Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2020;9(3):589-94

Pseudo-Outbreak of *Stenotrophomonas maltophilia* Isolated from Kidney Stones and Attributed to Inadequate Sterilization: Investigation of Molecular Typing and Clonal Relationship

Reyhan Yis[1], Selma Tosun[2], Emine Deniz Bayram[1], Ozlem Gamze Gulfidan[1], Tansu Degirmenci[3], Bulent Gunlusoy[3], Baris Otlu[4], Cigdem Kuzucu[1]

[1]Izmir Bozyaka Training and Research Hospital, Department of Medical Microbiology, Izmir, Turkey
[2]Izmir Bozyaka Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology, Izmir, Turkey
[3]Izmir Bozyaka Training and Research Hospital, Department of Urology, Izmir, Turkey
[4]Inonu University Faculty of Medicine, Department of Medical Microbiology, Malatya, Turkey

Received 06 January 2020; Accepted 11 February 2020
Available online 18.05.2020 with doi: 10.5455/medscience.2020.09.9223

Abstract

Stenotrophomonas maltophilia is a gram-negative bacterium. Hospitals can be a source of *S. maltophilia* because it adheres to nonliving surfaces and forms a biofilm. This study was performed to investigate the clonal relationship between *S. maltophilia* isolates obtained from kidney stone samples. Samples of kidney stones taken from patients and surrogate samples from nephroscopes, cleaning solution, disinfectant solution were included in the study. The clonal relationship between isolates was determined by PFGE. *S. maltophilia* was isolated from 34 of 94 kidney stone samples sent from the urology operating room between July 2017 and January 2018. A total of 26 *S. maltophilia* strains (21 from kidney stone samples, three from nephroscopes, and two from urine culture) were isolated. PFGE showed that the 21 kidney stone isolates and the 3 *S. maltophilia* isolates obtained from the nephroscope belonged to the same clone. The two urine culture isolates showed no clonal relationship to the outbreak isolates and were considered sporadic. Molecular typing confirmed that this pseudo-outbreak was attributed to inadequate disinfection of the nephroscopes. After disinfection protocols were reviewed and revised as needed, especially regarding the removal of organic material from nephroscopes after use, no further bacterial growth was detected from kidney stone specimens obtained with nephroscopes.

Keywords:*Stenotrophomonas maltophilia*, pseudo-outbreak, inadequate sterilization, molecular typing, clonal relationship

Introduction

Stenotrophomonas maltophilia is a gram-negative bacterium commonly found in the environment, water sources, plants, and animals [1]. In humans it causes various infections such as pneumonia [2], bacteremia [3], urinary tract infection [4], and soft tissue infections in immunodeficient patients [5]. In hospitals, sources of *S. maltophilia* can include taps, water systems, sinks, irrigation solutions, nebulizers, central venous catheters, ventilators, endoscopes, hemodialysis fluids, contaminated disinfectants, hand soaps, patient files, and inadequate disinfection practices [1,6]. Biofilm production allows bacteria to adhere to inanimate surfaces and exhibit resistance to environmental factors, phagocytic activity, and antimicrobials [7]. Hospital outbreaks and pseudo-outbreaks of *S. maltophilia* resulting from improper disinfection and decontamination practices have been reported previously [2,6,8]. Various molecular methods are used to evaluate genetic similarity between clinical isolates and environmental sources, especially during outbreaks [1]. Pulsed field gel electrophoresis (PFGE) is a widely used method for determining clonal relationships between isolates in outbreak investigations [1,3,9].

After repeatedly isolating *S. maltophilia* from kidney stone samples sent from the urology operating room to the microbiology laboratory of our center, this study was performed to investigate the outbreak, determine its source, and evaluate the clonal relationship between the isolates obtained during the outbreak.

***Corresponding Author:** Reyhan Yis, Izmir Bozyaka Training and Research Hospital, Department of Medical Microbiology, Izmir, Turkey, E-mail:reyhanyis@yahoo.com

Materials and Methods

This study was conducted following the approval of Izmir Bozyaka Training and Research Hospital Ethics Committee of Clinical Research (Date: 27.03.2019, Session No: 2019/03, Decision No: 03], in a training and research hospital with 567 beds and 13 active operating rooms. The Department of Urology is a training ward with 37 beds, and the urology team performs approximately 3600 operations annually in the main operating room, including outpatient procedures. In our center, kidney stone surgeries are performed by percutaneous nephrolithotomy (PCNL), which accounts for approximately 5% of all surgeries performed in the urology operating room. The urology operating room is equipped with three nephroscopes that can be used in these operations. The kidney stone removal procedure performed in the urology department of our hospital involves first making a percutaneous nephrostomy in the kidney pelvis through the posterior lateral abdominal wall for urine drainage. The nephroscope is positioned via a guide wire in the renal calyx and the stones are broken using a pneumatic lithotriptor and removed. The removed stones are sent to our laboratory to evaluate for bacterial growth.

S. maltophilia growth was detected in cultures of 34 of the 94 kidney stone samples sent from the urology operating room to the microbiology laboratory between July 4, 2017 and January 2018. After meeting with the Hospital Infection Control Committee, the records of the S. maltophilia-positive patients were retrospectively examined, and it was decided to take culture samples from the nephroscopes and disinfectants used in the urology operating room. In the first stage of the investigation, 10 samples were obtained in total, four each from the nephroscope in use and a disinfected nephroscope (both outer surfaces and inner surfaces of nephroscope, nephroscope forceps, nephroscope guide wire), one sample of the 10% povidone-iodine solution used as a pre-wash, and one sample of the disinfectant solution (Derdevice Plus PAA).

Samples were obtained from nephroscope surfaces using a moistened with sterile saline cotton swab and inner surfaces by flushing with 5 mL of sterile saline. For the solutions, 5-ml samples were collected in sterile glass tubes. The samples were inoculated on sheep blood agar and eosin-methylene blue agar. No growth was detected in any of the plates after the incubation period; however, the kidney stone samples sent to the laboratory continued to yield S. maltophilia, so the same sampling process was repeated. The samples were again inoculated on sheep blood agar and eosin-methylene blue agar. Identification and antibiotic susceptibility testing of the isolated bacteria were done using an automated system (Phoenix; Diagnostic Instrument Systems, Becton Dickinson). Antibiotic susceptibility testing was also repeated using the Kirby-Bauer disk diffusion method. The isolates were stored at -80°C until analysis.

Determination of Clonal Relationship

The clonal relationship between the isolates was studied using a modified version of the PFGE protocol used by Durmaz et al. [10] *XbaI* enzyme was used to cut the chromosomal DNA and a CHEF-DR II system (Bio-Rad, Nazareth, Belgium) was used to perform electrophoresis with a total run time of 20 h with initial switch time of 5 s and final switch time of 30 s. Band profiles obtained by agarose gel electrophoresis were photographed under a UV transducer. The band profiles obtained by both genotyping methods were analyzed using the Gel Compar version 6.6 software program (Applied Maths, Kourtrai, Belgium). The Dice Similarity Coefficient was used for band analysis and Unweighted Pairwise Grouping Mathematical Averaging (UPGMA) method was used for clustering analysis. Based on the similarity coefficients of the isolates, strains with over 95% similarity were accepted as the same clone [10].

Results

Twenty-one of the stored isolates were available in the stock culture. These plus three isolates obtained from nephroscope samples (both outer and inner surface of disinfected nephroscope and disinfected nephroscope forceps) and two *S. maltophilia* isolates obtained from urine cultures of two inpatients in the urology ward during the time period in question resulted in a total of 26 isolates analyzed. The two inpatients with S. maltophilia-positive urine cultures had no history of kidney stone surgery in the urology department and thus did not have kidney stone samples processed in the laboratory. Both patients were admitted to the urology ward for prostate needle biopsy, and urine cultures were done during their stay in hospital.

After *S. maltophilia* growth was repeatedly detected in kidney stone samples, urine samples were also sent for the patients undergoing PCNL in the urology ward. Fifteen of 21 patient's urine samples were sent but there was no growth *S. maltophilia* in urine cultures.

Analysis of the temporal distribution of the cases showed that of 34 patients' kidney stone samples, *S. maltophilia* was detected in six in July, one in August, one in September, two in October 10 in November, two in December, and 10 in January 2018 (Figure 1). A total of 26 *S. maltophilia* isolates were investigated in our study, including 21 kidney stone isolates, 3 of 10 environmental specimens (both outer and inner surface of disinfected nephroscope and forceps), and two isolates obtained from urine culture. Antibiotic susceptibility of the isolates was evaluated according to EUCAST criteria and 24 isolates (kidney stone samples, nephroscope samples) showed trimethoprim-sulfamethoxazole resistance in the automated system results and disk diffusion (MIC value: >4 mg/L, zone diameter 16< mm). The two strains isolated from urine in December 2017, unrelated to the other samples, were found to be susceptible to trimethoprim-sulfamethoxazole according to automated system and disk diffusion results (MIC value: ≤4 mg/L, zone diameter ≥16 mm).

PFGE showed that the 21 kidney stone isolates and the three *S. maltophilia* isolates obtained from the nephroscopes belonged to the same clone. The two urine culture isolates showed no clonal relationship to the outbreak isolates, they were unique and were considered sporadic. Dendogram belong to PFGE band patterns of all the *S. maltophilia* isolates is shown in Figure 2.

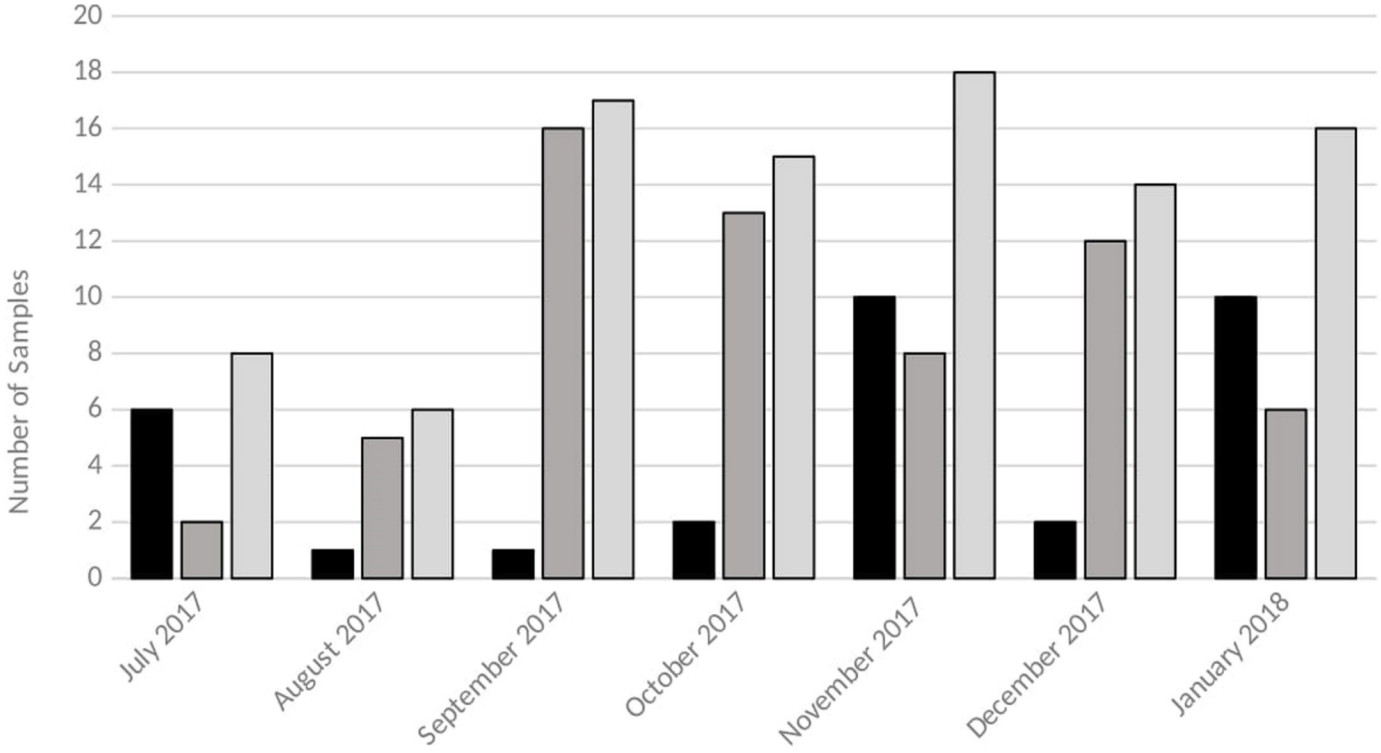

Figure 1: Monthly distribution of kidney stones with and without growth.

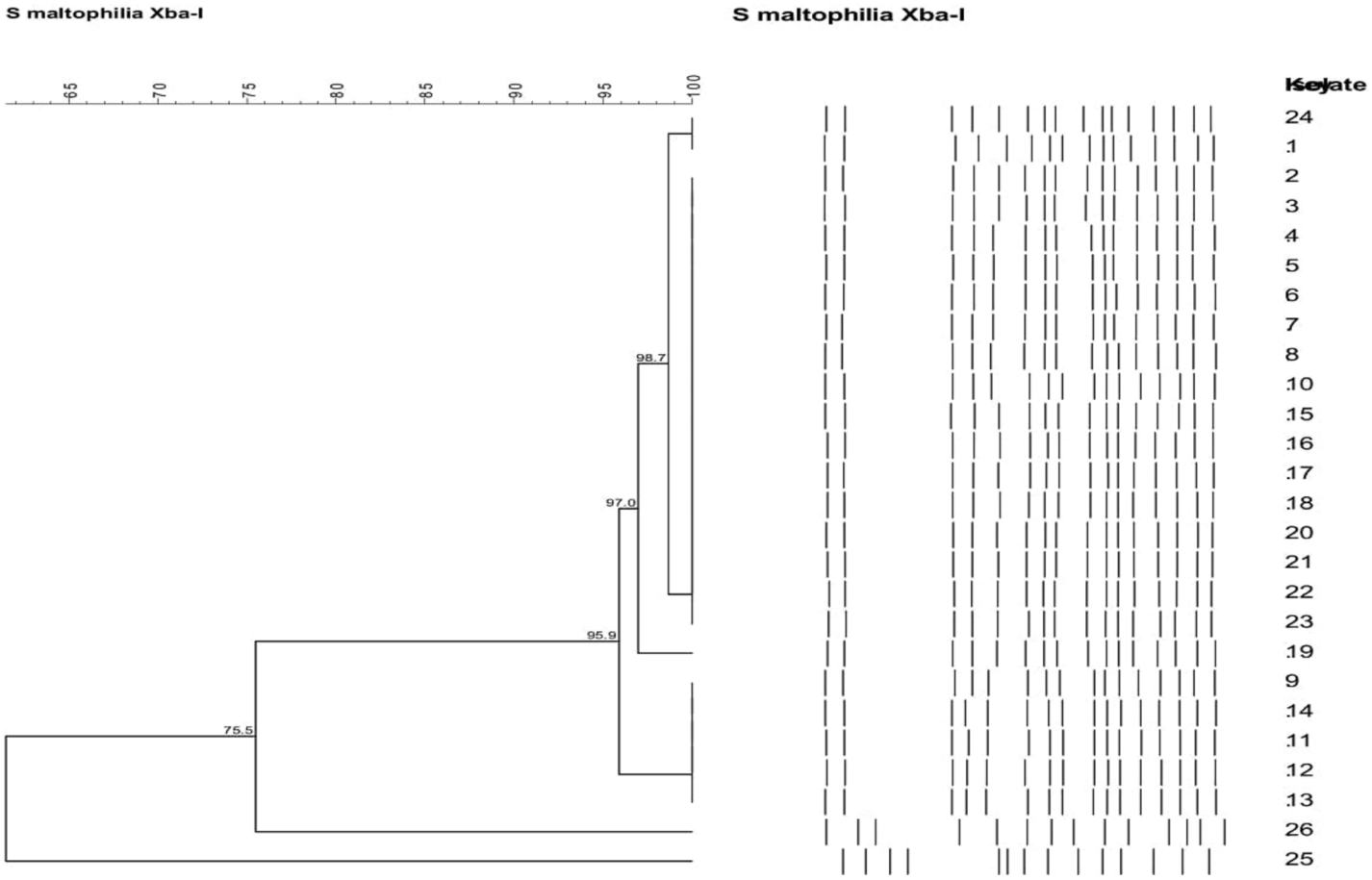

Figure 2: Dendogram of PFGE band patterns obtained by using UPGMA method in *S. maltophilia* isolates; (1-21: isolates produced from kidney stones, 22-24: isolates produced in samples taken from nephroscopes, 25-26: isolates produced in urine culture of two patients).

Discussion

Most instruments used in invasive procedures are reusable, and outbreaks and pseudo-outbreaks of *S. maltophilia* have been reported as a result of contamination of these devices from various sources [11,12]. Due to the reuse of lumened instruments such as endoscopes in urology and other surgical units, appropriate sterilization or high-level disinfection is especially important [13,14]. The isolation of the same *S. maltophilia* strain from both kidney stones and environmental samples obtained in the urology operating room (nephroscope outer and inner surfaces, and forceps) suggested that the problem stemmed from errors in the disinfection/ sterilization of the materials used. The most important step in effective high-level disinfection or sterilization is effective cleaning of organic and inorganic residues from instrument surfaces [14]. We learned that the general practice in our urology operating room prior to the pseudo-outbreak was to expose the used nephroscope to 10% povidone-iodinated or disinfectan solution for 5 min before using with the next patient. Organic and inorganic material can be removed from instruments manually or mechanically with detergents and enzymatic solutions. Numerous hospital outbreaks of various microorganisms have been reported due to insufficient cleaning and disinfection of reusable surgical instruments [9,15]. Without appropriate sterilization and disinfection, forceps used to collect stone fragments can cause outbreaks by carrying bacterial contamination from stones on their surfaces [9]. *S. maltophilia* is a biofilm-producing bacterium often resistant to disinfectants. The ongoing isolation of this organism from the nephroscope and particularly the forceps used to remove kidney stones in the urology operating room of our hospital despite regular disinfection procedures highlights the need to eliminate organic and inorganic residue from instruments before disinfection.

Our findings revealed that only one of our nephroscopes was contaminated with S. maltophilia, not all three. Firstly, there were no positive cultures from anephroscope that had been used after disinfection, whereas sampling of an unused, disinfected nephroscope yielded positive cultures. Moreover, the rate of positive cultures was not stable, but showed a cyclic fluctuation. This also explains why no growth was detected after the first nephroscope sampling while the second sampling yielded positive cultures. PCNL was performed in 94 patients in the examined time period and kidney stone samples of all the patients were sent to the laboratory. Growth of S. maltophillia was detected in 34 of those samples, which is consistent with the rate expected if one of the three nephroscopes in use was contaminated. Figure 1 shows the culture results of kidney stone samples sent to our laboratory between July 1, 2017 and January 31, 2018.

The most likely scenario behind this pseudo-outbreak is that the forceps were contaminated by infected stones (struvites) or stones carrying bacteria on their surfaces and probably contaminated the inner and outer surfaces of the nephroscope while passing through it, and the bacteria survived by forming a biofilm in the lumen. Although we were able to isolate bacteria from forceps and nephroscope parts, Kayabaş et al. [9] reported that only samples from forceps produced positive cultures in patients with positive urine cultures for P. aeruginosa, and stated that it may not be possible to detect microbiological growth in environmental samples from easily cleaned large-lumened instruments.

S. maltophilia shows a particular affinity for pulmonary epithelial cells. Because it can attach to plastic, teflon, and glass, *S. maltophilia* is capable of colonizing and forming biofilms on medical devices and human implants. This ability is one of the main reasons that *S. maltophilia* is commonly detected in hospital infections [1,16]. Biofilm formation on moist surfaces such as sinks, catheters, hospital water distribution systems, respiratory tubes, and dialysis equipment can result in the direct or indirect transmission of *S. maltophilia* to patients [1]. Biofilm accumulates inside lumened instruments, both holding the bacteria in place and creating a suitable environment for *S. maltophilia* to proliferate [6]. In the present case, inadequate mechanical and enzymatic cleaning allowed the bacteria to survive in a biofilm layer formed on the inner and outer surfaces of the nephroscope, resulting in the pseudo-outbreak detected in kidney stone samples sent to our laboratory. The fact that 15 patients had negative urine culture despite S. maltophilia-positive kidney stone cultures supports that this was a pseudo-outbreak. In addition, PFGE showed that the 21 kidney stone isolates and the three isolates obtained from the nephroscope belonged to the same *S. maltophilia* clone, while two positive urine cultures were sporadic, unrelated and unique. This confirmed that the source was nephroscope contamination and was not due to an actual outbreak of infection in patients.

S. maltophilia is intrinsically resistant to many antibiotics such as carbapenems, cephalosporins, macrolides, fluoroquinolones, and aminoglycosides. Increasing resistance to trimethoprim-sulfamethoxazole, which is frequently used in treatment, has also been reported in recent years. Using broad-spectrum antibiotics due to multiple drug resistance increases rates of *S. maltophilia* colonization and infection [1]. Interestingly, although the two sporadic *S. maltophilia* isolates obtained in urine culture were found to be susceptible to trimethoprim-sulfamethoxazole, those originating from a biofilm layer (the *S. maltophilia* strains isolated from kidney stone samples and nephroscope parts) showed resistance to this drug.

Although the risk of endoscopy-related infection is estimated to be very low, more health-related infections are associated with contaminated endoscopes than any other medical device. However, most of the recent outbreaks and pseudo-outbreaks have been caused by the use of damaged or defective bronchoscopes or contaminated equipment such as washing tanks, tubes, antibacterial filters, and cleaning brushes. Most publications report pseudo-outbreaks in which organisms were isolated from bronchoscopy specimens because of colonization or contamination of the bronchoscope rather than patient-to-patient transmission [17,18]. *S. maltophilia* pseudo-outbreaks associated with the use of bronchoscopes may be related to its propensity for colonizing the respiratory tract [8,11,19,20]. Other than the experience we describe in the present article, there have been no other reports of nephroscope-related outbreak or pseudo-outbreak of S. maltophilia.

Determining the source is key in the effective control of outbreaks. Molecular techniques can be used to investigate the epidemiology of outbreak strains and confirm their clonality [15, 21-24]. The relationship between exogenous reservoirs and the significance of cross-contamination during outbreaks can be documented with these techniques [25]. In recent years, epidemiological studies conducted to determine the local and global spread of epidemic

isolates have utilized methods such as plasmid analysis, ribotyping, polymerase chain reaction (PCR)-based typing and PFGE [26, 27]. In particular, PFGE is still the gold standard genotyping method for many microorganisms and has become a standard method for determining the genetic relationship between isolates in molecular epidemiological methods [28]. Yetkin et al. [22] examined the clinical and epidemiological features of nosocomial P. aeruginosa infections and evaluated clonal relationships of the isolates, emphasizing the spread in hospitals. In another study conducted in İnönü University, an outbreak originating from forceps was identified using PFGE [9]. In our study, PFGE was used to determine the genetic relationship between isolates from the kidney stones of 21 patients and 3 isolates from nephroscope samples, and all 24 *S. maltophilia* isolates were found to belong to the same clone.

As a result, this pseudo-outbreak was attributed to inadequate sterilization/disinfection, and the personnel who performed disinfection after the pseudo-outbreak period were provided the necessary training in instrument disinfection from an infection-control nurse. Disinfection protocols were reviewed and revised as needed, especially regarding the removal of organic material from nephroscopes after use followed by cleaning in an enzymatic solution. This study emphasizes the critical role of pre-cleaning and enzymatic cleaning before high-level disinfection. After taking these remedial measures, no further bacterial growth was detected from kidney stone specimens obtained with nephroscopes and sent to our laboratory.

Conflict of interest
The authors declare that they have no competing interest.

Financial Disclosure
All authors declare no financial support.

Ethical approval
This study was conducted following the approval of Izmir Bozyaka Training and Research Hospital Ethics Committee of Clinical Research (Date: 27.03.2019, Session No: 2019/03, Decision No: 03],

References

1. Brooke JS. Stenotrophomonas maltophilia: An Emerging Global Opportunistic Pathogen. Clin Microbiol Rev. 2012;25:2-41.

2. Guyot A, Turton JF, Garner D. Outbreak of Stenotrophomonas maltophilia on an intensive care unit. J Hosp Infect. 2013;85:303-7.

3. Gulcan H, Kuzucu C, Durmaz R. Nosocomial Stenotrophomonas maltophilia cross-infection: Three cases in newborns. Am J Infect Control. 2004;32:365-8.

4. Vartivarian SE, Papadakis KA, Anaissie EJ. Stenotrophomonas (Xanthomonas) maltophilia Urinary Tract Infection. A disease that is usually severe and complicated. Arc Intern Med. 1996;156:433-5.

5. Sakhnini E, Weissmann A, Oren I. Fulminant Stenotrophomonas maltophilia soft tissue infection in immunocompromised patients: An outbreak transmitted via tap water. Am J Med Sci. 2002;323:269-72.

6. Stigt JA, Wolfhagen MJ, Smulders P, et al. The identification of Stenotrophomonas maltophilia contamination in ultrasound endoscopes and reproduction of decontamination failure by deliberate soiling tests. Respiration. 2015;89:565-71.

7. Abbott IJ, Slavin MA, Turnidge JD, et al. Stenotrophomonas maltophilia emerging disease patterns and challenges for treatment. Expert Rev Anti Infect Ther. 2011;9:471-88.

8. Rial MB, Fernandez VL, Delgado MN, et al. A pseudo- outbreak of Pseudomonas putida and Stenotrophomonas maltophilia in a bronchoscopy unit. Respiration. 2016;92:274-8.

9. Kayabas U, Bayraktar M, Otlu B, et al. An outbreak of Pseudomonas aeruginosa because of inadequate disinfection procedures in a urology unit: A pulsed-field gel electrophoresis-based epidemiologic study. Am J Infect Control. 2008;36:33-8.

10. Durmaz R, Otlu B, Koksal F, et al. The optimization of a rapid pulsed-field gel electrophoresis protocol for the typing of Acinetobacter baumannii, Escherichia coli, Klebsiella spp. Jpn J Infect Dis. 2009;62:372-7.

11. Waite TD, Georgiou A, Abrishami M, et al. Pseudo-outbreaks Stenotrophomonas maltophilia on an intensive care unit in England. J Hosp Infect. 2016; 92:392-6.

12. Guy M, Vanhems P, Dananche C, et al. Outbreak of Pseudomona saeruginosa and Stenotrophomonas maltophilia infections related to contaminated bronchoscope suction valves Lyon, France 2014. Euro Surveill. 2016;14;21.

13. Smith's Textbook Endourology 3rd Edition, Edited by Arthur D Smith, Gopal Badlani, Glenn M Preminger, Louis R Kavoussi (2012). Wiley Blackwell, UK. (Carol Olsen; Care and sterilization of instruments page 3-9.)

14. Rutala WA, Weber DJ, and The Healthcare Infection Control Practices Advisory Committee (HICPAC). Guideline for Disinfection and Sterilization in Healthcare Facilities 2008.

15. Corne P, Godreuil S, Jean Pierre H, et al. Unusual implication of biopsy forceps in outbreaks of Pseudomonas aeruginosa infections and pseudo-infections related to bronschoscopy. J Hosp Infect. 2005;61:20-6.

16. Pompilio A, Crocetta V, Confalone P, et al. Adhesion to and biofilm formation on IB3-1 bronchial cells by Stenotrophomonas maltophilia isolates from cystic fibrosis patients. BMC Microbiol. 2010,10:102.

17. Cosgrove SE, Ristaino P, Caston-Gaa A, et al. Caveat emptor: the role of suboptimal bronchoscope repair practices by a third-party vendor in a pseudo-outbreak of Pseudomonas in broncho alveolar lavage specimens. Infect Control Hosp Epidemiol. 2012;33:224-9.

18. Kenters N, Huijskens EGW, Meier C, et al. Infectious diseases linked to cross-contamination of flexible endoscopes. Endosc Int Open. 2015;3:259-65.

19. Ece G, Erac B, Limoncu MH, et al. Stenotrophomonas maltophilia Pseudo-outbreak at a University Hospital Bronchoscopy Unit in Turkey. West Indian Med J. 2014;63:59-61.

20. Ahn GY, Yu FN, Jang SJ, et al. Pseudo-outbreak of Stenotrophomonas maltophilia due to contamination of bronchoscope. Korean J Lab Med. 2007;27:205-9.

21. Zawacki A, O'Rourke E, Potter-Bynoe G, et al. An outbreak of Pseudomonas aeruginosa pneumonia and blood stream infection associated with intermittent otitis externa in a health care worker. Infect Control Hosp Epidemiol. 2004;25:1083-9.

22. Yetkin G, Otlu B, Cicek A, et al. Clinical, microbiologic, and epidemiologic characteristics of Pseudomonas aeruginosa infections in a University Hospital, Malatya, Turkey. Am J Infect Control. 2006;34:188-92.

23. Ayan M, Kuzucu C, Durmaz R, et al. Analysis of three outbreaks due to Klebsiella species in a neonatal intensive care unit. Infect Control Hosp Epidemiol. 2003;24:495-500.

24. Gungor S, Ozen M, Akinci A, et al. A Chryseobacterium meningosepticum outbreak in a neonatal ward. Infect Control Hosp Epidemiol. 2003;24:613-7.

25. Thuong M, Arvaniti K, Ruimy R, et al. Epidemiology of Pseudomonas aeruginosa and risk factors for carriage acquisition in an intensive care unit. J Hosp Infect. 2003;53:274-82.

26. Kitchel B, Rasheed JK, Patel JB, et al. Molecular epidemiology of KPC-producing Klebsiella pneumoniae isolates in the United States: Clonal expansion of multilocus sequence type 258. Antimicrob Agents Chemother. 2009;53:3365-70.

27. Diancourt L, Passet V, Verhoef J, et al. Multilocus sequence typing of Klebsiella pneumoniae nosocomial isolates. J. Clin. Microbiol. 2005;43:4178-82.

28. Won SY, Munoz-Price LS, Lolans K, et al. Centers for Disease Control and Prevention Epicenter Program, Emergence and rapid regional spread of Klebsiella pneumoniae carbapenemase producing Enterobacteriaceae. Clin Infect Dis. 2011;53:532-40.