

Criteria of time and antibiotic susceptibility in the elimination of duplicates when calculating resistance frequencies

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Received 5 February 2003; returned 2 April 2003; revised 23 April 2003; accepted 23 April 2003

We studied the effects of various systems of eliminating repeat isolates on the absolute number and susceptibility of *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Staphylococcus aureus* isolates over a 2 year period. The criterion of time is objective and reproducible, whereas that of variation in antibiotic susceptibility detects variations in the susceptibility of microorganisms that acquire resistance during treatment, but may be affected by methodological errors in determining the antibiotic susceptibility. These tools are useful in the control of multi-resistant bacteria and enable the true situation regarding antibiotic resistance in each geographical area to be determined.

Keywords: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*

Introduction

Shannon & French¹ recently published an article on the importance of the different criteria used to eliminate repeat isolates in determining the antibiotic susceptibility frequency. In our study, we compare various criteria for the elimination of repeat isolates and their effect on the susceptibility frequency of microorganisms usually associated with nosocomial infections.

Materials and methods

Microorganisms studied

Pseudomonas aeruginosa, *Staphylococcus aureus* and *Acinetobacter baumannii*.

Period studied

Clinical isolates from the University General Hospital of Elche (450 beds), covering a population of 250 000 inhabitants, on the Mediterranean coast in the Southeast of Spain, between 1 January 2000 and 31 December 2001.

Criteria applied

(i) *Elimination of repeats by time*. We calculated the effect on susceptibility frequencies of eliminating isolates obtained from the same patient in an interval of time of less or equal to 0, 7, 15, 21 and 30 days. For this calculation we used the Viresist software program, which uses the time series methodology (ARIMA models) to calculate the evolution of

microbial susceptibility by analysing the monthly data on susceptibility, antibiotic consumption and hospital stays.²

(ii) *Elimination of repeats by variation in antibiotic susceptibility*. We calculated the effect on susceptibility frequencies of using the Wider system (Soria Melguizo, Spain), which is a microdilution system with lyophilized antibiotics, semi-automatic interpretation and ATCC strains for quality control. We eliminated isolates from the same patient that, irrespective of the time elapsed between isolations, had the same antibiogram against a group of important antibiotics selected specifically for each microorganism.³

Antibiotics selected

The following groups of antibiotics were selected. *S. aureus*: oxacillin, erythromycin, ciprofloxacin, fosfomicin, rifampicin, trimethoprim/sulfamethoxazole and vancomycin; *P. aeruginosa*: ceftazidime, cefepime, piperacillin/tazobactam, imipenem, ciprofloxacin and tobramycin; *A. baumannii*: ceftazidime, cefepime, piperacillin/tazobactam, imipenem, ciprofloxacin, tobramycin and ampicillin/sulbactam.

Results

Absolute number of isolates

The relationship between the number of isolates and number of patients is shown in Table 1. It can be seen that the distribution for the three microorganisms is very similar.

(i) *Elimination of repeats by time*. The absolute number of isolates decreased by 20–30% when isolates obtained from the same patient

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Table 1. Relation between the number of isolates and number of patients

	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>A. baumannii</i>
Average isolation rate			
No. of isolates tested/no. of patients tested	1.6 (622/389)	1.6 (886/554)	1.8 (87/48)
Frequency of single isolation			
No. of patients with single isolations/no. of patients tested	0.7 (435/622)	0.7 (620/886)	0.7 (61/87)

Table 2. Variation in the absolute number of isolates and the percentage of susceptibility according to the different criteria applied

Species	All	7 days	15 days	21 days	30 days	Variation in antibiotic susceptibility
Number of <i>P. aeruginosa</i> isolates	622	479 (77.0%)	428 (68.8%)	427 (68.6%)	414 (66.5%)	532 (85.5%)
Number of <i>S. aureus</i> isolates	886	724 (81.7%)	699 (78.9%)	693 (78.2%)	624 (70.4%)	688 (77.6%)
Number of <i>A. baumannii</i> isolates	87	61 (70.1%)	53 (60.9%)	48 (55.2%)	47 (54.0%)	68 (78.2%)
Susceptibility <i>P. aeruginosa</i> /ceftazidime	91.5	92.3	93.0	92.7	93.2	91.0
Susceptibility <i>P. aeruginosa</i> /imipenem	88.4	89.8	90.6	91.6	92.0	86.5
Susceptibility <i>P. aeruginosa</i> /ciprofloxacin	80.9	80.6	81.1	82.2	83.1	78.9
Susceptibility <i>P. aeruginosa</i> /amikacin	92.9	92.3	95.1	95.5	95.6	88.9
Susceptibility <i>S. aureus</i> /oxacillin	87.9	89.6	90.1	90.9	90.5	91.1
Susceptibility <i>A. baumannii</i> /imipenem	59.8	65.6	73.6	74.5	79.2	73.5

in an interval of <7 days were eliminated. Extending the period of time, a slower decrease, reaching 40–45%, was seen to occur. This is due to the fact that these microorganisms colonize various sites in the same patient (lungs, wounds, etc.).⁴ The data are summarized in Table 2.

(ii) *Elimination of repeats by variation in antibiotic susceptibility.* This results in a smaller reduction in the number of isolates (15–20%), especially in the case of *P. aeruginosa* and *A. baumannii*, probably due to a more rapid generation of resistance⁵ or the transmission of resistant strains among the patients.^{6,7} Application of this criterion to *S. aureus* demonstrates that this microorganism exhibits more stable antibiotic patterns.

Variation in the percentages of antibiotic susceptibility

(i) *Elimination of repeats by time.* When the period of time for eliminating isolates is increased, the percentage of susceptible strains also increases. This indicates that there are many more isolates when the patient is infected by microorganisms that are resistant to antibiotics. The data are shown in Table 2.

(ii) *Elimination of repeats by variation in antibiotic susceptibility.* Application of this criterion results in a decrease in the percentage of antibiotic susceptibility, since it detects the microorganisms that acquire resistance during treatment.

Discussion

Determining the way to eliminate duplicate microorganisms from the same patient is a complex process involving many factors, such as the absolute number of isolates, type of microorganism, type of patient, antibiotic treatments started and microbiological cultures processed. Traditionally, it has been suggested that the first isolate from each

patient should be considered, but nowadays new software packages available make it possible to consider other courses of action.

If we consider the criterion of time in the elimination of isolates, as the period of time for eliminating isolates from the same patient increases, there is a decrease in the number of isolates and an increase in the percentage of susceptibility, since there is a greater number of samples from patients with resistant microorganisms. This is an objective procedure that is reproducible in different laboratories, but it does not detect changes in antibiotic susceptibility caused by the generation of resistance during antibiotic treatments if they take place over a short period of time.

The criterion of variation in antibiotic susceptibility is more useful to detect changes in the susceptibility of resistant bacteria subjected to multiple antibiotic treatments, but it is less objective and less reproducible owing to methodological errors in determining the antibiotic susceptibility.

Use of these parameters as a complement to molecular biology techniques could be of value in the control of the transmission of resistant strains and detection of outbreaks.⁸ Although they are difficult to study, specialized systems^{2,3,5} capable of analysing a large number of data and applying complex statistical methodology have made this task easier, thereby enabling a better understanding of the resistance of the main pathogenic bacteria.⁹

Acknowledgements

This study was supported by grant FIS 00/0002/05 from the Health Research Fund.

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