JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1987, p. 567–568
0095-1137/87/030567-02$02.00/0

Vol. 25, No. 3

Modification of Grimont Biotyping System for Epidemiologic Studies with Nosocomial *Serratia marcescens* Isolates

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Received 25 August 1986/Accepted 10 December 1986

A modification of the Grimont biotyping system for *Serratia marcescens* permitted the rapid testing of nosocomial strains by a plate-disk assimilation technique instead of with individual substrate tubes.

In a previous communication (6), we reported the analysis of epidemiologic markers of nosocomial *Serratia marcescens* isolates, which included the biotyping system described by Grimont and Grimont (2). Since the preparation and inoculation of the biotyping substrates contained in individual tubes is time consuming, we designed a plate assimilation technique using substrate-impregnated disks, similar to the technique described by Rosenthal for *Pseudomonas* species (5).

(Part of this study was presented at the 86th Annual Meeting of the American Society for Microbiology, Washington, D.C., 23 to 28 March 1986 [J. Sifuentes and D. H. M. Gröschel, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C281, p. 374].)

A total of 102 nosocomial isolates of *S. marcescens* were obtained from five different hospitals: 37 from Instituto Nacional de la Nutricion, Mexico City, Mexico; 32 from the University of Virginia, Charlottesville, Va.; 19 from S. Alvarez-Elcoro, Veterans Administration Medical Center, Johnson City, Tenn.; 9 from C. C. Sanders, Creighton University School of Medicine, Omaha, Nebr.; and 5 from the collection of R. P. Wenzel, from Norfolk General Hospital, Norfolk, Va. The identification of all strains was reconfirmed as described previously (6).

Biotyping was performed on agar slants by the method of Grimont and Grimont (2). For the plate technique, a mineral base medium (KH_2PO_4, 2 g; K_2HPO_4, 3 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 3 g; $(NH_4)_2SO_4$, 1 g; Bacto-agar [Difco Laboratories, Detroit, Mich.], 15 g; bromothymol blue [0.2% aqueous solution], 25 ml; distilled water to 1 liter) was prepared and poured into 150-mm plastic petri dishes. Filter paper disks (BBL Microbiology Systems, Cockeysville, Md.) were impregnated with 0.1-ml portions of 10% solutions of the carbon sources (D-glucose, DL-carnitine, lactose, *m*-erythritol, benzoic acid, 3-hydroxybenzoic acid, D-quinic acid, and trigonelline), dried at 35°C, and kept at 2 to 4°C for no longer than 15 days. The inocula for plates and slants were prepared, and pigment and tetrathionate reductase production were tested as previously described (6). Agar slants and plates were inoculated in duplicate from the same preparation, and each plate was inoculated with a swab as described for diffusion antimicrobial susceptibility testing (4). After placement of the disks, the plates were incubated at 30°C and inspected for growth after 4, 7, and 14 days. The results of biotyping were interpreted by the method of Grimont et al. (3). Agreement between the two biotyping methods was tested with the kappa index, and the statistical significance was calculated with the Z value (1).

The results of biogrouping by the original slant method of Grimont and Grimont (2) are shown in Table 1. The new plate-disk method showed 97% agreement with slant biogrouping and 89% agreement with slant biotyping. The discrepancies (3%) observed in biogrouping were due to differences in assimilation of substrates. One strain of biogroup A 5/8 failed to assimilate quinic acid and was assigned to biogroup TCT, and another strain of this biogroup showed slight growth with erythritol and was assigned to biogroup A 2/6. A biogroup A 4 strain failed to assimilate *m*-hydroxybenzoic acid and was interpreted as belonging to biogroup A 2/6. DL-Carnitine assimilation showed less reproducibility; five strains carnitine positive in tubes were negative in the disk test, and for three strains, this changed the biotype assignment.

TABLE 1. Biogroups of nosocomial isolates of *S. marcescens* determined by the method of Grimont and Grimont (2)

Source of isolates	No. of strains tested	No. of strains in biogroup:					
		A 1	A 2/6	A 3	A 4	A 5/8	TCT
Instituto Nacional de la Nutricion	37		1	1		35	
University of Virginia	32	5	5	5	7	5	5
Veterans Administration Medical Center, Johnson City, Tenn.	19	6				13	
Creighton University	9	1				6	2
Norfolk General Hospital	5					4	1

Biogrouping of *S. marcescens* by the disk assimilation method in combination with MIC data is an easily performed and inexpensive tool for studying the epidemiology of nosocomial infections in small hospitals and in third world countries. Predominant biogroups of nosocomial *S. marces-*

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cens were seen in isolates from four of five hospitals, and biogroups A 5/8 and A 1 were associated with multiple antibiotic resistance.

We conclude that the described modification of the Grimont biotyping system is useful for the analysis of nosocomial *S. marcescens* outbreaks by small-hospital and infection control laboratories.

We thank B. Mitchell-Cowardin for technical assistance and J. J. Calva for assistance with the statistical analysis.

J. S.-O. performed this work as a Consejo Nacional de Ciencia y Technologia (Mexico) Fellow at the University of Virginia.

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