MOLECULAR IDENTIFICATION AND ANTIMICROBIAL RESISTANCE PATTERN OF SEVEN CLINICAL ISOLATES OF *Nocardia* spp. IN BRAZIL

Larissa Anuska Zeni CONDAS(1), Márcio Garcia RIBEIRO(1), Marisol Domingues MURO(2), Augea Palmira Castagna de VARGAS(3), Tetsuhiro MATSUZAWA(4), Katsukiyo YAZAWA(4), Amanda Keller SIQUEIRA(1), Tatiana SALERNO(1), Gustavo Henrique Batista LARA(1), Rafaela Mastrangelo RISSETI(1), Karen Spadari FERREIRA(5) & Tohru GONOI(4)

SUMMARY

*Nocardia* is a ubiquitous microorganism related to pyogranulomatous infection, which is difficult to treat in humans and animals. The occurrence of the disease is on the rise in many countries due to an increase in immunosuppressive diseases and treatments. This report of cases from Brazil presents the genotypic characterization and the antimicrobial susceptibility pattern using the disk-diffusion method and inhibitory minimal concentration with E-test® strips. In summary, this report focuses on infections in young adult men, of which three cases were cutaneous, two pulmonary, one neurological and one systemic. The pulmonary, neurological and systemic cases were attributed to immunosuppressive diseases or treatments. Sequencing analysis of the 16S RNA segments (1491 bp) identified four isolates of *Nocardia farcinica*, two isolates of *Nocardia nova* and one isolate of *Nocardia asiatica*. *N. farcinica* was involved in two cutaneous, one systemic and other pulmonary cases; *N. nova* was involved in one neurological and one pulmonary case; and *Nocardia asiatica* in one cutaneous case. The disk-diffusion antimicrobial susceptibility test showed that the most effective antimicrobials were amikacin (100%), amoxicillin/clavulanate (100%), cephalxin (100%) and ceftriaxone (100%), while isolates had presented most resistance to gentamicin (43%), sulfamethoxazole/trimethoprim (43%) and ampicillin (29%). However, on the inhibitory minimal concentration test (MIC test), only one of the four isolates of *Nocardia farcinica* was resistant to sulfamethoxazole/trimethoprim.

KEYWORDS: Nocardiosis; *Nocardia*; Opportunistic disease; Antimicrobial susceptibility test.

INTRODUCTION

Nocardiosis is a chronic and severe pyogranulomatous disease caused by the environmentally ubiquitous actinomycete of the *Nocardia* genus1. Nocardiosis is an emerging disease in humans and animals worldwide1,8,13,26,32,34. According to BAIO et al. (2013) nocardiosis is a neglected disease, particularly in patients with some degree of immunosuppression3. For many years, since Edmond Nocard’s first description of the pathogen in 1888, its diagnosis was based on phenotypic methods17. To date, 102 species of *Nocardia* have been discovered using molecular methods, of which seven have been reclassified and 90 currently stand on the list of prokaryotic names with standing nomenclature23. Among these species, at least 25 are pathogenic to humans and animals1,8,13,17,29. *N. asteroides*, *N. brasiliensis*, *N. farcinica*, *N. nova*, *N. cyriacigeorgica* and *N. veterana* are the main species related to nocardiosis in humans11,14,17.

Either tegumentary injury or the inhalation of bacteria is considered the most common route of transmission of *Nocardia* spp. in humans2. Clinically, the main manifestations of human nocardiosis are pneumonia, encephalitis, lymphadenitis, lymphangitis and cutaneous tissue lesions3. Therapy consists of a prolonged course, its success depending on the species of bacteria, the virulence of the strain, the organs affected, the time of evolution and the health status of the susceptible individual(s)3. *Nocardia* spp. is refractory to conventional antimicrobial therapy. Sulfonamides potentiated by trimethoprim, minocycline, aminoglycosides (amikacin, gentamicin) and cephalosporins (ceftriaxone, ceftriaxone, cephalaxin) alone or in combination, based on *in vitro* tests4, are the choices of treatment for human and animal nocardiosis20,24,34.

The purpose of this case report is to present the species of *Nocardia* involved in human nocardiosis in Brazil, and their respective drug susceptibility pattern.

MATERIAL AND METHODS

Isolates. Seven strains of *Nocardia* spp., identified in three Hospitals from different states of Brazil (one in Rio Grande do Sul, one in São Paulo and five in Paraná), were isolated from clinical cases of nocardiosis. The strains were isolated from different specimens (bronchial wash, cutaneous and organ fragments) in defibrinated sheep blood agar (5%), Sabouraud strains were isolated from different specimens (bronchial wash, cutaneous strains were isolated from different specimens (bronchial wash, cutaneous...
10 days. Colonies suggestive of the genus *Nocardia* were evaluated by dry, convex, adherent, and white to orange color aspects. After 48 to 72 hours post-inoculation, colonies were submitted to Gram and Kinyoun stain. Gram-positive, filamentous to cocobacillary, partially acid-fast organisms were identified as *Nocardia*.

**Molecular identification.** Molecular analysis was carried out in the Medical Mycology Research Center, Chiba, Japan. Genomic DNA for sequencing was performed according to KAGEYAMA et al., 2004. The 16S rRNA gene was amplified by PCR using a DNA thermal cycler (TaKaRa Bio Inc., Chiba, Japan) under the following conditions: 35 cycles at 94 °C for 60 seconds for denaturation, 60 °C for 60 seconds for primer annealing, and 72 °C for 120 seconds for primer extension. PCR primers were the prokaryotic 16S rRNA universal primer pairs, 8F and 491R, 520F and 1100R, and 926F and 1542R. DNA sequences were determined with an automatic sequence analyzer (ABI PRISM™ 3130; Applied Biosystems, Japan), using the same primers and a dye terminator cycle sequencing kit (Applied Biosystems). Near-complete 16S rRNA gene sequences consisting of approximately 1400 bases pairs were obtained. The sequence of the 16S rRNA gene was compared to the GenBank database using BLAST, and 16S rRNA sequences of related *Nocardia*-type strains were retrieved from the database. These sequences were submitted to GenBank/JBIJ/EMBL.

**In vitro drug susceptibility tests.** All strains were examined using the disk-diffusion test and minimum inhibitory concentration test (MIC test) based on E-test™ (E-test™, AB biodisk, BioMérieux, Dalvägen, Sweden), according to the procedures described by GLUPCKZYNISKI et al. 2006 and NCCLS 2006. Isolates resistant to three or more antimicrobials were considered multi-resistant.

The study of drug susceptibility in Actinomycetes is more laborious because this group of bacteria usually grows in clumps. Due to this adherent characteristic during bacterial growth, the observed irregular broth turbidity makes it difficult to measure the optimum inoculum concentration. Naturally, the precise concentration of unit colony formation is essential for the correct interpretation of both antimicrobial tests.

The isolates were briefly subcultured twice in blood agar (5%) to ensure their purity. After 48 hours, they were inoculated in brain-heart-infusion at 37 °C for another 48 hours. Sterile glass beads were added at the time the strains were vortexed to decrease the formation of clumps and subsequently obtain a more accurate optical density (OD) equivalent to a 0.5 McFarland standard to disk-diffusion test and 1.0 McFarland standard for E-test.

All of the strains were submitted to a disk-diffusion test and the inhibition zones interpreted following standards according to BAUER et al., 1966 and AMBAYE et al. 1997. The antibiotics selected were amikacin (30 μg), ampicillin (10 μg), amoxicillin/clavulanate (20 μg/10 μg), cefotaxime (30 μg), cephalzone (75 μg), ceftriaxone (30 μg), cephalexin (30 μg), cefuroxime (30 μg), cefalolin (30 μg), imipenem (10 μg), gentamicin (10 μg), mafenicoline (30 μg) and sulfamethoxazole/trimethoprim (25 μg).

In the MIC test, a maximum of five E-test strips were attached to Mueller Hinton agar and were then incubated at 37 °C. The results were recorded after 48 - 72 hours because of the organisms’ fastidious growth. The following antibiotics were used: amikacin (0.016 - 256 μg/mL), ampicillin (0.016 - 256 μg/mL), amoxicillin/clavulanate (0.016 - 256 μg/mL), ceftriaxone (0.016 - 256 μg/mL), gentamicin, sulfamethoxazole-trimethoprim (0.002 - 32 μg/mL) and imipenem (0.002 - 32 μg/mL).

The similarities between the results from the disk-diffusion test and E-test were analyzed using the Kappa agreement index. According to the values obtained, the agreement analysis between the tests followed the criteria established by THrusfield (1995). All statistical analysis was done using Bioestat v.5.0 and SPSS v.14 packages. The Chi-squared and Fisher’s exact tests were used to analyze whether the resistance percentages of *Nocardia* spp. were normally distributed between strains for each antibiotic tested with the disk-diffusion method. The level of significance for this test was *p < 0.05*.

**RESULTS**

All strains were taken from young adult males between 28 and 35 years of age. Three patients displayed cutaneous manifestations (exhibiting fistulous mycetomas due to traumatic inoculation), while two showed pulmonary, one neurological and one systemic. Two individuals with pulmonary symptoms, one with neurological, and one with systemic were co-infected with immunosuppressive diseases, or were undergoing/had undergone prolonged treatments with chemotherapy or corticotherapy. Of all the patients, four died due to the severity of their disease (these were pulmonary, neurological and systemic cases), two recovered (cutaneous manifestations), and one case had no documented outcome (cutaneous manifestation) (Table 1).

The microbiological culture of samples showed dry, convex, strongly adherent, whitish to orange-brown colonies with a powdery surface after 48-96 hours of incubation at 37 °C. Gram and modified Ziehl-Neelsen stains showed thin, branched filaments sometimes fragmented in cocobacillary forms, suggestive of the *Nocardia* genus.

The sequence of the 16S rDNA gene enabled the identification of four strains - *N. farcinica*, two *N. nova*, and one *N. asiatica* - based on its 99.6% or higher sequence similarity to the reference sequence in GenBank (DDBJ/GenBank/EMBL), using BLAST as recommended by CLSI, 2008. The access number for the isolates in GenBank is as follows: IFM 11128/AB 633331 - *N. nova*; IFM 11096/AB 630965 - *N. farcinica*; IFM 11231/AB 636474 - *N. farcinica*; IFM 11232/AB 636475 - *N. farcinica*; IFM 11099/AB 630966 - *N. nova*; IFM 11100/AB 630967 - *N. asiatica*; IFM 11285/AB 638765 - *N. farcinica*.

In this study, *N. farcinica* was observed in two cutaneous, one systemic and other pulmonary cases; *N. nova* was present in one neurological manifestation and one pulmonary; and *Nocardia asiatica* in one cutaneous case (Table 1).

The in vitro drug susceptibility test, based on disk-diffusion, is presented in Table 2. Based on the disk-diffusion test, *Nocardia* spp. isolates were sensitive to amikacin, amoxicillin/clavulanate, cephalaxin, cefalolin, cefotaxime and minocycline. However, around 50% of the *Nocardia* spp. isolates were resistant to ampicillin, gentamicin and sulfamethoxazole/trimethoprim. Of the seven isolates, two were...
multi-resistant to three or more antimicrobials. One isolate of *N. farcinica* was resistant to ampicillin, cefoperazone and gentamicin; and the other isolate of *N. farcinica* was resistant to ampicillin, sulfamethoxazole/trimethoprim, gentamicin and cefuroxime.

The minimum inhibitory concentration of tested antimicrobials showed suitable breakpoints, with all the isolates being susceptible to the tested antimicrobials (Table 3) except for one isolate of *Nocardia farcinica*, which was resistant to sulfamethoxazole/trimethoprim.

### Table 3

Minimum inhibitory concentrations (μg/mL) and susceptibility proportion estimates of *Nocardia* spp. isolated from seven case reports. UNESP, Botucatu, SP, Brazil

| Antimicrobials | MIC<sub>S</sub> (μg/mL) | MIC<sub>P</sub> (μg/mL) | %Susceptible (Overall n=7) |
|----------------|-----------------|-----------------|-----------------------------|
| Amikacin       | 0.20            | 0.40            | 100                         |
| Amoxicillin/   | 0.5             | 3               | 100                         |
| clavulanate    |                 |                 |                             |
| Ampicillin     | 0.25            | 1               | 100                         |
| Cephalaxin     | 1               | 2               | 100                         |
| Ceftriaxone    | 1.5             | 2               | 100                         |
| Gentamicin     | 1               | 2               | 100                         |
| Imipenem       | 0.75            | 1.5             | 86                          |
| Sulfamethoxazole/ | 1.5       | 3               | 100                         |
| trimethoprim   |                 |                 |                             |

*MIC<sub>S</sub> and MIC<sub>P</sub>; values are concentrations at which ≥ 50% and ≥90% of isolates are inhibited by antimicrobials.

The agreement between the tests was considered low by the statistical analysis.

### DISCUSSION

These findings reinforce that molecular techniques are a reliable, suitable and quick method for the diagnosis of species of *Nocardia* genus taken from a human origin. Phenotypic evaluations could be performed to identify the *Nocardia* species, combining tests such as the hydrolysis of organic compound (adenine, xanthine, hypoxanthine, casein, esculin and tyrosine), carbohydrate assimilation, antimicrobial susceptibility pattern, citrate utilization and acetamid and arylsulfatase utilization, among others<sup>4,17,36</sup>. However, they are usually laborious, time-consuming, and require experience in evaluating the results. For this reason, molecular methods emerged as an alternative that can be used on several different
body fluids and tissue samples, and can also be used for identifying strains that are difficult to grow in a conventional medium\(^8\).\(^{29}\).

The 16S rRNA gene is highly conserved with constant regions and, to date, its complete sequence of approximately 1400 bp has a considerably large database on GenBank, which allows for the identification of most Nocardia species\(^{29}\). However, because 16S rRNA shows minimal variation and is also present in different numbers of copy variants, this gene should be sequenced in its total 1400 bps and follow the standards of CLSI, which recommends a similarity higher than 99.6%. Nowadays, other researchers are reporting on the sequencing of housekeeping genes with a better discriminatory power, even with partial gene sequencing\(^{16,29}\).

The incidence of human nocardiosis has increased in the last two decades across several countries, particularly in patients affected by immunosuppressive therapies or diseases\(^{3,13}\). In Brazil, the 22 cases of human nocardiosis, predominantly displaying pulmonary symptoms, were most prevalent in adult males (59.2%) between the ages of 21 and 84. Most cases were related to immunosuppressed conditions (69.9%), such as transplants, corticotherapy and being HIV-positive\(^{19}\). These findings agree with similar observations that described immunocompromised conditions in humans affected by nocardiosis\(^{10,19,21,30}\), reinforcing the opportunistic behavior of the Nocardia genus\(^1\). However, it is important to stress that cutaneous presentations are not always associated to a previous health condition, indicating possible transmission through cutaneous trauma, as suggested by other studies\(^6\). In this study, nocardiosis only affected men between the ages of 28 and 35. This is consistent with the results of other studies, which have also identified similar occurrences of nocardiosis in young adult males, indicating the occupational risk of human infection by Nocardia species in mainly immunocompromised patients exposed to the agent within their environment\(^{10,21,34}\).

The clinical picture of human nocardiosis is diverse, though cutaneous and pulmonary manifestations are present in the majority of cases\(^3\). In the last few years, N. farcinica has been the most isolated species in reviews of different kinds of patient infection, whether with or without immunosuppression, representing around 22% and 45% of cases respectively\(^{19,20,32,34}\). Evidence shows that N. farcinica is widely distributed in the environment, has a high potential of virulence and is closely associated to a great number of fatal nocardiosis cases, including those with systemic dissemination\(^6\). Cases related to N. farcinica confirmed the severity of the disease in immunosuppressed patients.

In other reports, N. nova has been associated to approximately 20% of isolates in the United States and was the most pathogenic species in Canada until 2008\(^{18,35}\). N. nova has been attributed to different clinical presentations in humans, mainly related to immunosuppressive conditions or trauma\(^6\).\(^{33,35}\). These findings reinforce this species’ pathogenic potential for humans, and the risks of developing systemic nocardiosis, resulting in death.

N. asiatica was recently identified in human nocardiosis in Brazil, and this is one of few reports worldwide\(^{5,21,22}\) which reinforces the necessity of identifying more isolates and understanding their epidemiology.

The lower efficacy of human strains to gentamicin and ampicillin in the disk-diffusion test is probably related to the pattern of low susceptibility of N. farcinica resistance, indicating that these are not recommended as therapy\(^{20,31}\). However, the high efficacy of cephalosporins against N. farcinica isolates and the high success rate of amoxicillin/clavulanate against N. nova could be justified by the variability in β-lactamase activity present in the bacteria’s cell wall\(^{20}\). In agreement with previous results, minocycline appeared to be highly effective as in vitro against the Nocardia species isolated in these reports\(^{1,31}\), however MUÑOZ et al. (2007) observed resistance to this drug in N. nova and N. cyriacigeorgica.

Sulfamethoxazole/trimethoprim presented a lower efficacy in disk-diffusion tests against the isolates and are perhaps, therefore, not appropriate for therapy. Similar observations with a larger number of clinical isolates were made by TREMBLAY et al. (2011), informing of the importance of establishing treatment protocols for in vitro tests. However, despite the worldwide resistance pattern to sulfonamides recorded in clinical isolates, few patients failed therapy with these drugs, suggesting that the inhibition breakpoint for sulfonamides still poses a challenge and varies among different laboratories\(^6\). This can result from the methodology suggested by CLSI in which sulfonamide breakpoints are based on 80% of inhibition of growth endpoint compared to the 100% inhibition of growth by other drugs. In future research, more isolates should have the antimicrobial profile analyzed using the same CLSI specifications, as performed in this study, and new breakpoints for Nocardia species should perhaps be considered. Moreover, the indiscriminate use of the drug for comorbidities can induce resistance of Nocardia to sulfonamides and molecular mechanisms, thus it should be analyzed\(^{8,19,26}\).

Some studies showed the applicability of the E-test for antimicrobial susceptibility testing for the analysis of actinomycete resistance\(^{10}\). However, disagreement between MIC and disk diffusion tests has been indicated in other papers\(^{5,23}\). These differences probably occur due to colony lumps formed during culture growth, which makes it difficult to obtain a precise McFarland scale, or an ideal count of colony-forming units\(^23\). So far, for Nocardia the broth dilution method is still favorable, in comparison to the E-test, in order to assure the significance of the minimum inhibitory concentration method.

Despite the small number of isolates, it was possible to notice that nocardiosis in Brazil mainly affects men and immunosuppressed patients with localized or disseminated infection. Clinical manifestations could vary depending on the species, virulence of isolates, and immunocompromised factors of the patients. The cases reported in this study were seen in patients with a higher comorbidity, predominantly associated with pulmonary or disseminated forms. Antimicrobial resistances of isolates reinforce the importance of prior in vitro tests before initiating therapy. Further investigation with a larger number of cases and isolates is necessary.

**RESUMO**

Identificação molecular e perfil de sensibilidade a antimicrobianos de sete isolados clínicos de Nocardia spp. no Brasil

Nocardia é um microorganismo ubiquitário relacionado a infecções piogranulomatosas, com difícil resolução tecidual em humanos e animais. A doença é mundialmente emergente devido ao aumento de doenças e tratamentos imunossupressores. Este relato de casos ocorridos no Brasil visa apresentar a identificação molecular dos isolados e o padrão...
de sensibilidade a antimicrobianos por disco-difusão e concentração inibitória mínima (CIM) através de fitas E-test®. Os casos ocorreram em homens, em idade adulta. Três quadros foram cutâneos, dois pulmonares, um neurologico e um sistêmico. O quadro respiratório, o neurológico e um sistêmico estavam associados à doença ou terapia imunossupressora. O sequenciamento do gene 16S rRNA (1491pb) possibilitou a identificação de quatro isolados de Nocardia farcinica, dois de Nocardia nova e um de Nocardia asiatica. N. farcinica foi observada em dois casos dermatológicos, um pulmonar e um quadro sistêmico, N. nova foi isolada de um caso neurológico e outro pulmonar; e N. asiatica em um caso dermatológico. O teste de disco-difusão mostrou que amicacina (100%), amoxicilina/clavulânato (100%), cefalexina (100%) e ceftiofur (100%) foram mais efetivos; enquanto gentamicina (43%), sulfametoaxazol/trimetropina (43%) e ampicilina (29%) foram menos efetivos. No entanto, no teste de concentração inibitória mínima (CIM), apenas um dos quatro isolados da espécie Nocardia farcinica mostrou-se resistente a sulfametoaxazol-trimetropina.

ACKNOWLEDGMENTS

To FAPESP Funding (Protocols 2009/56037-1 and 2010/53494-5) and Mycology Research Center of University of Chiba - Japan.

REFERENCES

1. Acha, PN, Szyfres B. Nocardiosis. In: Acha, PN, Szyfres B. Zoonosis y enfermedades transmisibles comunes al hombre y a los animales, Vol.I. Washington: Organización Panamericana de la Salud; 2003. p. 212-92.
2. Agterof MJ, Van Der Bruggen T, Tersmette M, Ter Borg EJ, Van Den Bosch JMM, Biesma DH. Nocardiosis: a case series and a mini review of clinical and microbiological features. Neth J Med. 2007;65:199-202.
3. Ambaye A, Kohner PC, Wolffan PC, Roberts KL, Roberts GD, Cockerill FR. Comparison of agar dilution, broth microdilution, disk diffusion, E-Test, and BACTEC radiometric methods for antimicrobial susceptibility testing of clinical isolates of the Nocardia asteroides complex. J Clin Microbiol. 1997;35:847-52.
4. Anzalone CL, Cohen PR, Tarrand JJ, Diwan AH, Prieto VG. Nocardia yamanushiensis in an immunocompromised patient presenting as an indurated nodule on the dorsal hand. Tumori. 2013;99:156-9.
5. Baio PV, Ramos JN, Dos Santos LS, Soriano MF, Ladeira EM, Souza MC, et al. Molecular identification of Nocardia isolates from clinical samples and an overview of human nocardiosis in Brazil. PLoS Negl Trop Dis. 2013;7(12):e2573.
6. Bauer Aw, Kirby W, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45:493-6.
7. Beaman BL & Beaman L. Nocardia species: host-parasite relationships. Clin Microbiol Rev. 1994;7:213-64.
8. Brown-Elliott BA, Brown JM, Convile PS, Wallace RJ. Clinical and laboratory features of the Nocardia spp. based on current molecular taxonomy. Clin Microbiol Rev. 2006;19:259-82.
9. Brown-Elliott BA, Biehlé J, Convile PS, Cohen S, Saboulette M, Sussland D, et al. Sulfonamide resistance in isolates of Nocardia spp. from a U.S. Multicenter Survey. J Clin Microbiol. 2012;50:670-2
10. Chedid MB, Chedid MF, Porto NS, Severo CB, Severo LC. Nocardial infections: report of 22 cases. Rev Inst Med Trop Sao Paulo. 2007;49:239-46.
11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; approved standard. NCCLS. 2006. Susceptibility testing of Mycobacteria, Nocardiae, and other aerobic Actinomycetes. 8th ed. Approved standard CLSI document M2-A9. Wayne, PA: 2006.
12. Clinical and Laboratory Standards Institute. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing; approved guideline. CLSI document MM18-A.Wayne, PA: 2008.
13. Condas LAZ, Ribeiro MG, Yazawa K, Vargas APC, Salerno T, Giuffrida R, et al. Molecular identification and antimicrobial susceptibility of Nocardia spp. isolates from bovine mastitis in Brazil. Vet Microbiol. 2013;167:708-12.
14. Convile PS, Brown JM, Steigerwalt AG, Lee JW, Byrer DE, Anderson VL, et al. Nocardia asteroides as a pathogen in North American Patients. J Clin Microbiol. 2003;41:2560-8.
15. Convile PS, Brown-Elliott BA, Wallace Jr RJ, Withefsky FG, Kozioł D, Geraldine S, et al. Multisite reproducibility of the Broth microdilution method for susceptibility testing of Nocardia species. J Clin Microbiol. 2012;50:1270-80.
16. Convile PS, Withefsky FG. Multiple Copies of the 16S rRNA gene in Nocardia nova isolates and implications for sequence-based identification procedures. J Clin Microbiol. 2005;2881-5.
17. Convile PS, Withefsky F, Nocardia, Rhodococcus, Gordonia, Actinomadura, Streptomyces, and other aerobic actinomycetes. In: Veralovio I, Carroll KC, Punke G, Jorgensen JH, Landry ML, Warnock DW. Manual of Clinical Microbiology. 10th ed. Washington: AMS Press; 2011.
18. Corti ME, Villalba-Fiotti MF. Nocardiosis: a review. Int J Infect Dis. 2003;7:243-50.
19. Farina C, Boirun P, Ferrai I, Provost F, Goglio A. Report of human nocardiosis in Italy between 1993 and 1997. Eur J Epidemiol. 2001;17:1019-22.
20. Glupczynski Y, Berhin C, Janssens M, Wauters G. Determination of antimicrobial susceptibility patterns of Nocardia spp. from clinical specimens by Etest. Clin Microbiol Infect. 2006;12:905-12.
21. Iona E, Giannoni F, Brunori L, Gennaro M, Mattei R, Fattorini L. Isolation of Nocardia asiatica from cutaneous ulcers of a human immunodeficiency virus-infected patient in Italy. J Clin Microbiol. 2007;45:2088-9.
22. Kagayama A, Poonwan N, Yazawa K, Mikami Y, Nishimura K. Nocardia asiatica sp. nov., isolated from patients with nocardiosis in Japan and clinical specimens from Thailand. Int J Syst Evol Microbiol. 2004;54:125-30.
23. Krumperman PH. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol. 1983;46:165-70.
24. List of prokaryotic names with standing in nomenclature. [Internet] 2012. Available from: www.bacterio.net/nocardia.html
25. Lowman W, Aithma N. Antimicrobial susceptibility testing and profiling of Nocardia species and other aerobic actinomycetes from South Africa: comparative evaluation of broth microdilution versus the Etest. J Clin Microbiol. 2010; 8:4534-40.
26. Muñoz J, Mirelis B, Aragón LM, Gutiérrez N, Sánchez F, Español M, et al. Clinical and microbiological features of nocardiosis 1997-2003. J Med Microbiol. 2007;56:545-50.
27. Quinn PJ, Carter ME, Markey BK, Carter GR. The Actinomycetes. In: ___. Clinical Veterinary Microbiology. London: Wolfe; 2004.
28. Ribeiro MG, Salerno T, Mattos-Guadali AL, Camello TCF, Langoni H, Siqueira AK, et al. Nocardiosis: an overview and additional report of 28 cases in cattle and dogs. Rev Inst Med Trop Sao Paulo. 2008;50:177-85.
29. Roth A, Andress S, Kroppenstedt RM, Harmsen D, Mauch H. Phylogeny of the genus *Nocardia* based on reassessed 16S rDNA gene sequences reveals underspeciation and division of strains classified as *Nocardia asteroides* into three established species and to unnamed taxa. J Clin Microbiol 2003;41:851-6.

30. Santos IS. Actinomictoses no Rio Grande do Sul: a propósito de 59 casos, atualizando Actinomicose, Nocardiose e Rodococose. [Dissertação]. Porto Alegre: Universidade Federal do Rio Grande do Sul, Faculdade de Medicina; 2010.

31. Saubolle MA, Sussland D. Nocardiosis: review of clinical and laboratory experience. J Clin Microbiol 2003;41:4497-4501.

32. Tan CK, Lai CC, Lin SH, Liao CH, Chou CH, Hsu HL, et al. Clinical and microbiological characteristics of Nocardiosis including those caused by emerging *Nocardia* species in Taiwan, 1998-2008. Clin Microbiol Infect. 2010;16:966-72.

33. Thrusfield M. Veterinary epidemiology. 2. ed. Cambridge: Blackwell Science; 1995.

34. Tremblay J, Thibert L, Alarie I, Valiquette L, Pépin J. Nocardiosis in Quebec, Canada, 1988-2008. Clin Microbiol Infect. 2011;17:690-6.

35. Wallace JR, Brown, BA, Tsukamura M, Brown JM, Grace O. Clinical and laboratory features of *Nocardia nova*. J Clin Microbiol. 1991;29:2407-11.

36. Wallace JR, Steele LC, Sumter G, Smith JM. Antimicrobial susceptibility patterns of *Nocardia asteroides*. Antimicrob Agents Chemother. 1988;2:1776-9.

Received: 12 January 2014
Accepted: 23 September 2014