a given species was expressed as the number of duplicate isolates relative to all isolates and compared by the chi-square test for trends. All isolates of a species from a patient, other than the first isolate, were considered duplicate isolates of that species, regardless of susceptibility profile, body source, or specimen type. All statistical analyses were performed with Epi-Info, version 6.0 (Centers for Disease Control and Prevention, Atlanta, Ga.). All tests were two-tailed, and a *P* value of <0.05 was considered statistically significant.

## RESULTS

From 1 January 2001 through 31 December 2003, there were a total of 7,311 isolates of *S. aureus* from 2,867 patients, 3,561 isolates of *K. pneumoniae* from 1,715 patients, and 4,078 isolates of *A. baumannii* from 1,747 patients. The trends of MRSA, CRKP, and IRAB isolation rates calculated from for all isolates and for the first isolates per patient over the 3-year study period are shown in Table 1. When all isolates were considered, the annual rates of MRSA isolation did not differ significantly over the 3-year period (79.9, 78.8, and 79.6%,

respectively; *P* = 0.86). When only the first isolate per patient was considered, the annual rates of MRSA isolation decreased significantly over time (70.2, 65.7, and 64.1%, respectively; *P* = 0.006). Annual rates of CRKP isolation did not differ significantly by either method (47.7, 64.6, and 48.4%, respectively, and *P* = 0.46 for all isolates; and 32.1, 45.5, and 29.2%, respectively, and *P* = 0.13 for first isolates alone). In contrast, the annual rates of IRAB isolation showed a significantly decreasing trend by both methods (30.9, 11.1, and 5.3%, respectively, and *P* < 0.0001 for all isolates; and 19.9, 8.1, and 3.8%, respectively, and *P* < 0.0001 for first isolates alone).

The trends of duplication rates calculated from results for all isolates of the three species over the 3-year study period are shown in Table 2. For *S. aureus*, the annual duplication rates of MSSA decreased significantly over time (39.6, 37.6, and 31.7%, respectively; *P* = 0.01), whereas the duplication rates of MRSA increased significantly (64.3, 67.8, and 68.9%, respectively; *P* = 0.004). For *K. pneumoniae* and *A. baumannii*, however, the annual duplication rates did not differ significantly for either susceptible or resistant isolates.

In the 2,867 patients from whom *S. aureus* was isolated, 1,425 (49.7%) had only one isolate, whereas 1,442 (50.3%) had two or more isolates. Of the 1,715 patients from whom *K. pneumoniae* was isolated, 1,122 (65.4%) had only one isolate,

TABLE 2. Trends of duplication rates calculated from results for all isolates of a given species from 2001 to 2003

Organism and resistance	Year and no. of isolates <sup>a</sup>			P value
	2001	2002	2003	
<i>S. aureus</i>				
Methicillin susceptible <sup>b</sup>	165/417 (39.6)	211/561 (37.6)	167/527 (31.7)	0.01
Methicillin resistant <sup>b</sup>	1,068/1,661 (64.3)	1,413/2,083 (67.8)	1,420/2,062 (68.9)	0.004
<i>K. pneumoniae</i>				
Cefotaxime susceptible	109/416 (26.2)	233/593 (39.3)	1,335/562 (24.0)	0.20
Cefotaxime resistant	234/379 (61.7)	783/1,083 (72.3)	352/528 (66.7)	0.26
<i>A. baumannii</i>				
Imipenem susceptible	519/950 (54.6)	657/1,235 (53.2)	686/1,244 (55.1)	0.75
Imipenem resistant	318/425 (74.8)	103/154 (66.9)	48/70 (68.6)	0.08

<sup>a</sup> Data represent the number of duplicate isolates relative to all isolates. Values in parentheses represent percentages.

<sup>b</sup> Trends over 3 years for which *P* values were <0.05 by the chi-square test for trends.

TABLE 3. Antibiogram changes among patients with duplicate isolates

No. of patients	Organism and no. of patients <sup>a</sup>		
	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>A. baumannii</i>
Without duplicate isolates	1,425 (49.7)	1,122 (65.4)	944 (54.0)
With duplicate isolates	1,442 (50.3)	593 (34.6)	803 (46.0)
All susceptible <sup>b</sup>	209 (14.5)	204 (34.4)	642 (80.0)
All resistant <sup>b</sup>	1,051 (72.9)	264 (44.5)	64 (8.0)
Susceptible to resistant <sup>b</sup>	127 (8.8)	62 (10.5)	63 (7.8)
Resistant to susceptible <sup>b</sup>	55 (3.8)	63 (10.6)	34 (4.2)
Total	2,867	1,715	1,747

<sup>a</sup> Data represent the number of patients. Values in parentheses represent percentages.

<sup>b</sup> Values in parentheses are the percentage of patients with duplicate isolates.

whereas 593 (34.6%) had two or more isolates. Of the 1,747 patients from whom *A. baumannii* was isolated, 944 patients (54.0%) had only one isolate, whereas 803 (46.0%) had two or more isolates. Among the patients who had duplicate isolates of a given species, 87.4% of patients with *S. aureus*, 78.9% with *K. pneumoniae*, and 87.9% with *A. baumannii* had the same antibiogram type of susceptible or resistant strain in each repeat isolate (Table 3). Of the 1,442 patients who presented with *S. aureus*, 127 (8.8%) initially presented with an MSSA and then presented with an MRSA, with the median time between these changes being 8 days (interquartile range [IQR], 5 to 27 days). In contrast, 55 of these patients (3.8%) initially presented with an MRSA, which later changed to an MSSA, with a median interval of 45 days (IQR, 10 to 94 days). For the 593 patients isolated with *K. pneumoniae*, 62 (10.5%) presented with isolates that changed from CSKP to CRKP, with a median interval of 8.5 days (IQR, 4 to 30 days), and 63 (10.6%) presented with isolates that changed from CRKP to CSKP, with a median interval of 30 days (IQR, 10 to 56 days). For the 803 patients isolated with *A. baumannii*, 63 (7.8%) presented with isolates that changed from ISAB to IRAB, with a median interval of 13 days (IQR, 6 to 42 days), whereas 34 (4.2%) presented with isolates that changed from IRAB to ISAB, with a median interval of 11 days (IQR, 6.5 to 27 days).

## DISCUSSION

Since clinicians may take frequent cultures from patients with unresolved infections, duplicate isolates are likely to be included in cumulative susceptibility reports. Depending on the susceptibility patterns of these duplicate isolates, the antibiogram may be skewed toward either susceptibility or resistance. The removal of duplicate isolates from susceptibility data would therefore lead to an antibiogram that more clearly reflects the susceptibility pattern of specific microorganism and/or antimicrobial combinations within a particular institution. Different methods of duplicate isolate removal, however, could lead to much confusion when antibiograms from different institutions are compared or when their data are combined, as is commonly done for regional and national surveys. To

date, there is limited information comparing different methods of duplicate isolate removal and their potential effects on susceptibility reports (8–10).

Reports on antimicrobial susceptibility have used different criteria regarding the time period to be used as the limit for an isolate to be considered a duplicate. An automated system that gathers information from a large number of hospitals in the United States has a limit of 5 days, after which repeat isolates are not considered duplicates (7). A report on antimicrobial resistance in microorganisms from blood cultures excluded the same microorganisms isolated within 7 days (6). In some institutions, a repeat isolate of a species from the same patient obtained within 7 days was not tested for antimicrobial susceptibility (1). Seven days, however, may be too short a cutoff period for a single episode of infection or colonization (8). Patients may remain in hospital for long periods of time or require treatments that necessitate readmission to the hospital. In a comparison of cutoff periods of 5, 30, and 365 days, one study suggested that 365 days was the best interval for classifying isolates as duplicates (8).

While molecular typing methods may be used as strict criteria for duplicate isolates, they are not currently used in clinical practice. An automated laboratory management program that was used to remove duplicate isolates defined duplicates as isolates from the same patient and of the same bacterial species and susceptibility category (10). However, these criteria are too complicated to apply widely. In attempting to define more appropriate and simple criteria, the NCCLS recently proposed and approved guidelines in which only the first isolate of a given species from an individual patient would be included in a cumulative antimicrobial susceptibility report (2, 3). After the NCCLS proposal appeared, one study compared various calculation methods for cumulative antimicrobial susceptibility (9). These methods calculated susceptibility in all isolates, the first isolate per patient, the most resistant or susceptible interpretation per patient, the average result, and the first isolate per episode (using a 7- or 30-day interval). The authors of that study agreed with the NCCLS guidelines because of their validity, simplicity, and lack of ambiguity (9).

In a comparison of annual MRSA rates over 6 years for all isolates and for the first isolate per patient (1) in which duplicate isolates were removed according to NCCLS guidelines, the MRSA rate was different from the rate for all isolates because MRSA isolates had more duplicates than MSSA isolates. In addition, removal of duplicates resulted in a significant difference in the MRSA rate in 4 of the 6 years. When removing duplicates, it should be considered that initial susceptibility could change in duplicates from the same patient. This effect may be not critical, however, because 91% of patients with duplicate isolates did not switch between MSSA and MRSA status but retained their original *S. aureus* strain. In addition, in 88% of patients with duplicate MRSA isolates, the isolates were phenotypically identical. Similar results were observed in our study; 87% of *S. aureus* isolates, 79% of *K. pneumoniae* isolates, and 88% of *A. baumannii* isolates did not change in susceptibility.

We compared annual resistance rates over 3 years calculated from results for all isolates and for the first isolate of a species per patient per year. These comparisons were applied to several resistant organisms of MRSA, CRKP, and IRAB, which

are currently recognized as causing problems in hospitalized patients. For MRSA, we found that the trends of annual rates differed depending on whether all isolates or the first isolate per patient was assayed. MRSA rates showed a significant decrease over time for the first isolate per patient and were unchanged over time for all isolates, a discrepancy caused by the different proportion of duplicate isolates per year. Annual duplication rates of MSSA decreased over time, whereas those of MRSA increased significantly, suggesting that MRSA rates for all isolates may be increasingly overestimated. In contrast, trends of annual CRKP and IRAB rates did not differ significantly between all isolates and first isolates per patient, because the annual duplication rates did not differ for either susceptible or resistant isolates of *K. pneumoniae* and *A. baumannii*.

In conclusion, our study suggested that trends of annual resistance rates could be different between those calculated from results for all isolates and for the first isolate of a species per patient per year, regardless of body source or susceptibility. The simplicity and lack of ambiguity of the method using the first isolate per patient could therefore remove the effect of duplicate isolates and be useful in determining cumulative antimicrobial susceptibility.

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