

## AN OUTBREAK OF *STREPTOCOCCUS DYSGALACTIAE* SUBSP *EQUISIMILIS* IN A HOSPITAL IN THE SOUTH OF BRAZIL

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### ABSTRACT

The beta-hemolytic group C streptococci (Lancefield's group) has been considered an emergent human pathogen, showing an important role as an opportunist agent, being responsible for nosocomial infections and outbreaks. This study is reporting the first outbreak of nosocomial infection caused by *Streptococcus dysgalactiae* subsp. *equisimilis* in Brazil. From January, 2002, to December, 2004, *S. equisimilis* was isolated in 67/207 (32.37%) samples from secretions of patients' infected wounds, interned at the Hospital of Sanitary Dermatology in the State of Paraná (HDSPR). The prevalence of this microorganism increased from 11/42 (26.19%) in 2002, 14/65 (21.54%) in 2003 to 42/100 (42.00%) in 2004. This increase was statistically significant ( $p=0.024$ ), and this microorganism became the most frequently isolated in these patients, overtaking the rates of isolation of *Pseudomonas aeruginosa*. The *S. equisimilis* grew in pure culture, as a unique microorganism, in six samples (2.9%) out of 207. Fresh feces of 15 animals (horses and sheep) living in the proximities of the hospital were also examined and three of them positive for *S. equisimilis*. The biochemical profile of the strains isolated from the patients and from the animals was the same. These animals might have been the source of the dissemination of the outbreak in the hospital. New studies will be necessary to confirm the genetic relationship between the strains isolated from patients and animals.

**Key words:** Nosocomial infection, Group C streptococci, *Streptococcus dysgalactiae* subsp. *equisimilis*, outbreak.

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### INTRODUCTION

Nowadays four species of Streptococcus group C are known: *S. dysgalactiae* subsp. *equisimilis*, *S. equi* subsp. *zooepidemicus*, *S. equi* subsp. *equi* and *S. dysgalactiae* subsp. *dysgalactiae*, the first three belonging to beta-hemolytic group C streptococci (GCBHS) (7).

The beta-hemolytic group C streptococci (Lancefield's group) is known as a common cause of infections in animals (26). Swine and horses are commonly infected by *S. equisimilis* (15), whereas humans are rarely infected. In horses and other domestic animals, *S. equisimilis* causes suppurative conditions (4).

Human infections with group C streptococci can lead to severe diseases, particularly in individuals with underlying

conditions such as cardiovascular disease, malignancy or immunosuppression (19). *S. equisimilis* is associated with pharyngitis (27) and a variety of localized and systemic suppurative infections, sometimes fatal in humans (1,2).

Lately the number of publications and notifications of serious diseases caused by GCBHS such as wound infections (8,23), septicemia (17), bacteraemia (16,18,20), septic arthritis (11,21), pneumonia (22,24) and streptococcal toxic shock syndrome (13) has increased. This microorganism has been considered an emergent human pathogen, playing an important role as an opportunist agent, as well as the cause of nosocomial infections and outbreaks (5,6,9,25).

This study aimed to detect the main pathogen involved in the development of infections in skin lesions in patients

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hospitalized in the Hospital of Sanitary Dermatology in the State of Paraná (HDSPR), which is a reference hospital for treatment of Hansen's patients in the state. The lesions, mainly located in the inferior members of the body, frequently occur in these patients, as a consequence of the lack of sensibility and necrosis, common to the disease. These lesions become easily colonized by microorganisms, which could attack and invade extensive areas, compromising the health of these patients.

## MATERIALS AND METHODS

The Bacteriology Laboratory of the Public Health Laboratory of the State of Paraná - LACEN, received 207 samples of secretions of infected wounds in the period between January, 2001 and December, 2004, most of them located in the inferior members of patients hospitalized in the Hospital of Sanitary Dermatology of the State of Paraná, PR, Brazil (HDSPR).

The HDSPR, former São Roque Hospital, is located in Piraquara, in the metropolitan region of the city of Curitiba, PR. HDSPR has 84 beds and is a reference hospital in sanitary dermatology in the state of Paraná. It integrates the Brazilian National Health Care System (SUS) and provides specialized assistance in the treatment of Hansen's disease. The patients included in the study were hospitalized at the moment of the analysis, but were free to transit in the hallways, restrooms and gardens.

Fresh feces belonging to horses ( $n = 10$ ) and sheep ( $n = 5$ ) from the Center for Production and Research of Immunobiologics (CPPI) situated in the surroundings of the HDSPR were also examined. The feces of these animals were collected intrarectally by a CPPI veterinarian, who wore sterile gloves and stored the samples shortly after in a Stuart's tube and forwarded to the bacteriology sector of LACEN.

The samples, both human and animal, were cultured on blood agar plates (tryptose blood Agar base, Difco) and MacConkey agar plates (MERCK), and incubated at 35°C in aerobic atmosphere for 18/24 hours.

The isolates of *S. dysgalactiae* subsp. *equisimilis* were presumptive identified based on their phenotypic characteristics on blood agar plates, after incubation at 35°C in aerobic atmosphere. The  $\beta$ -hemolytic, catalase-negative, Gram-positive cocci arranged in chains were tested with antisera-coated latex beads (Streptococcal Grouping Kit<sup>R</sup>, Oxoid, Basingstoke, England). Biochemical testing such as deamination of arginine, hydrolysis of esculin, production of acid from sorbitol, trehalose, lactose and ribose and Voges-Proskauer test, were performed for the characterization of the species (7).

The prevalence (%) of *Pseudomonas aeruginosa* and *S. dysgalactiae* subsp. *equisimilis* in the years 2002, 2003 and 2004 were compared using the nonparametric qui-square test for independent samples. The level of significance was set at

0,05. The project of study was approved by the Human Being Research Ethics Committee (CEP – 0285/06).

## RESULTS

The following results of biochemical tests let to the identification of *Streptococcus dysgalactiae* subsp. *equisimilis*: bacitracin resistance, negative Camp test and Voges-Proskauer reactions, positive arginine and esculin reactions, and ability to ferment ribose, lactose and trehalose, but not sorbitol. The production of acid from ribose, trehalose and sorbitol are important tests for the characterization of these isolates.

A miscellany of microorganisms were recovered from the patients samples. *Pseudomonas aeruginosa* was found in 80/207 (38.7%) of the examined samples, followed by *S. equisimilis* in 67/207 (32.4%), *Staphylococcus aureus* in 51/207 (24.6%), *Morganella morganii* in 46/207 (22.2%), *Proteus mirabilis* in 34/207 (16.4%), *Streptococcus* of the Group G in 16/207 (7.7%), *Acinetobacter baumannii* in 15/207 (7.2%), *Proteus vulgaris* in 14/207 (6.8%) and *Streptococcus pyogenes* in 3/207 (1.5%). Other detected bacteria were *Acinetobacter spp.*, *Proteus spp.*, *Corynebacterium spp.*, *Echerichia coli*, *Shewanella putrefaciens*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterobacter agglomerans* (Table 1).

In 2004, the examined samples presented an increased in isolation of *S. equisimilis*. The prevalence increased from 11/42 (26.19%) in 2002, 14/65 (21.54%) in 2003 to 42/100 (42.0%) in 2004. This increase was statistically significant ( $p = 0.024$ ), and this microorganism became the most frequently isolated in these patients, overtaking the rates of isolation of *Pseudomonas aeruginosa*. The prevalence of *S. dysgalactiae* subsp. *equisimilis* increased significantly along the years 2002 through 2004 ( $\chi = 7,47$ ; GL = 2;  $p = 0.024$ ), differently from *Pseudomonas aeruginosa*, which had not presented a significant increase ( $\chi = 0.53$ ; GL = 2;  $p = 0.77$ ), as shown in Table 2.

In six samples (2.9%) out of 207 the *S. equisimilis* grew in pure culture, as a unique microorganism. In the other samples the growth was polymicrobial. Among feces of animals that live in the proximities of the hospital, three were positive for *S. equisimilis*. The biochemical profile of the strains isolated from the patients was the same found in the animals.

## DISCUSSION

The diversity of microorganisms is variable in different areas, among hospitals in the same city or even inside units of the same hospital. Therefore it is extremely important for the hospitals to follow the increase of the main bacteria involved in cases of hospital infections.

This study reports for the first time in Brazil, an outbreak of nosocomial infection caused by *S. equisimilis*. This microorganism corresponded to 42.00% of the total isolates

**Table 1.** Microorganisms isolated from lesions sample of patients wounded between January/2002 and December/2004.

Microorganism	Jan/02-Dec/02 n=42 samples	Jan/03-Dec/03 n=65 samples	Jan/04-Dec/04 n=100 samples	Total n=207 samples
<i>Pseudomonas aeruginosa</i>	18 (42.85%)	24 (36.92%)	38 (38.00%)	80 (38.65%)
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	11 (26.19%)	14 (21.54%)	42 (42.00%)	67 (32.37%)
<i>Staphylococcus aureus</i>	13 (30.95%)	15 (23.07%)	23 (23.00%)	51 (24.64%)
<i>Morganella morganii</i>	9 (21.42%)	16 (24.61%)	21 (21.00%)	46 (22.22%)
<i>Proteus mirabilis</i>	7 (16.67%)	15 (23.07%)	12 (12.00%)	34 (16.42%)
<i>Streptococcus Group G</i>	0 (0.00%)	7 (10.77%)	9 (9.00%)	16 (7.73%)
<i>Acinetobacter baumannii</i>	9 (21.42%)	5 (7.69%)	1 (1.00%)	15 (7.25%)
<i>Proteus vulgaris</i>	6 (14.28%)	5 (7.69%)	3 (3.00%)	14 (6.76%)
<i>Streptococcus pyogenes</i>	0 (0.00%)	2 (3.07%)	1 (1.00%)	3 (1.45%)

**Table 2.** Prevalence (%) of *Pseudomonas aeruginosa* and *S. dysgalactiae* subsp. *equisimilis* in 2002, 2003 and 2004 ( $\chi$ ;  $\alpha$  = 0.05)

	n	<i>Pseudomonas aeruginosa</i>	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>
Jan/02-Dec/02	42	18 (42.85%)	11 (26.19%)
Jan/03-Dec/03	65	24 (36.92%)	14 (21.54%)
Jan/04-Dec/04	100	38 (38.00%)	42 (42.00%)
$\chi$	-	>0.05 ( $\chi$ = 0.53; p = 0.77)	<0.05 ( $\chi$ = 7.47; p = 0.024)

found in the secretions of lesions of Hansen’s patients at the Hospital of Sanitary Dermatology in the State of Paraná in 2004, and grew in pure culture in six samples (2,9%), demonstrating an important pathogenic potential. The literature shows that *S. equisimilis* can cause serious infections, and sometimes fatal ones such as pneumonia, sinusitis, septicemia, endocarditis, osteomyelitis, meningitis, among others (1).

In the UK, in 1994, seven patients were affected in an outbreak of *S. equisimilis* occurred in a Maternity Unit in Durham (9). Other outbreaks were reported by Efstratiou, occurred in burns and maternity units, hospital wards, outpatient clinics, army camps and religious communities (6). Thirty-three confirmed cases of puerperal fever caused by this microorganism occurred in three hospitals inside and around Chelmsford (25). These data show the importance of the correct identification of the bacteria causing the infection, because streptococci of Lancefield group C are relatively infrequent causative agents of infection in humans (2).

The course of this disease is usually acute and serious. In most cases, it affects patients with underlying conditions, however, the infection may occur in healthy individuals as well (14,19).

The bacteria can be carried on the skin, mucous membranes, and in lymphoid tissues of healthy individuals (12). These microorganisms are opportunistic and usually gain entry to the body through injuries. Pathogenic streptococci do not survive for long outside the animal body, it is believed that the transmission is through direct contact or from aerosols (3).

According to Goldmann *et al.* (10), the precise route of transmission is unknown; but it is believed that streptococci can be carried on the hands, shed from the nose, or aerosolized by flatus.

Teare *et al.* (25) showed that most of the cases of an outbreak occurred in a ward, where toilet seats and a shower might have aided transmission, although insufficient data was obtained to exclude the role for person-to-person spread.

The results of the present study indicate that the animals surrounding the hospital might have been the source of dissemination of the infection. The spread of these microorganisms could have occurred through dust or aerosols, by animal feces exposed on the soil. The proximity of these animals to the hospital may perhaps explain the high prevalence of these microorganisms in the secretions of wounds from these patients.

New studies are being conducted to verify the origin of the contamination, as well as to confirm the genetic relationship between the strains isolated from patients and animals, for better understanding of the dissemination route of these microorganisms and to confirm the cloning nature of the strain isolated.

## RESUMO

### Surto causado por *Streptococcus dysgalactiae* subsp. *equisimilis*, em um hospital, no Sul do Brasil

O estreptococo beta-hemolítico do grupo C de Lancefield tem sido considerado patógeno humano emergente, mostrando importante papel como agente oportunista,

implicado algumas vezes em infecções hospitalares e surtos. Este estudo está relatando o primeiro surto de infecção hospitalar causado pelos *Streptococcus dysgalactiae* subsp. *equisimilis* no Brasil. De janeiro de 2002 a dezembro de 2004, isolou-se *S. equisimilis* em 67/207 (32,37%) das amostras de secreções de lesões de feridas coletadas de pacientes internados no Hospital de Dermatologia Sanitária do Paraná (HDSPR). A prevalência deste microrganismo aumentou de 11/42 (26,19%) em 2002, 14/65 (21,54%) em 2003 para 42/100 (42,00%) em 2004. Este aumento foi estatisticamente significativo ( $p=0.024$ ), tornando este microrganismo o mais freqüentemente isolado nos pacientes, ultrapassando as taxas de isolamento de *Pseudomonas aeruginosa*. Em seis amostras (2,9%) entre as 207 examinadas, *S. equisimilis* cresceu em cultivo puro, como único microrganismo. Também foram examinadas fezes frescas de 15 animais (cavalos e ovelhas) que vivem nas proximidades do hospital, e três amostras foram positivas para *S. equisimilis*. O perfil bioquímico encontrado entre os isolados dos pacientes foi o mesmo encontrado entre os isolados das fezes dos animais. Acredita-se que estes animais possam ter sido a fonte de disseminação do surto no hospital. Novos estudos serão necessários para confirmar o relacionamento genético entre os isolados dos pacientes e animais.

**Palavras-chave:** Infecção hospitalar, Estreptococos do grupo C, *Streptococcus dysgalactiae* subsp. *equisimilis*, surto.

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