Zoonotic Sporotrichosis in Rio de Janeiro, Brazil: A Protracted Epidemic yet to Be Curbed

To the Editor—The zoonotic transmission of sporotrichosis seems to be rare worldwide. However, since 1998, an increasing number of cases have been reported in humans in Rio de Janeiro, Brazil, the vast majority of them associated with contact with cats affected by the same condition [1, 2]. From 1998 through 2004, there were 759 humans and 1503 cats diagnosed with this mycosis by Sporothrix schenckii isolation in biological specimens at the Evandro Chagas Clinical Research Institute, Oswaldo Cruz Foundation (IPEC/Fiocruz). This represents an enormous increment vis-a-vis previous findings, because during the previous 12 years, there were only 13 cases in the same reference center [2].

From 2005 through 2008, 804 human patients were diagnosed with sporotrichosis, corresponding to an annual increase of 85%. The most affected population remains unchanged, with a predominance of women aged 40–49 years who are engaged in domestic duties and are from deprived social strata. Close contact with cats, either with clinically evident disease or with no symptoms, was reported in 91% of the human cases. Bites and/or scratches were reported by 68% of these patients, suggesting such lesions as the putative mean of transmission of the fungus.

The clinical picture [3] comprised 66% of presentations belonging to the lymphocutaneous form, 25% of the fixed form, and 9% of patients with disseminated lesions (ie, disseminated cutaneous forms, with or without extracutaneous lesions).

Also worthy of notice is the apparent overlapping with other infectious conditions, such as human immunodeficiency virus infection (14 patients), tuberculosis (3), leprosy (2), and human T-lymphotropic virus infection (2). Among those patients infected by human immunodeficiency virus, 36% presented the disseminated severe form of the disease.

The drug of choice to treat these patients has been oral itraconazole \( (n = 514; 64\% \text{ of patients}) \), and terbinafine was used in 184 (23%) patients. Amphotericin B was very seldom used (6 patients). Almost 2% of clinically cured patients had clinical relapses (reemergence of their lesions), whereas 11% \( (n = 90) \) of the patients did not need to be treated, because of spontaneous cure. Patients were followed from 3–6 months after the end of therapy. Nine percent of the patients were lost to follow-up. Six patients were hospitalized, with 2 deaths. Irrespective of the drug regimens, 89% of the cases were cured.

It is still not certain how the infectious agent has been disseminated throughout the Rio de Janeiro municipality and its outskirts, but it is beyond reasonable doubt that the close interaction with cats represents a key form of transmission of the fungus. Felines have very close contact with contaminated soil and organic matter and constitute a reservoir of this agent [4, 5]. An improper destination given to ill or dead cats was mentioned by 71% of their current/former owners (most cats were just abandoned or died without receiving a proper burial or cremation). Such nonhygienic practices most likely foster the sustained dissemination of the mycosis, contributing to its current epidemic (en route to endemization?) status, which has yet to be curbed in Rio de Janeiro’s metropolitan area.

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Genetic Determinants of Antibiotic Resistance in Diarrheagenic Klebsiella pneumoniae Subspecies ozaenae: An Emerging Enteropathogen in Senegal

To the Editor—Diarrheal diseases are common in developing countries. They are usually caused by bacteria like Salmonella, Shigella, Escherichia coli, Campylobacter, and Vibrio cholerae. Members of the genus Klebsiella are found as normal flora in the human intestinal tract; some strains are considered to be enteropathogenic and are able to cause diarrhea both in immunocompetent and immunocompromised individuals [1, 2]. Although this pathogen is responsible for diarrhea, the molecular mechanism of its pathogenesis remains unclear. Virulence factors includ-
ing fimbriae, iron uptake systems, lipopolysaccharide, capsular serotype, and toxins involved in the pathogenicity of *Klebsiella pneumoniae* have been found on horizontally acquired DNA regions, such as pathogenicity islands, plasmids, transposons, and bacteriophages [3, 4]. Horizontal transmission of genes among bacteria, including antimicrobial resistance genes, can also occur via transduction, transformation, or conjugation.

We report here the genetic determinants of resistance and virulence in diarrheagenic *K. pneumoniae* subspecies *ozaenae* isolated in Senegal. We isolated 2 strains of *K. pneumoniae* subspecies *ozaenae* from adults with diarrhea in 2 hospitals in Dakar, one an urban hospital and the other a university teaching hospital—based infectious diseases clinic. The first isolate was obtained in January 1998 from a human immunodeficiency virus–negative person, the second in May 2000 from a human immunodeficiency virus type 1–infected patient with a CD4 lymphocyte count <200 cells/μm³. These subjects were randomly selected to be part of a case-control study. *K. pneumoniae* subspecies *ozaenae* was considered to be the sole etiologic agent of diarrhea because it was obtained as a pure culture in nonselective, solid, bromocresol-purple. Strains were identified with the API 20E system (Biomerieux). Antibiotic susceptibility was tested by disc diffusion method, according to Clinical and Laboratory Standards Institute recommendations.

Strains were resistant to amoxicillin (minimum inhibitory concentration [MIC], 128 μg/mL), amoxicillin-clavulanic acid (MIC, 64 μg/mL), ticarcillin (MIC, 256 μg/mL), trimethoprim-sulfamethoxazole (MIC, 256 μg/mL), tetracycline (MIC, 256 μg/mL), chloramphenicol (MIC, >8 μg/mL), ofloxacin (MIC, 64 μg/mL), and ciprofloxacin (MIC, 64 μg/mL). No extended-spectrum β-lactamases were detected. This resistance towards the first-line (tetracyclines and trimethoprim-sulfamethoxazole) and the second-line drugs (quinolones) and the absence of an extended-spectrum β-lactamase allow the use of broad-spectrum cephalosporins to treat diarrheal illness in these cases.

DNA was extracted using a kit (Qiagen), according to the manufacturer’s recommendations, and was stored at −20°C. Polymerase chain reaction (PCR) for the detection antimicrobial resistance genes and integrons was performed with primers described elsewhere (GenBank accession numbers AB187515, FJ616991, AF070235, and X53796) [5–8]. *bla*<sub>TEM1</sub>, *cat1*, *tetB*, and *qnrS* genes encoding ampicillin, chloramphenicol, tetracycline, and quinolone resistance, respectively, were present in all strains. Both strains hosted class 1 and class 2 integron. The characterization of class 1 integron revealed an atypical cassette array with an insertion sequence, *oxa*<sub>30</sub>-*aadA1-IS1*. In class 2 integrons, the organization of cassette arrays revealed the same 3 cassettes as were found in Tn7 (*dfra1- sat1- aadA1-orfX*). The 3′ segment was absent in class 2 integrons. The atypical class 1 integron (*oxa*<sub>30</sub>-*aadA1-IS1*) was described in the *Shigella* resistance locus in the chromosome, as demonstrated in other strains of *Shigella flexneri* and *Shigella dysenteriae* with worldwide origins [9, 10]. The presence of the same integron content in *Shigella* species suggests that *K. pneumoniae* subspecies *ozaenae* isolates may have acquired the atypical class 1 integron by horizontal transfer with other resistance determinants; specifically conjugal transfer, which requires cell-to-cell contact, making gene flux easier in the gastrointestinal tract.

To determine whether the resistance determinants were transferable, we performed a conjugative experiment on selective medium containing nalidixic acid (50 μg/mL) plus trimethoprim (100 μg/mL). All antimicrobial drug resistances were transferred at once from *K. pneumoniae* subspecies *ozaenae* to an *E. coli* strain resistant to nalidixic acid. Plasmid analysis revealed that the isolates and transconjugants contained a single plasmid of ≥100 Kb. The PCR analysis of plasmid DNA and transconjugants confirmed the presence of all resistance genes and class 1 and class 2 integrons, suggesting the plasmidic location of these resistant determinants.

This is the first description of *K. pneumoniae* subspecies *ozaenae* associated with diarrhea in Senegal. Guerin et al [2] reported a case of bloody diarrhea caused by *K. pneumoniae* but did not find virulence genes and presented the possibility of the presence of new virulence genes. The ferric dicitrate uptake system (fec) in *Shigella* species is known to play an important role in bacterial virulence [11]. To determine whether the ferric dicitrate transport system (fec) was involved in *K. pneumoniae* subspecies *ozaenae* virulence, we successful performed PCR with use of primers described by Pan et al [9]. Because all relevant markers of the *Shigella* resistance locus pathogenicity island were present in *K. pneumoniae* subspecies *ozaenae*, we thought that a horizontal gene transfer, a process that is well known to contribute to microbial evolution, could have possibly occurred between *Shigella* and *K. pneumoniae*.

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