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Fatal diarrhea caused by multidrug resistant *Pseudomonas aeruginosa* in a foal from Rio de Janeiro, Brazil*

Diarreia fatal causada por Multidrug resistant *Pseudomonas aeruginosa* em um potro no Rio de Janeiro, Brasil

Bruno Penna,* Aline Pinna,* Carlos R. A. Ribeiro,* Priscila da Silva Pinto,* Walter Lilenbaum*

Abstract

The present study evaluated the susceptibility pattern of one *Pseudomonas aeruginosa* isolate obtained from a deceased foal following an acute case of diarrhea. The isolate was identified by reference methods and tested with a panel of 27 antimicrobial agents. *Pseudomonas aeruginosa* was confirmed in pure culture and the isolate demonstrated resistance to all tested antimicrobials. Since it was a unique case in this herd, we suggest that contamination did not happen by horizontal transmission. The multidrug resistance was unexpected, associated to the virulence of the isolate, that killed the foal in five days, despite treatment.

Keywords: *Pseudomonas aeruginosa*, foal, diarrhea, multidrug resistance.

Resumo

O presente estudo avaliou o padrão de suscetibilidade de uma amostra de *Pseudomonas aeruginosa* isolada de um potro falecido por consequência de um caso de diarreia aguda. A amostra foi identificada por métodos de referência, e testada para a suscetibilidade a um painel de 27 agentes antimicrobianos. *Pseudomonas aeruginosa* foi isolada em cultura pura e demonstrou resistência a todos os antibióticos testados. Como foi um caso único no rebanho, sugerimos que a contaminação do potro não aconteceu através de transmissão animal-animal. Foi realmente inesperado o perfil de suscetibilidade a antibióticos (multirresistente), associada à virulência do isolado, que matou o potro em cinco dias, apesar do tratamento.

Palavras-chave: *Pseudomonas aeruginosa*, potro, diarreia, *multidrug resistance.*

Introduction

Pseudomonas aeruginosa is a Gram negative microrganism that can be found in animal hosts and environment (Janam et al., 2011). This bacteria are known to be a major agent of infections in human hospitals, and has also been reported as opportunistic agent in animals (Kidd et al., 2011). In horses, it has been associated to endometritis leading to reduced fertility (Kidd et al., 2011). Nevertheless, few studies regarding systemic disease have been reported.

P. aeruginosa can be resistant to all classes of antimicrobial agents making it especially difficult to treat patients with compromised immune systems (Penna et al., 2010). Due to the presence of several drug efflux systems and porins, it is intrinsically resistant to a wide range of antimicrobials including benzylpenicillins, aminobenzylpenicillins, carboxypenicillins, 1st and 2nd generation cephalosporins, chloramphenicol and tetracyclines. It also forms biofilms which are impervious to antimicrobials, further complicating therapy (Rubin et al., 2008). Major classes of antimicrobial used for systemic treatment of infections include anti-pseudomonal penicillins, 3rd and 4th generation cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones. Unfortunately, resistance to these drugs is frequently encountered in clinical practice. Due to highly variable resistance patterns, empiric therapy may result in inappropriate treatment. Thus, antimicrobial susceptibility testing should be a crucial step to select appropriate therapy. Multidrug resistant *P. aeruginosa* (MDRPA) were defined as strains resistant to piperacillin, ceftazidime, imipenem and gentamicin (Gales et al., 2001; Paramythiotou et al., 2004).

The aim of this article was to report a case of fatal diarrheia in a foal and demonstrate the susceptibility pattern of this particular *Pseudomonas aeruginosa* strain**.**

Case report

A five-month-old male foal was presented with an acute diarrhea. The foal did not present any prior history of diarrhea (bacterial or otherwise) or any other clinical condition. At the moment of the first evaluation the animal presented dehydration, apathy and also anorexia. After collection of feces for bacteriology, supportive therapy was employed with fluid and electrolytes reposition. Additionally, antibiotic therapy was installed, associating sulphametoxazol + trimethoprim (15mg/kg BID) to ceftiofur (4.4mg/kg SID). By the third day, Ceftriaxone (50mg/kg BID) was included, since no clinical response was observed. Despite all efforts, foal died on the fifth day. There were no other

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**Veterinary Bacteriology Laboratory, Fluminense Federal University, 101Prof. Hernani Mello Street, office 309. Zipcode 24210-130, Niteroi, RJ, Brazil
Correspondence: tel.: 55(21) 26292435 Hernani Mello Street, office 309. Zipcode 24210-130, Niteroi, RJ, Brazil fax: 55(21) 26292432.
E-mail: pennanet@gmail.com

animal presenting diarrhea, as well as no history of pseudomonal endometritis in mares on the farm.

Bacteriology from feces sample confirmed the presence of *Pseudomonas aeruginosa* in pure culture. It was identified (morphological characteristics, biochemical standard tests) and tested for susceptibility to antimicrobial drugs (CLSI, 2008). After bacterial growth, smears were made, Gram-stained and examined microscopically. Since sample presented morphology of Gram-negative rod it was transferred to MacConkey's agar and Pseudomonas agar (Merck – New Jersey, USA). Isolate in pure culture was identified on the basis of colony morphology, Gram-staining, pigment production (pyocyanine and pyoverdin), oxidase test, motility, aerobic fermentation of glucose, lactose, maltose and mannitol, arginine and lysine utilization, nitrate reduction, urease and DNAse production, ONPG (orthonitrophenyl-beta-D-galactopyranoside) test, acetamide and esculin hidrolysis, and susceptibility to polimyxin, according to reference methods (Koneman et al., 2005).

The isolate showed *in vitro* resistance to all of the 27 tested drugs, i.e. Ceftriaxone, Cephalotin, Cephalexin, Ceftazidime, Cefadroxil, Cefaclor, Cephapirin, Cefotaxime, Ceftiofur, Amoxicicllin, Amoxicillin associated to Clavulanic acid, Ampicilin, Ampicilin associated to sulbactam, Piperacilin, Piperacilin associated to Tazobactam, Imipenem, Amikacin, Gentamicin, Tobramycin, Enrofloxacin, Ciprofloxacin, Norfloxacin, Tetraciclin, Doxiciclin, Erythromycin, Clarythromycin and Azythromycin.

Discussion

Treatment of *P. aeruginosa* infections is challenging, since some strains present multi-resistance. That isolate presented intrinsic resistance and acquired resistance to antimicrobial agents that are commonly used, and met the criteria of multidrug resistant *P. aeruginosa* (MDRPA), defined as resistance to piperacillin, ceftazidime, imipenem and gentamicin.

Since it was a unique case in this herd, we suggest that foal contamination did not happen by horizontal transmission. The mare did not presented with any signs of endometritis caused by *P. aeruginosa* before or after the foaling. Also, it did not presented any signs even after the foal died. Since to our knowledge there are no reports of mares being asymptomatic carriers of *P. aeruginosa* in the uterus or the vagina, a transmission from the mare to the foal is not the most likely hypothesis although it can not be rulled out. Indeed, it is a lot more probable considering an environmental source in that case, since *P. aeruginosa* is a classic opportunistic microorganism known for its high capacity to survive in the environment (Janam et al., 2011), what have been recently demonstrated in reproductive disorders in horses (Kidd et al., 2011). It was really unexpected the antibiotic susceptibility profile (multidrug resistant), associated to the virulence of the isolate, that killed the foal in five days, despite the treatment. In addition, the fact that this multidrug resistant bacterium was acquired from the environment is extremely alarming.

Conclusion

Although a single case report, does not illustrate a major problemthese data shows that multidrug resistance *Pseudomonas aeruginosa* is present in our environment and should be considered when facing cases of acute diarrhea in foals. also, sanitary measurements should be considered in these cases.

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