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First Case of Human Infection Due to *Pseudomonas fulva*, an Environmental Bacterium Isolated from Cerebrospinal Fluid▽

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We report the first case of human infection due to *Pseudomonas fulva*. *P. fulva* caused acute meningitis following the placement of a drainage system in a 2-year-old female. Additionally, the isolate displayed a VIM-2 carbapenemase in a class 1 integron context.

CASE REPORT

A 2-year-old female was brought to our center after 30 days of treatment in another center due to a history of fever, malaise, and partial food rejection. A sample of urine was taken for a bacteriological culture, and empirical treatment with amoxicillin was begun. The urinalysis showed pathological sediment with more than 25 leukocytes per field, pyuria, and bacteriuria. The urinary culture yielded $>10^5$ CFU of *Proteus penneri* per ml. Based on the susceptibility test result, amoxicillin was discontinued and a 1-week course of trimethoprim-sulfamethoxazole (TMP-SMX) therapy was prescribed. Since she did not recover, she was hospitalized in the same center for evaluation. After 48 h, she presented a poor general state with signs of meningismus. Therapy with ceftriaxone (150 mg/kg/day) was started. A computerized axial tomography (CAT) test, performed after lumbar puncture, revealed ventricular dilatation. The cerebrospinal fluid (CSF) cytochemical analysis was normal, and the CSF culture was negative.

Due to a torpid evolution and because the patient presented facial paralysis, she was referred to Ricardo Gutierrez Children's Hospital, where a new brain CAT was performed showing progressive hydrocephalus. In order to drain the CSF, an external catheter was placed in the lateral ventricle. A presumptive diagnosis of meningitis with a bad evolution or tuberculous meningitis was made. Empirical treatment with vancomycin (60 mg/kg/day) (intravenous [i.v.]), cefotaxime (220 mg/kg/day) (i.v.), and tuberculostatic drugs (isoniazid, 5 mg/kg/day; rifampin, 10 mg/kg/day; streptomycin, 15 mg/kg/day; pyrazinamide, 20 mg/kg/day) was started. Since the lumbar puncture was traumatic, CSF chemistry and cell count were not carried out; however, the CSF culture obtained by lumbar puncture (for bacteria, fungi, and mycobacteria) was negative.

After 9 days of having the catheter drainage in place, the patient showed no neurological sign improvement. The catheter was changed, and a CSF sample for bacterial culture was taken with negative results. The CSF cell count was 8 leukocytes/mm^3, and protein and glucose contents were 17 mg/dl and 70 mg/dl, respectively. Vancomycin was suspended, and the patient continued with ceftriaxone, and despite a negative result for the detection of *Mycobacterium tuberculosis* by PCR, the patient continued on tuberculosis drugs until the culture was finished.

As indicated by neurosurgery, a nuclear magnetic resonance test was carried out, showing a lesion that occupied the midbrain and pons consistent with a probable ependymoma.

On the eighth day after the new drainage system was placed, the patient was in very poor condition; therefore, internal ventriculoperitoneal shunting and performing a brain biopsy of the mass were considered. A new CSF sample was taken and sent for bacterial culture. The CSF cell count was 218 leukocytes/mm^3, with 98% neutrophils, and protein and glucose contents were 52 mg/dl (glycemia, 112 mg/dl) and 63 mg/dl, respectively.

The CSF Gram stain showed no bacteria. After 24 h of incubation, nonfermenting Gram-negative bacilli grew in the CSF culture. The microorganism grew well on Columbia agar with 5% sheep blood and chocolate agar. It was oxidase negative, motile, arginine dehydrolase positive, lysine decarboxylase negative, and nitrate reduction negative and did not grow at 42°C. It was identified as *Pseudomonas putida* (99%) by the Vitek 2 system. With the preliminary report of nonfermenting Gram-negative bacilli and after the patient had been submitted to 27 days of treatment with third-generation cephalosporins, the antibiotic therapy was changed to meropenem (120 mg/kg/day) (i.v.) plus TMP-SMX (20 mg/kg/day) (i.v.).

The drainage system was removed, and the ventricular tip was sent for culture. It was cultured by using a semiquantitative

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TABLE 1. Biochemical identification of *Pseudomonas fulva* isolate

Test	Result[a] for:				
	Our isolate	*P. fulva*	*P. putida*	*P. oryzihabitans*	*P. luteola*
Oxidase activity	−	+[b]	+	−	−
Motility	+	+	+	+	+
Production of water-insoluble yellow pigment on tryptic soy agar	+	+	−	+	+
Production of water-soluble fluorescent pigment on King B	−	−	+	−	−
Nitrate reduction	−	−	−	−	V
Arginine hydrolysis	+	+	+	V	+
Esculin hydrolysis	−	−	−	−	+
Oxidation of:					
Glucose	+	ND	+	+	+
Xylose	+	ND	+	+	+
Mannitol	−	ND	V	+	+ or (+)
Maltose	−	ND	V	+	+
Lactose	−	ND	V	V	V

[a] +, positive; + or (+), positives and late positives together totalling 90% or more; −, negative; V, variable; ND, not done. Data for *Pseudomonas fulva* are from reference 27. Data for *Pseudomonas putida*, *Pseudomonas luteola*, and *Pseudomonas oryzihabitans* are from reference 3.
[b] The positive oxidase test result is based on only three strains studied by Uchino et al. (27).

roll-plate method in addition to the qualitative method by immersing the catheter tip in brain heart infusion (BHI) with subsequent incubation at 37°C and subcultured on Columbia agar with 5% sheep blood and chocolate agar. After 24 h of incubation, the same organism that had been developed in the CSF was isolated. With the report of resistance to carbapenems, third-generation cephalosporins, gentamicin, and TMP-SMX by the Kirby-Bauer method, meropenem and TMP-SMX were discontinued and colistin (5 mg/kg/day) (i.v.) together with piperacillin-tazobactam (300 mg/kg/day) (i.v.) therapy was started to obtain the final result of antibiotic susceptibility by MIC (Etest; AB Biodisk, Solna, Sweden).

With these MICs (piperacillin-tazobactam, 64 μg/ml; colistin, 0.5 μg/ml; and ciprofloxacin, 0.25 μg/ml), it was decided to increase the piperacillin-tazobactam dose to 400 mg/kg/day i.v. and to continue with the same dose of colistin.

After 2 days of treatment, colistin was discontinued and ciprofloxacin (40 mg/kg/day) (i.v.) was added.

A new external drainage system was placed. The CSF recovered through the drainage system yielded a positive result for the same microorganism, and the cytochemical analysis yielded the following results: glucose, 42 mg/dl (glycemia, 101 mg/dl), protein, 51 mg/dl, and 10,000 leukocytes/mm^3, with 99% neutrophils.

The drainage system was removed, and the ventricular tip was sent for culture. It was positive for the same microorganism.

Colonies on nutrient agar were smooth, entire, and flat to convex and had a water-insoluble yellow pigment after an incubation of 24 h at 35°C. The organism was identified using standard biochemical tests (3, 27) and API 20 NE (bioMérieux, Marcy L'Etoile, France). The biochemical tests of the isolate and related microorganisms are shown in Table 1. The strain was identified by API 20 NE as *Pseudomonas putida* (96.6%) (biocode 0143451).

PCR amplification of the 16S rRNA was performed in order to identify the correct species. The PCR product of the 16S rRNA gene, using the primers described by Weisburg et al. (30), was obtained with *Taq* DNA polymerase according to the manufacturer's specifications (Promega). Sequencing was performed on both DNA strands using ABIPrism 3100 BioAnalyzer equipment at the Utah State University sequencing facility. The sequences were analyzed with BLAST V2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/). The sequenced analysis revealed a 99% identity with the sequences corresponding to the 16S RNA ribosomal genes of *P. fulva* (GenBank accession no. FJ972539), *P. fulva* NRIC 0180^T (GenBank accession no. AB046998), *Pseudomonas* sp. Acj 106 (GenBank accession no. AB480754), and *Pseudomonas* sp. 3zhy (GenBank accession no. AM411621). With a lower score, but still with high identity, the sequence revealed 99% identity with the sequence of the 16S RNA ribosomal gene corresponding to *P. putida* (GenBank accession no. AB294558). In order to achieve the most accurate identification of the organism, and taking into account the importance of the correct identification of the isolate, the case being the first human infection due to *P. fulva*, we decided to perform DNA-DNA hybridization.

DNA-DNA hybridization was carried out using the method of Ezaki et al. (6). The level of DNA-DNA similarity of the isolate showed a high value with *P. fulva* NRIC 0180^T (100%) and low values with strains of related *Pseudomonas* species, ranging from 41 to 63% similarity (*P. parafulva* AJ 2129^T, 63%; *P. cremoricolorata* NRIC 0181^T, 60%; *P. putida* IFO 14164^T, 41%).

Susceptibility to 10 antimicrobial agents was determined by agar dilution (Mueller-Hinton agar; Difco, BBL, United States) in duplicate assays following the Clinical and Laboratory Standards Institute (CLSI) recommendations (4). The MICs of imipenem (MSD, NJ) and meropenem (AstraZeneca, United Kingdom) were determined in the absence and in the presence of 0.4 mM EDTA-Na_2 (ICN, United States). A ≥3-dilution decrease produced by the carbapenem/EDTA combination compared with the result for unsupplemented carbapenem was considered suggestive of the presence of metallo-β-lactamase (MBL) (11) (Table 2). Other antibiotics tested were ceftazidime (GlaxoSmithKline, United Kingdom) and cefepime (Bristol-Myers Squibb, NJ), assayed both alone

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TABLE 2. Antibiotic susceptibility (MIC) of *Pseudomonas fulva* clinical isolate

Antimicrobial agent	MIC (μg/ml)	Interpretation[c]
Ceftazidime	32	R
Ceftazidime plus lithium clavulanate[a]	32	R
Cefepime	8	S
Cefepime plus lithium clavulanate[a]	8	S
Piperacillin/tazobactam	128	R
Imipenem	32	R
Imipenem plus EDTA[b]	0.5	S
Meropenem	32	R
Meropenem plus EDTA[b]	2	S
Amikacin	16	S
Gentamicin	128	R
Ciprofloxacin	0.25	S
Aztreonam	16	I
Colistin	1	S

[a] Clavulanic acid, 10 μg/ml (final concentration).
[b] EDTA, 0.4 mM (final concentration).
[c] S, sensitive; R, resistant; I, intermediate.

and in combination with lithium clavulanate (4 μg/ml) (Roemmers, Argentina); aztreonam (Bristol-Myers Squibb, NJ); gentamicin and amikacin (Richet, Argentina); ciprofloxacin (Roemmers, Argentina); piperacillin-tazobactam (Wyeth, United States); and colistin (Sigma, United States). The inoculated agar plates were incubated overnight at 35°C. The MIC results were interpreted using CLSI categories (5) The MICs for the *P. fulva* isolate are shown in Table 2.

For metallo-β-lactamase (MBL) screening by the disk diffusion assays, a double-disk assay using EDTA/SMA disks (1,900/750 μg per disk, respectively) (Britania, Argentina) placed 15 mm (center to center) from one disk of imipenem was used (11). By means of this assay, synergism between carbapenem and EDTA/SMA disks was observed and a putative MBL present in *P. fulva* was also indicated.

To detect carbapenem enzymatic activity, a modification of the assay originally developed by Masuda et al. (16) and further modifications (14, 15) was used. Crude ß-lactamase extracts of this isolate displayed carbapenem hydrolysis in a bioassay. Addition of EDTA to the test (14) revealed the presence of an MBL as responsible for the carbapenemase activity.

β-Lactamase preparation and analytical isoelectric focusing (IEF) were performed as previously described (17). β-Lactamase bands were visualized by the iodometric method described by Labia & Barthélémy (9) using penicillins and cephalosporins as substrates (17). Crude preparations from bacteria possessing β-lactamases of known isoelectric points (pI) were used as standards: TEM-1 (pI 5.4), PER-2 (pI 5.4), SHV-2 (pI 7.6), P99 (pI 7.8), CTX-M-2 (pI 7.9), and SHV-5 (pI 8.2). IEF showed that the isolate produced a unique β-lactamase with ESBL activity at pI 5.4.

Due to the EDTA inhibition observed by disk diffusion and MIC, the strain was tested for MBL genes by PCR. Isolation of total DNA was performed as described previously (17). The strain was analyzed for the presence of the bla_{VIM}, bla_{IMP}, and bla_{SPM} genes, using previously described primers (10, 23). Additionally, according to IEF results, the strain was tested by PCR for the bla_{TEM}, bla_{GES}, and bla_{PER} ß-lactamase genes (2, 17, 22). Thermal cycler T-gradient and $MgCl_2$, deoxynucleoside triphosphate, specific primers, and *Taq* polymerase were supplied by Biometra, Göttingen, Germany, and Invitrogen, United States, respectively. PCR elongation times and temperatures were adjusted to the expected size of the PCR product and to the nucleotide sequence of the primers, respectively. Nucleotide sequencing and sequence analysis of the amplicon were performed as previously described.

P. fulva showed specific amplification for bla_{VIM}. According to the IEF results, putative overlapped ß-lactamase bands (such as PER-2, GES, and TEM) were ruled out by PCR.

Since almost all the bla_{VIM} genes currently reported are integron located (10, 24, 28), PCRs were performed using primers directed against the 5′ and 3′conserved segments of class 1 integrons (12). *P. fulva* was found to produce two class 1 integrons of 1.6 and 2.0 kb. When the primers 5′-CS/VIM-R were used, an amplicon of 475 bp was observed, which is indicative of the localization of the gene bla_{VIM} as the first cassette in the class 1 integron structure. Additionally, the VIM-F/3′-CS primer combination produced a 1.5-kb amplicon, suggesting that bla_{VIM} was part of the larger of the integrons detected. Nucleotide sequences were completely homologous to the bla_{VIM-2} coding gene.

With antibiotic susceptibility results, colistin (5 mg/kg/day) (i.v.) was added again.

Subsequently, biopsy of the tumor was performed; the diagnosis was a primitive neuroectodermal tumor.

Several CSF cultures (five samples) performed during a 14-day course of treatment were negative, but the patient had a poor clinical course and died.

During the taxonomic study of *Pseudomonas* strains isolated from rice and petroleum fields and oil brine in Japan, Iizuka and Komagata (7) isolated a water-soluble-pigment- and a water-insoluble-pigment-producing strain, included them in the fluorescent group, and named them *P. fulva* and *Pseudomonas straminea* (7). Recently, seven *P. fulva* strains obtained from culture collections were recharacterized by Uchino et al. (27). Additionally, *P. fulva* was isolated from the gills of *Geukensia demissa*, a freeze-tolerant salt marsh bivalve mollusc. These bacteria could perform the same function as hemolymph ice-nucleating proteins by limiting ice formation to extracellular compartments (13).

Only one report of *P. fulva* isolation from the sputum of a patient with cystic fibrosis is mentioned in the literature; however, the authors did not report the clinical significance of this finding (29).

P. fulva has been isolated from banana rhizospheric soil (19). In addition, together with *P. putida*, it has been the most frequently isolated species in rice seed samples obtained from provinces in the Philippines (31). Interestingly, as *P. fulva* was antagonistic to fungal or bacterial pathogens of rice, rice seed is an important source of biological control agents (31).

Also, *P. fulva* is known to be involved in plant pathogenic fungi elimination during the slow filtration process used in soilless tomato cultures (25). However, *P. fulva* has never been isolated from CSF. Furthermore, this isolate carried the bla_{VIM-2} gene cassette.

Notably, during the year prior to the isolation of *P. fulva*,

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four *P. putida* isolates producing the bla_{VIM-2} gene in a class 1 integron context were detected at Ricardo Gutierrez Children's Hospital (21). The analysis of the integron structures revealed the presence of the bla_{VIM} gene cassette as the first cassette in all of them but with highly diverse variable regions. These isolates were from different clinical samples (two from CSF, one from peritoneal fluid, and one from urine), and all of them exhibited different pulsed-field gel electrophoresis (PFGE) profiles (21). Since the bla_{VIM-2} genes were found to be on mobile gene cassettes inserted into integrons (8, 10, 24, 26), our findings suggest that the spread of these MBL genes at Ricardo Gutierrez Children's Hospital could have been due to the movement of the resistant genes among different clones. The occurrence of bla_{VIM-2} in different clones and in different *Pseudomonas* species is indicative of the presence of horizontal gene transfer. Moreover, another study has revealed the presence of bla_{VIM-11}, a novel variant of the metallo-β-lactamase bla_{VIM} family which differs from bla_{VIM-2} in a unique mutation, in a *Pseudomonas aeruginosa* clinical isolate in the same medical center (22).

Metallo-ß-lactamase-producing *P. putida* isolates were first detected in 2002 in Ricardo Gutierrez Children's Hospital. About 80% of the strains of *P. putida* isolated in the period 2002 to 2008 showed resistance to carbapenem due to the metallo-β-lactamase production. PCR showed that all of the metallo-β-lactamase producers had bla_{VIM-2} but none had bla_{IMP}.

Detection of bla_{VIM-2} in *Pseudomonas* spp. in South America was initially reported by the SENTRY Antimicrobial Surveillance Program (18) and included one *Pseudomonas fluorescens* isolate in Chile and three *P. aeruginosa* isolates in Venezuela. In addition, we have reported the first occurrence of bla_{VIM-2} in *P. putida* in Latin America (1) and the first bla_{VIM-11} in a *P. aeruginosa* isolate worldwide (22).

Although the rest of the phenotypic characteristics were identical to those of *P. putida* (Table 1), the oxidase-negative reaction and the yellow pigment aroused suspicion that this was another *Pseudomonas* species different from *P. putida*. While these features are common to *Pseudomonas luteola* and *Pseudomonas oryzihabitans*, other biochemical tests excluded them (Table 1).

The positive oxidase test result in *P. fulva* is based on only three strains studied by Uchino et al. (27); however, our strain was oxidase negative.

In addition, a feebly negative oxidase reaction is cited in the original description of *P. fulva* in the second edition of *Bergey's Manual of Systematic Bacteriology* (20).

Thus, the variable oxidase reaction may be a phenotypic characteristic of this species.

The substrate utilization profile (malate positive, phenylacetate negative, malonate negative) was in agreement with that specified by Uchino et al. for *P. fulva* (27). The system API 20 NE did not allow approaching the final identification because *P. fulva* is not in the database, and the DNA hybridization was definitive for organism identification.

The isolation of *P. fulva* represents the first case of human infection by this organism and shows that a microorganism that is part of the environmental microbiota can cause severe infections and acquire resistance genes under selective pressure in the hospital.

Nucleotide sequence accession number. The sequence of the *P. fulva* partial 16S rRNA gene has been submitted to GenBank under the accession number GU244412.

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REFERENCES

1. **Almuzara, M. N., M. Radice, N. de Gárate, A. Kossman, A. Cuirolo, G. Santella, A. Famiglietti, G. Gutkind, and C. Vay.** 2007. VIM-2-producing Pseudomonas putida, Buenos Aires. Emerg. Infect. Dis. **13:**668–669.
2. **Arlet, G., and A. Phillipon.** 1991. Construction by polymerase chain reaction and intragenic DNA probes for three main types of transferable beta-lactamases (TEM, SHV, CARB) [corrected]. FEMS Microbiol. Lett. **66:**19–25.
3. **Blondel-Hill, E., D. A. Henry, and D. P. Speert.** 2007. *Pseudomonas*, p. 770–802. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC.
4. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, M7-A7, 7th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Clinical and Laboratory Standards Institute.** 2008. Performance standards for antimicrobial susceptibility testing; M100-S18, 18th informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA.
6. **Ezaki, T., Y. Hashimoto, and E. Yabuuchi.** 1989. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int. J. Syst. Bacteriol. **39:**224–229.
7. **Iizuka, H., and K. Komagata.** 1963. New species of *Pseudomonas* belonging to fluorescent group. (Studies on the microorganisms of cereal grains. Part V). Nippon Nogeikagaku Kaishi **37:**137–141.
8. **Iyobe, S., H. Kusadokoro, J. Ozaki, N. Matsumura, S. Minami, S. Haruta, T. Sawai, and K. O'Hara.** 2000. Amino acid substitutions in a variant of IMP-1 metallo-β-lactamase. Antimicrob. Agents Chemother. **44:**2023–2027.
9. **Labia, R., and M. Barthélémy.** 1979. L'enzymogramme des betalactamases: adaptation en cel de la methode iodometrique. Ann. Microbiol. (Paris). **130:**295–304. (In French.)
10. **Lauretti, L., M. L. Riccio, A. Mazzariol, G. Cornaglia, G. Amicosante, R. Fontana, and G. M. Rossolini.** 1999. Cloning and characterization of bla_{VIM}, a new integron-borne metallo-ß-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob. Agents Chemother. **43:**1584–1590.
11. **Lee, K., Y. S. Lim, D. Yong, J. H. Yum, and Y. Chong.** 2003. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-ß-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J. Clin. Microbiol. **41:**4623–4629.
12. **Levesque, C., L. Piche, C. Larose, and P. H. Roy.** 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. **39:**185–191.
13. **Loomis, S. H., and M. Zinser.** 2001. Isolation and identification of an ice-nucleating bacterium from the gills of the intertidal bivalve mollusc *Geukensia demissa*. J. Exp. Mar. Bio. Ecol. **261:**225–235.
14. **Marchiaro, P., V. Ballerini, T. Spalding, G. Cera, M. A. Mussi, J. Morán-Barrio, A. J. Vila, A. M. Viale, and A. S. Limansky.** 2008. A convenient microbiological assay employing cell-free extracts for the rapid characterization of Gram-negative carbapenemase producers. J. Antimicrob. Chemother. **62:**336–344.
15. **Marchiaro, P., M. A. Mussi, V. Ballerini, F. Pasteran, A. M. Viale, A. J. Vila, and A. S. Limansky.** 2005. Sensitive EDTA-based microbiological assays for detection of metallo-ß-lactamases in nonfermentative gram-negative bacteria. J. Clin. Microbiol. **43:**5648–5652.
16. **Masuda, G., S. Tomioka, and M. Hasegawa.** 1976. Detection of β-lactamase production by Gram-negative bacteria. J. Antibiot. (Tokyo) **29:**662–664.
17. **Melano, R., A. Corso, A. Petroni, D. Centrón, B. Orman, A. Pereyra, N. Moreno, and M. Galas.** 2003. Multiple antibiotic-resistance mechanisms including a novel combination of extended-spectrum ß-lactamases in a *Klebsiella pneumoniae* clinical strain isolated in Argentina. J. Antimicrob. Chemother. **52:**36–42.
18. **Mendes, R. E., M. Castanheira, P. Garcia, M. Guzman, M. A. Toleman, T. R. Walsh, and R. N. Jones.** 2004. First isolation of *bla*VIM-2 in Latin America: report from the SENTRY Antimicrobial Surveillance Program. Antimicrob. Agents Chemother. **48:**1433–1434.
19. **Naik, P. R., N. Sahoo, D. Goswami, N. Ayyadurai, and N. Sakthivel.** 2008. Genetic and functional diversity among fluorescent pseudomonads isolated from the rhizosphere of banana. Microb. Ecol. **56:**492–504.
20. **Palleroni, N. J.** 2005. Genus *Pseudomonas*, p. 323–379. *In* D. J. Brenner, N. R. Krieg, J. T. Staley, and G. M. Garrity (ed.), Bergey's manual of systematic bacteriology, 2nd ed. Springer, New York, NY.
21. **Pasteran, F., D. Faccone, L. Guerriero, A. Limansky, M. Rapoport, M.**

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Galas, A. Corso and Whonet Collaborative Group. 2008. Abstr. XIII Jornadas Argentinas de Microbiología, Rosario, Santa Fe, Argentina, abstr. 305.
22. **Pasteran, F., D. Faccone, A. Petroni, M. Rapoport, M. Galas, M. Vazquez, and A. Procopio.** 2005. Novel variant (blaVIM-11) of the metallo-ß-lactamase blaVIM family in a GES-1 extended-spectrum-ß-lactamase-producing *Pseudomonas aeruginosa* clinical isolate in Argentina. Antimicrob. Agents Chemother. **49:**474–475.
23. **Pasteran, F., M. Rapoport, A. Petroni, D. Faccone, A. Corso, M. Galas, M. Vazquez, A. Procopio, M. Tokumoto, and V. Cagnoni.** 2006. Emergence of PER-2 and VEB-1a in *Acinetobacter baumannii* strains in the Americas. Antimicrob. Agents Chemother. **50:**3222–3224.
24. **Poirel, L., T. Naas, D. Nicolas, L. Collet, S. Bellais, J. D. Cavallo, and P. Nordmann.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-ß-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob. Agents Chemother. **44:**891–897.
25. **Renault, D., F. Déniel, E. Benizri, D. Sohier, G. Barbier, and P. Rey.** 2007. Characterization of *Bacillus* and *Pseudomonas* strains with suppressive traits isolated from tomato hydroponic-slow filtration unit. Can. J. Microbiol. **53:**784–797.
26. **Riccio, M. L., N. Franceschini, L. Boschi, B. Caravelli, G. Cornaglia, R. Fontana, G. Amicosante, and G. M. Rossolini.** 2000. Characterization of the metallo-ß-lactamases determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of bla_{IMP} allelic variants carried by gene cassettes of different phylogeny. Antimicrob. Agents Chemother. **44:**1229–1235.
27. **Uchino, M., O. Shida, T. Uchimura, and K. Komagata.** 2001. Recharacterization of *Pseudomonas fulva* Iizuka and Komagata 1963, and proposals of *Pseudomonas parafulva* sp. nov. and *Pseudomonas cremoricolorata* sp. nov. J. Gen. Appl. Microbiol. **47:**247–261.
28. **Walsh, T., M. Toleman, L. Poirel, and P. Nordmann.** 2005. Metallo-β-lactamases: the quiet before the storm? Clin. Microbiol. Rev. **18:**306–325.
29. **Warwick, S., B. Duke, S. Soleimanian, and D. Wareham.** 2008. Abstr. 18th European Congress of Clinical Microbiology and Infectious Diseases, abstr. P-1971.
30. **Weisburg, W. G., S. M. Barns, D. A. Pelletier, and L. D. Lane.** 1991. 16S-ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173:**697–703.
31. **Xie, G. L., A. Soad, J. Swings, and T. W. Mew.** 2003. Diversity of Gram negative bacteria antagonistic against major pathogens of rice from rice seed in the tropic environment. J. Zhejiang Univ. Sci. **4:**463–468.

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