**ABSTRACT**

**Objective:** Stenotrophomonas maltophilia is an opportunistic pathogen found predominantly in the environment and hospital setting. Invasive procedures and treatment methods, instruments used for diagnosis and irrational antibiotic use play major roles in the spread of this pathogen. The study aimed to evaluate consecutive S maltophilia isolation from bronchoalveolar lavage samples during bronchoscopy procedure during a week.

**Methods:** Four patients consecutively had S maltophilia isolated during bronchoscopy between September 8 and 15, 2012. The identification of the isolates and their antibiotic susceptibility were studied by automated Vitek version 2.0 (Biomerieux, France) system. The clonal relationship between the isolates was studied by enterobacterial repetitive intergenic consensus (ERIC) polymerase chain reaction (PCR).

**Results:** Four consecutive S maltophilia isolates had identical band patterns and showed clonal relatedness.

**Conclusion:** Bronchoscopy is a common invasive procedure that is utilized in chest diseases departments and intensive care units (ICUs). Contamination may take place due to inappropriate use and cause spread of infectious pathogens. In the current study, we detected consecutive S maltophilia strains with identical band patterns isolated within a week. After appropriate disinfection and cleaning procedures, no further isolation was detected.

**Keywords:** Bronchoscopy, invasive procedure, pseudo-outbreak, *Stenotrophomonas maltophilia*

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**RESUMEN**

**Objetivo:** Stenotrophomonas maltophilia es un patógeno oportunista que se encuentra predominantemente en el medio ambiente y entorno de los hospitales. Los procedimientos invasivos y los métodos de tratamiento, los instrumentos utilizados para el diagnóstico y tratamiento, así como el uso irracional de antibióticos, desempeñan un importante papel en la propagación de este patógeno. Este estudio persigue evaluar el aislamiento consecutivo de S maltophilia de las muestras de lavado broncoalveolar durante el procedimiento broncoscópico en el período de una semana.

**Métodos:** A cuatro pacientes se les aisló S maltophilia consecutivamente en broncoscapias realizadas entre el 8 y el 15 de septiembre de 2012. La identificación de los aislamientos y su sensibilidad a los antibióticos fueron estudiados por el sistema automatizado Vitek 2 (Biomerieux, Francia). La relación clonal entre los aislamientos fue estudiada mediante consenso intergénico repetitivo enterobacteriano (ERIC) en conjunción con la reacción en cadena de la polimerasa (PCR).

**Resultados:** Cuatro aislados consecutivos de S maltophilia tenían patrones de banda idénticos e exhibían conexidad clonal.

**Conclusión:** La broncoscopia es un procedimiento invasivo común que se aplica en los departamentos de enfermedades torácicas, y las unidades de cuidados intensivos (UCI). La contaminación puede ocu-
INTRODUCTION

Stenotrophomonas maltophilia is an opportunistic pathogen that is prevalent in the environment and hospital setting. S. maltophilia, like Pseudomonaceae family members, is a Gram negative, aerobic bacillus, which does not produce pigment under usual circumstances and in routine culture media. Some strains may produce a grey colour in deoxycholate containing agar. This isolate does not produce beta-haemolysis on blood agar. Most of the isolates create obvious colonies on trypti-case soya agar or on blood agar at 37 °C in 24 hours. The bacterium carries enzymes like catalase, lipase, esterase, and hyaluronidase and oxidizes lactose, glucose, xylose and maltose (1). This strain is usually detected as a nosocomial pathogen especially in the hospital setting, intensive care units and especially in patients with underlying disorders requiring immnosuppression. Invasive procedures, treatment methods and diagnostic instrumentation and irrational antibiotic use may have caused an increase in isolation of this microorganism (2, 3). Stenotrophomonas maltophilia is frequently isolated from the oropharynx and sputum sample of the adult population and from the environment. This pathogen mostly causes urinary tract infection and wound infection. Recently, there has been an increase in bacteraemia caused by S. maltophilia. Bacteraemia source may be the respiratory, gastrointestinal and urinary systems and intravascular catheterization; but in most cases, the route of infection is not clear (4). Bronchoscopy is an invasive and safe procedure that is usually carried out in intensive care units and has rather less complications. Nonetheless, inappropriate use of bronchoscopes and contamination with the infectious agents cause spreading of infection (5, 6).

The aim of this study was to evaluate consecutive S. maltophilia isolation from bronchoalveolar specimens in a one-week period and investigate the source and clonal relationship among these isolates by molecular methods at the Bronchoscopy Unit of the Medicalpark Hospital, Izmir University School of Medicine.

SUBJECTS AND METHODS

Izmir University School of Medicine Medicalpark Hospital is a tertiary care centre with 244 beds. Four patients had S. maltophilia strains isolated consecutively from bronchoalveolar specimens between September 8 and 15, 2015, at the Bronchoscopy Unit. The identification and antimicrobial susceptibility of these isolates were studied by automated Vitek version 2.0 (Biomerieux, France). The environmental cultures were also taken. The clonal relationship was done by enterobacter-

ational repetitive intergenic consensus (ERIC) polymerase chain reaction (PCR) using specific ERIC-2 primers (7). The components of the 50 µl master mix are shown in the Table.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
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</thead>
<tbody>
<tr>
<td>10X enzyme buffer</td>
<td>5 µl (1x)</td>
</tr>
<tr>
<td>MgCl2</td>
<td>5 µl (2.5mM)</td>
</tr>
<tr>
<td>dNTPs</td>
<td>5µl (200 µM)</td>
</tr>
<tr>
<td>ERIC-2</td>
<td>1 µl (25 pmol)</td>
</tr>
<tr>
<td>Taq polymerase</td>
<td>0.5 µl (2.5 U)</td>
</tr>
<tr>
<td>Sterile deionized water</td>
<td>28.5 µl</td>
</tr>
<tr>
<td>DNA</td>
<td>5 µl</td>
</tr>
</tbody>
</table>

The amplification was as follows: one cycle (94 °C, 1 minute); 30 cycles (94 °C, 1 minute, 45 °C, 1 minute, 72 °C, 2 minutes) and 72 °C, 5 minutes. The amplified products were imaged by using 0.5 µg/ml ethidium bromide containing gel prepared in 1% agarose having 1X TBE buffer (Sigma, USA) under 120 volts for 45 minutes and under ultraviolet light. Gene Ruler™ 100 bp DNA ladder (Fermentas, Lithuania) was used in detecting the size of the bands.

RESULTS

The four consecutive S. maltophilia isolates were clonally related and showed identical band pattern (Figure). Environmental cultures did not yield S. maltophilia isolates.

Figure: Enterobacterial repetitive intergenic consensus (ERIC) polymerase chain reaction (PCR) gel image of four consecutive S. maltophilia isolates.

M: Marker (100 bp); 1: Patient #1; 2: Patient #2; 3: Patient #3; 4: Patient #4
DISCUSSION

Stenotrophomonas maltophilia is an aerobic, nonfermentative Gram-negative bacteria and it is an opportunistic pathogen that can cause serious infections in intensive care units (4). Contamination due to S maltophilia can cause endophthalmitis in cataract cases or infection from contaminated lock solutions in long-term central venous access (8, 9).

Behnia et al isolated two A baumannii isolates from bronchoalveolar specimens and four S maltophilia strains from patients with pneumonia who were on mechanical ventilation support at the intensive care unit in two months. Consecutive isolates were evaluated and it was found that the bronchoscope was contaminated. Disinfection was provided with strict decontamination procedures (10). Similarly, in our study, we detected S maltophilia from the bronchoalveolar lavage specimens of consecutive patients and showed clonal relatedness by molecular methods. After applying serious decontamination and disinfection procedures at the Bronchoscopy Unit, no further isolation was detected.

Brooks et al reported increased S maltophilia isolation in bronchoscopy cultures in August 2003 (11). The authors evaluated 306 bronchoscopy procedures from January 2001 to August 2003. Sixty-seven bronchial lavage samples revealed S maltophilia. The disinfection procedures were monitored once again according to the manufacturer’s instructions. Disposable brush use and alcohol application to the bronchoscope at the last step of cleaning solved the problem (11). In our study, we also detected S maltophilia isolation in four consecutive patients within a one-week period and after strict disinfection procedures, alcohol application and by hanging the instrument in a dry closet, no further isolates were detected.

Ahn et al detected S maltophilia strains after bronchoscopic procedures at Chosun University in 2006 over a one-week period (12). The strains showed identical band patterns. The authors estimated that one of the bronchoscopes got contaminated and further infection was avoided by strict cleaning and disinfection. In our study, the bronchoscope also got contaminated and we detected increased S maltophilia isolation from the bronchoalveolar lavage specimens. The strains showed identical band patterns in molecular analysis. After disinfection and cleaning procedures, no further isolates were observed.

Invasive diagnostic procedures and irrational antibiotic use policies play an important role in S maltophilia epidemics. S maltophilia pseudo-outbreaks at bronchoscopy units may happen because of a large number of patients undergoing bronchoscopy and lack of appropriate cleaning, disinfection and drying with alcohol. In our study, we detected four S maltophilia isolates from patients undergoing bronchoscopy at our hospital during a one-week period. Identical band patterns were shown from these isolates by ERIC PCR. Strict cleaning and disinfection procedures were followed and no further isolation was detected. More attention should be paid to this microorganism as it can cause epidemics and infections in intensive care units and operating theatres where invasive procedures take place. Appropriate disinfection procedures should be maintained and the staff should be continuously monitored and educated.

REFERENCES