

Pseudo-outbreak of *Serratia marcescens* in a tertiary care hospital

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SUMMARY

The aim of this study was to describe a pseudo-outbreak due to *Serratia marcescens* associated with laboratory contamination, and also the epidemiologic and laboratory methods used to determine the source of contamination.

An apparent increase in positive culture results for *Serratia marcescens* was observed in the Clinical Microbiology Laboratory of Kocaeli University Hospital between September and November 2007. An outbreak investigation including retrospective and prospective studies using chart review, environmental sampling and random arbitrary polymorphic DNA-polymerase chain reaction (RAPD-PCR) of randomly selected isolates were performed by the Infection Control Committee.

Nine out of 67 strains belonged to a real infection. *S.marcescens* was also isolated from saline solution in the specimen processing area of the laboratory. It was recognized that the technician has been using the same stock saline solution for processing specimens without changing the injector. RAPD patterns of the clinical isolates were identical to the pattern of saline strain. The contaminated saline solution was discarded, the technician was trained and no additional cases of suspected contamination have been observed.

This pseudo-outbreak emphasize the importance of the observation of specimen processing procedures and the training of laboratory workers.

KEY WORDS: Pseudo-outbreak, Pseudoinfection, *Serratia marcescens*

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INTRODUCTION

A pseudo-outbreak has been defined as the recovery of the same microorganism from cultures of multiple patients who are not infected or colonized with the organism.

It may be due to contamination of specimens, laboratory error, or changes in surveillance techniques. Such outbreaks are often time-consuming and costly, may lead to unnecessary treatment or hospital admission of cases and often result in a loss confidence in the credibility of the laboratory (Shears, 1996).

Serratia marcescens is a gram-negative rod that is motile, rarely ferment lactose, and produces an extracellular DNase. It is widespread in the environment and can survive in disinfectants, some of which have been the sources of outbreaks (Donnenberg, 2005).

In this report, we describe a pseudo-outbreak with *S. marcescens* at a university hospital, which lasted three months prior to the identification and elimination of the source.

MATERIALS AND METHODS

Our University Hospital is a 650 bed tertiary care hospital in Kocaeli, Turkey. We detected a significant increase in the numbers of *S. marcescens* isolates over a 3-month period that led us to suspect of an outbreak. Thereafter, an investigation by the Infection Control Team was launched.

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Patient records were reviewed to determine who had the real infection. Various environmental samples were taken, including liquid hand washing soaps and surface disinfectants from different clinics. At the same time, we conducted a microbiological analysis of possible laboratory sources of contamination. The cultures of needles, forceps and saline solution were performed. The automated VITEK-2 system (bioMérieux, France) was used for bacterial identification and antibiotic susceptibility testing.

Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was used for genotypic characterization of the *S.marcescens* isolates. Oligonucleotide primers PSA-5'-AGCGGGC-CAA-3' and PSB-5'-ACGGCCGACC-3' were used. Amplification was accomplished with 40 cycles of variable annealing temperatures such as 30 s at 30°C, 30 s at 45°C followed by 2 min of extension at 72°C and 20 s of denaturation at 95°C. PCR products were run on 0.9% agarose gel at 10 v/cm², visually inspected and interpreted on a UV lamp. The genotypes were characterized as identical (identical banding pattern), highly related (one mismatch in banding pattern), or unrelated.

RESULTS

Description of the outbreak

In all 67 isolates of *S.marcescens* resistant to ampicillin, ampicillin/sulbactam, cephazoline, cefuroxime, cephixime and tetracycline were detected in the cultures from various specimens of both hospitalized and outpatients between September and November 2007. This number was found to be higher than in the past months (Figure 1). The mean isolation number was 4 isolates per month before the outbreak period. Nine of the 67 isolates were from real infection. *S. marcescens* strains were isolated from 30 tracheal aspirate samples, 9 catheters, 5 urinary samples, 1 bronchoalveolar lavage fluid and 22 other clinical samples.

A pseudo-outbreak was suspected due to several reasons. First of all, most of the patients did not have any clinical signs and symptoms. Secondly, hospitalized patients were followed in different wards. Third, *S. marcescens* with an identical antibiotic resistance pattern were also isolated from the cultures of outpatients. Besides, laboratory contamination was not initially suspected as a

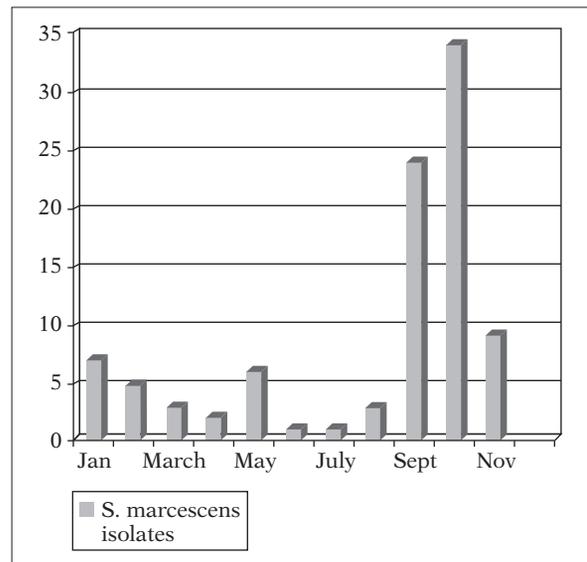


FIGURE 1 - Number of *S. marcescens* isolates recovered between January and November 2007.

cause of the pseudo-outbreak because gram-negative bacilli were observed in the gram staining of some clinical samples.

Environmental sampling

Two of 7 cultures from liquid hand-washing soaps yielded *Pseudomonas aeruginosa* and one of 34 disinfectants yielded *Pseudomonas putida*. *S.marcescens* was isolated from the saline solution used in the specimen processing area of microbiology laboratory.

One month before the occurrence of outbreak, a new technician had joined the microbiology laboratory staff. Therefore, the technician was observed and questioned about sample handling and sterilization-disinfection procedures. It became apparent that the technician had used the same saline stock solution for sampling during the 4 months period and did not change the injector needle between different samples. We recognized that the technician also used the same saline solution to prepare the samples for gram staining.

Decontamination procedures

The contaminated saline solution was discarded and the technician was trained to use a different injector and saline solution for each sample. On the following days *S. marcescens* was not isolated from the cultures.

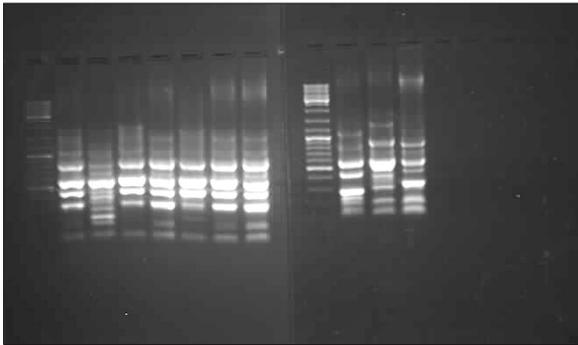


FIGURE 2 - RAPD patterns of the isolates.

The soaps and disinfectants in which *Pseudomonas* isolated were also discarded and it was recommended to use fewer soaps and disinfectants and to have samples of them cultured by the Infection Control Team periodically.

Genotyping of the *S. marcescens* isolates

Ten randomly selected *S.marcescens* isolates, one from saline solution, 9 from different clinical samples and one isolated after decontamination were genotyped by RAPD-PCR. A single *S.marcescens* strain was present in 5 patients and it was also identical to the strain from saline solution (lanes 1, 3-7, Figure 2).

DISCUSSION

Approximately 11% of all nosocomial outbreaks are believed to represent pseudo-outbreaks (Shears,1996) that become apparent due to a discrepancy in the patient's clinical condition and the culture result. Pseudo-outbreaks due to different organisms including gram-negative and positive bacteria, mycobacteria, fungi and parasites have been described (Verweij *et al.*, 1997; Grinbaum *et al.*, 2003; Corne *et al.*, 2005; Ohsaki *et al.*, 2007; Wadhwa *et al.*, 2005; Grigis *et al.*, 2000; Silva *et al.*, 2003; Medeiros *et al.*, 2007; Wang *et al.*, 2008). Most pseudo-outbreaks are caused by errors in collecting, handling or processing of specimens (Shears, 1996; Verweij *et al.*, 1997). The present study reported a *S.marcescens* pseudo-outbreak caused by laboratory contamination.

Antimicrobial susceptibilities were similar in our *S.marcescens* isolates. Although antibiotyping is used successfully for epidemiological investiga-

tions, it was not useful in our study because the strains with the same susceptibility pattern were different by genotyping.

During the outbreak investigation, sampling was performed from soaps and disinfectants and some of them yielded *P. aeruginosa* and *P. putida*. Although these bacteria were not the source of the pseudo-outbreak, the detection of this contamination was important for infection control. Fortunately, there was no increase in infections caused by *Pseudomonas*. Saline solution contaminated with *S.marcescens* was detected as the source of the pseudo-outbreak. The contaminated saline solution was discarded and no additional cases of suspected contamination have been observed.

This pseudo-outbreak emphasizes the importance of periodic observation of specimen processing procedures, the education of laboratory workers and surveillance procedures.

Part of the study was reported as a poster presentation at the "18th European Congress of Clinical Microbiology and Infectious Diseases , Barcelona, Spain".

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