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Outbreak of Hospital Infection from Biofilm-embedded Pan Drug-resistant *Pseudomonas aeruginosa*, Due to a Contaminated Bronchoscope

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Abstract

Background: Colistin-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*) has been defined as pandrug-resistant (PDR) strain. Outbreaks of PDR *P. aeruginosa* especially in pulmonary tract infections due to contaminated bronchoscopes have rarely been reported. The emergence of pandrug-resistant strains in both CF (Cystic Fibrosis) and non-CF clinical isolates over recent years remains of a great concern. Hospital wards contaminated with PDR *P. aeruginosa* infection, must be shut down until their eradication. Health Authorities must be informed immediately and infection control strategies must be implemented.

Aim: To report such an outbreak and modify the infection control strategy in an academic hospital in Ankara Turkey.

Methods: From October to December 2013, PDR-*Pseudomonas aeruginosa* were identified from bronchial cultures of 15 patients who had undergone bronchoscopy prior to the infection. Three batches of surveillance cultures were obtained from the environmental objects and healthcare workers related to the procedures. Pulsed-field gel electrophoresis (PFGE) was used for bacterial typing. Antimicrobial susceptibility was assessed by disc diffusion and E-test methods.

Findings: A total of 70 specimens were obtained during the first surveillance operation. One Colistin-resistant *P. aeruginosa* was isolated from a bronchoscope. Although the disinfection protocols for bronchoscope were revised and implemented, seven additional bronchial cases were identified thereafter. The pathogen was identified from two subsequent surveillance cultures and was not eliminated until Ethylene oxide sterilization was added to the disinfection protocol. PFGE revealed that all 15 isolates from the patients and the three isolates from the

bronchoscope shared a common pattern with minor variance. XbaI restriction enzyme turned out better than SpeI in interpreting bacterial pulse types with BioNumerics 6.0. The most suitable cut off value for SpeI was above 80% Dice similarity while for XbaI above 95% Dice similarity with BioNumerics 6.0.

Conclusion: The outbreak of "Colistin" pan drug-resistant *Pseudomonas aeruginosa* was caused by a contaminated bronchoscope and was terminated by the implementation of a revised disinfection protocol for bronchoscope.

Keywords Pulsedfield gel electrophoresis; DNA finger printing; *Pseudomonas aeruginosa*; Bronchoscope; Nosocomial infection

Abbreviation

BAL: Bronchial Aspirate Lavage; ENT: Ear, Nose, Throat; ICU: Intensive Care Units; MDR: Multi Drug Resistant; PDR: Pan Drug-Resistant; ICPS: Infection Control Practitioners; OPA: Ortho-Phthalaldehyde; ETO: Ethylene Oxide; CF: Cystic Fibrosis

Introduction

Pandrug-resistance in *Pseudomonas aeruginosa* is defined by the US CF foundation consensus guidelines as resistance to colistin or all agents, and the emergence of pandrug-resistance (PDR) strains in both CF and non-CF clinical isolates over recent years remains of great concern [1].

Endoscopes are an important diagnostic and therapeutic instrument in modern medicine. However, if endoscopes were not subjected to a sufficiently high level of disinfection, they may cause outbreaks of healthcare-acquired infections (HCAIs). Almost all forms of endoscopes have been reported to be

involved in outbreaks of HCAs due to inadequate disinfection [2-5]. Among the different kinds of endoscopes, bronchoscope and gastrointestinal endoscopes have been most frequently reported as bearing pathogens. [6,7] Bacteria, Mycobacteria, fungi and viruses have all been reported as causing outbreaks [7-9]. However, outbreaks caused by Pan drug-resistance microorganisms on bronchoscopes have been rarely reported [7]. Colistin resistant *Pseudomonas aeruginosa* has been known as Pan drug-resistance bacteria. Due to life threatening of PDR *Pseudomonas aeruginosa* in old ages and immuno-compromised patients, endoscopes must be precisely controlled time to time.

This article reports an outbreak of pulmonary tract infections caused by (Colistin) "PDR" *Pseudomonas aeruginosa* that had never been identified previously in Turkey's hospitals. Laboratory investigation by molecular methods was performed and confirmed that this outbreak had been caused by a contaminated bronchoscope.

Methods

Turkey, Ankara "M" Hospital is a regional private, academic hospital in central Ankara, Turkey. The bronchoscopy department is operated by one attending doctor, bronchoscopy room is equipped with one rigid bronchoscope, and the bronchoscopy procedure is usually performed on an outpatient setting and an average of eight patients per week undergoes this procedure. The disinfection protocol for bronchoscope in the hospital was cleaning surface of bronchoscope tube with sterile gauze soaked in 70% ethanol or immersion of the scope in detergent/disinfectant solution. The method below was introduced by FDA-CDC,"1", and we modified it as "2" [10-13].

Table 1. Demographics and clinical characteristics of the 15 cases with pulmonary tract infections owing to *Pseudomonas aeruginosa* and the sampling dates for the isolates from one bronchoscope (M=Male, F=Female) BSCOPE=Bronchoscope.

Source	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13	Case 14	Case 15	BSC OPE 1	BSC OPE 2	BSC OPE 3
Age (Years)	64	44	67	59	58	48	59	66	69	48	59	38	59	48	69			
Sex	M	F	F	F	F	M	M	M	M	M	M	M	F	M	M			
Date of Bronchoscopy	Oct -17	22- Oct	26- Oct	30- Oct	01- Nov	07- Nov	13- Nov	17- Nov	23- Nov	24- Nov	25- Nov	25- Nov	26- Nov	29- Nov	01- Dec	23- Nov	29- Nov	03- Dec

Hence, an outbreak of PDR (Colistin-resistant) *P. aeruginosa* bronchial infection related to this procedure was highly suspected. Therefore, an investigation started on the following day.

On 13th November 2013, the first investigation was commenced and some interventions were conducted and PDR *P. aeruginosa* was isolated (described below); however, seven additional cases of bronchial infection caused by Colistin-

1-Opening of all joints of the bronchoscopes, washing by tap-water, immersing in the diluted detergent containing enzymes (3M Rapid Multi-Enzyme Cleaner, including protease, amylase, lipase, and cellulose; 3M distilled water ¼ 1:100) for 15 min, and then immersion in 0.55% Ortho-phthalaldehyde (OPA) produced in last two weeks for 10 min (OPA strips were used prior to each immersion of bronchoscope for confidence from its effectiveness, according manufacturer instruction). Final rinse with sterile distilled water was then executed to remove OPA residues.

2-Above protocol plus hypochloric acid solution and ethylene oxide (ETO) weekly sterilization was added to the disinfection protocol for bronchoscope 15 minutes for each of them).

The outbreak

On 13 October 2013, one Colistin-resistant *P. aeruginosa* isolate was identified from the bronchial culture of a patient (case 1, Table 1) who developed bronchial infection after a bronchoscopy procedure in the bronchoscopy department. Similar isolates were subsequently identified from the bronchial specimens of the patients visiting the same department.

On 13 November 2013, the microbiology staff informed the infection control practitioners of the emergence of this infection. The number of bronchial infection due to this microorganism had reached a total of seven cases. Once informed, the infection control practitioners immediately reviewed the medical charts of these seven patients, and found that all seven patients had had a bronchoscopy procedure before developing a bronchial infection.

resistant *P. aeruginosa* were identified subsequently (29th November, the second infection control).

On 3rd December 2013, the third infection control interventions (described below) were conducted, and no new case was found. The date of these cases and the genotype of the isolates are shown in Table 1 and Figure 1.

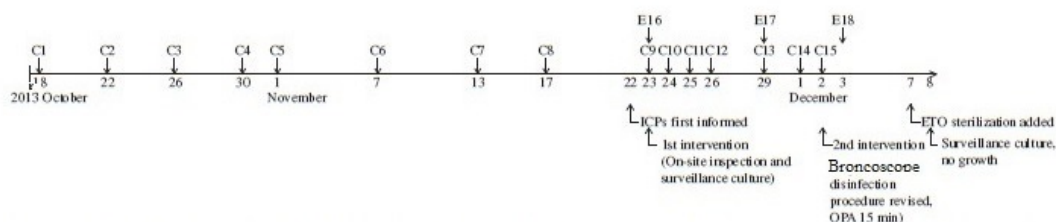


Figure 1. Epidemic curve and pulsed field Gel Electrophoresis genotypes of *Pseudomonas aeruginosa* Isolates from patients (C1-C15) and bronchoscope (E16-E18) during three intervention; ICPS: Infection Control Practitioners; OPA: Ortho-Phthalaldehyde; ETO: Ethylene Oxide.

Investigation and Interventions

The epidemiological curve of infected cases suggested exposure to a common source [9,10,14].

On-site inspection of the routine operation and disinfection procedures for the bronchoscopy found no inappropriateness. To identify the potential source of contamination, we conducted a surveillance culture for environmental objects and healthcare workers (HCWs) that were involved with the procedure of bronchoscopy.

The environmental objects included operating tables, taps, surfaces of washing tank, brushes of washing room, distilled water before and after rinsing, detergent enzyme for cleaning, hypochloric acid, OPA, bronchoscope and otoscopes, and their storage boxes. Samplings from the hands of HCWs involved in the bronchoscopy procedure were also obtained. A total of 70 samples were taken, comprising 48 samples from the environment, 17 samples from instruments, and 5 samples from the hands of HCWs.

All specimens from the surfaces of the suspected subjects described above, including the hands of HCWs, were obtained by swabbing the surface with sterile cotton swabs immersed in culture broth. For each scope, three specimens were taken. The surfaces of the eye piece and joint (after being released from the scope) were sampled as described above. The external channel of the scopes was flushed with sterile distilled water, which was concentrated by centrifugation and cultured.

Liquid samples that contained antimicrobial substances were processed with a 0.45 μ filter and the dregs were cultured; the other suspected fluids were centrifuged and cultured.

All of the instruments associated with the procedure of bronchoscopy were thoroughly cleaned and disinfected. The enzyme solution used for cleaning, despite manufactory guideline about OPA reusability till two weeks, for minimizing our research variables the OPA for disinfection were discarded and fresh solutions provided. The personnel responsible for the disinfection of bronchoscope were asked to implement the disinfection policy strictly. At this point, the bronchoscope was in use as usual.

Sterilization of bronchoscopes in details

Sterilization in the surgical field has been the primary modality in preventing spread of infections. We followed Igor Naryzhny Sterilization method [15]. As such, we proceeded to change reprocessing of our Olympus bronchoscope from automated high-level disinfection (HLD) to combined HLD followed by ETO gas sterilization of all bronchoscopes, as well as additional pre-cleaning steps. To help reduce visible bio-burden, each bronchoscope was pre-cleaned (First Step Bedside Pre-Clean Kit; Cygnus Medical, Branford, Conn) immediately after patient use before transport for reprocessing. The bronchoscope was then brought to the cleaning room where it was manually cleaned with multi-enzyme detergent/cleaner (CST-404C Surg-ENZ; Moorestown, NJ) using manufacturer recommendations. After this, the bronchoscope was placed into a washer/disinfector for reprocessing (System 83 Plus; Custom Ultrasonics, Ivyland, Pa) using a high level disinfectant (MetriCide OPA Plus; Metrex Research, Orange, Calif). All recommendations and guidelines provided by the endoscope manufacturer, as well as the manufacturers of the cleaning/disinfecting solutions and equipment, were closely followed. After HLD, the bronchoscope was then brought to the surgical sterilization facility. The Pentax proposed guidelines for ETO gas sterilization of bronchoscopes were closely followed along with, as suggested by Pentax, the guidelines of the sterilizer manufacturer. Igor Naryzhny institution had already equipped with 4 dual-cycle 100% ethylene oxide gas sterilizers (Steri-Vac Sterilizer/Aerator 8XL; 3M, St. Paul, Minn) for use on surgical equipment including endoscopes used during surgical procedures. Guidelines recommended by the Association for the Advancement of Medical Instrumentation (AMMI) as well as the manufacturers of the equipment were strictly followed. Hypochloric acid sterilization was fulfilled for 15 minutes as our novel method. Each bronchoscope was placed into a disposable wrap (Halyard; Alpharetta, GA), then placed into the sterilizer chamber along with surgical equipment. The sterilization process consisted of machine startup, a 1-hour automated sterilization with 3M 100% ETO single-use cartridge and a 12-hour aeration cycle, totaling at least 15 to 16 hours (this did not include HLD time). The bronchoscope was then brought to the bronchoscopy department where it was removed from the sterilized wrapper and visually inspected. If not immediately used, the bronchoscope was hung in a non-sterile storage cabinet (Model

20000; Custom Ultrasonics, Ivyland, Pa) with medical air aeration.

If the bronchoscope was not used in 5 days, it underwent reprocessing with HLD as described above. Due to lack of guidelines, we began culturing each bronchoscope specifically for PDR on a monthly basis 4 months after initiating the sterilization process. Culturing of all bronchoscopes was performed at the same time, therefore a bronchoscope may have been cultured either after HLD but before ETO sterilization, immediately after ETO sterilization or any time after ETO or additional HLD before its use; (I, timing of culture was random within each bronchoscope sterilization cycle) As previously identified as the culprit for contamination and potential nidus for organic debris, the elevator mechanism of the bronchoscope was cultured in the up and down position (ESwab Collection Kit; ACL Laboratories, Rosemont, Ill). Culturing was performed under sterile technique, which included disinfection of the counter and the use of gowns, face masks/shields, hair covering, and sterile gloves. After appropriate preparation, the outer tip of the bronchoscope was sanitized with an alcohol pad using caution to not wipe the elevator mechanism and lens face at the distal end that was to be sampled with the ESwab; the bronchoscope was air dried before sampling. The bronchoscope was placed in a tray with sterile pad/liner for sampling. The ESwab was dipped in the medium of the transport container to pre-moisten, and excess fluid was pressed from the ESwab inside the inner walls of the container. The ESwabs were held above the red mark on the shaft as that was the part of the shaft that was broken off and discarded; the shaft below the red mark was not touched. One ESwab was used for both the up elevator and the down elevator positions. The inside of the elevator mechanism, recess and channel in the down position were sampled. Next, the same ESwab was used to sample the elevator mechanism and recess in the up position, as well as to scrub the face of the lens. The ESwab was then placed into the transport tube, which was tightened and labeled as indicated. After culturing, the specimen was sent to ACL Laboratories and the bronchoscope was reprocessed with HLD. At that time, awaiting the results of cultures did not preclude the use of the bronchoscopes [15].

Laboratory investigations

All the clinical isolates of *P. aeruginosa* as well as those from surveillance cultures were identified by standard methods of the Clinical and Laboratory Standards Institute (CLSI), and further characterized by molecular methods. Antibiotic susceptibility testing was done by two methods, namely disc diffusion and E-test. Susceptibility to the following antibiotics was tested using a disc method: Carbenicillin (Cb), piperacillin-tazobactam (Tzp), ceftazidime (Caz:30 mg), imipenem (Imp:10 mg), meropenem (Mem:10 mg), gentamicin (Gn:10 mg), tobramycin (Tb), netilmicin (Net), amikacin (Ak:30 mg), ciprofloxacin (Cip), colistin (Ks: 8 mg), minimum inhibitory concentrations (MICs) of ertapenem, imipenem, meropenem and colistin were also

assessed with E-test strips (AB Bio disk, Bio Merieux). Interpretation was according to CLSI breakpoint concentrations (CLSI guidelines M100S26) [11].

Pulsed-field gel electrophoresis (PFGE) of the XbaI, SpeI (New England Bio labs, Beverly, MA, USA) macro-restricted genomic DNA, was performed to delineate the genetic relatedness of the isolates using procedures described previously [12,16]. PFGE patterns were interpreted according to the criteria suggested by Tenover et al. and the genotypes were designated in alphabetical order [17]. PFGE patterns with one to three band differences from an existing genotype were defined as subtypes of that genotype and were labeled with Arabic number suffixes. Two isolates were considered to be indistinguishable, highly related, or distinct if they had the same subtype (no band difference), the same genotype (one to three band differences), or different genotype (four or more band differences), respectively. Two epidemiologically non-related *P. aeruginosa* isolates (Figure 2, C1 and C2) were used as control "Marker" strains.

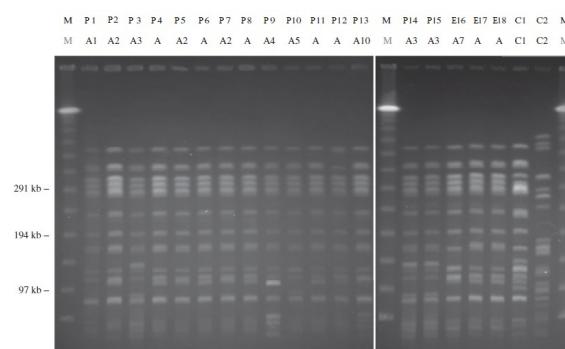


Figure 2. Pulsed-field gel electrophoresis patterns of XbaI-digested genomic DNA of *Pseudomonas aeruginosa* isolates. Lane M is the standard. Lanes P1-P15, isolates from patients 1-15 with the same order as those in Table 2. Lanes E16-E18, isolates from bronchoscope stages 1, 2, 3 also with the same order as those in Table 2. Lanes C1 and C2 are epidemiological non-related isolates. Band sizes in kilo base pairs are shown on the left.

Biofilm detection

Biofilm existing was evaluated by two laboratory methods plus electron microscopy method as follows.

ATP consumption detection by bio-luminescence

Final washing solutions at the end of each interventional stage was used for evaluating presence or absence of living bacteria, according to protocol of BioThema Isogen (Product number 266-11).

Table 2. Laboratory investigation of the 18 *Pseudomonas aeruginosa* isolates analyzed

Isolate	PFGE Genotype	Antibiotic Susceptibility Testing							MIC ($\mu\text{g/mL}$)		
		AMI	GM	COL	CRO	CAZ	IPM	ERT	IPM	MER	ERT
Case 1	A1	S	S	R	I	R	S	S	0.5 (S)	0.125 (S)	2.0 (S)
Case 2	A2	S	R	R	R	R	S	I	1.0 (S)	1.0 (S)	4.0 (I)
Case 3	A3	S	R	R	R	R	S	R	2.0 (S)	2.0 (S)	16 (R)
Case 4	A	S	R	R	R	R	S	I	2.0 (S)	2.0 (S)	4.0 (I)
Case 5	AZ	S	R	R	R	R	S	I	1.0 (S)	1.0 (S)	4.0 (I)
Case 6	A	S	R	R	R	R	S	I	1.0 (S)	1.0 (S)	8 (R)
Case 7	AZ	S	R	R	R	R	S	R	4.0 (S)	2.0 (S)	16 (R)
Case 8	A	S	R	R	R	R	S	R	2.0 (S)	0.125 (S)	32 (R)
Case 9	A4	S	R	R	S	S	S	I	0.2 (S)	0.5 (S)	1.0 (S)
Case 10	A5	S	R	R	I	R	S	S	1.0 (S)	1.0 (S)	0.5 (S)
Case 11	A	S	R	R	R	R	S	R	2.0 (S)	1.0 (S)	8 (R)
Case 12	A	S	R	R	R	R	I	R	4.0 (S)	2.0 (S)	16 (R)
Case 13	A10	S	R	R	R	R	S	R	4.0 (S)	2.0 (S)	16 (R)
Case 14	A3	S	R	R	R	R	S	R	2.0 (S)	2.0 (S)	8 (R)
Case 15	A3	S	R	R	R	R	S	R	4.0 (S)	2.0 (S)	12 (R)
Scope 1	A7	S	R	R	R	R	S	I	2.0 (S)	1.0 (S)	4.0 (I)
Scope 2	A	S	R	R	R	R	S	I	2.0 (S)	1.0 (S)	8 (R)
Scope 3	A	S	R	R	R	R	S	R	2.0 (S)	2.0 (S)	32 (R)

PFGE: Pulse Field Gel Electrophoresis; **MIC:** Minimum Inhibitory Concentration; **AMI:** Amikacin; **GM:** Gentamicin; **Col:** Colistin; **CRO:** Ceftriaxone; **CAZ:** Ceftazidime; **IPM:** Imipenem; **ERT:** Ertapenem; **MER:** Meropenem; **S:** Susceptible; **I:** Intermediate; **R:** Resistant

Crystal violet method

By above method, final washing water of three interventional stage was used for evaluating biofilm formation as follow; after washing with distilled water, 100 micro-liter of washing water was place in micro-plate and air fixed, micro-plate was stained with 110 micro-liter of 0.4% aqueous crystal violet solution for 45 minutes, after wards each well was washed four times with 350 micro-liter of sterile distilled water and immediately destined with 200 micro liter of 95% ethanol. After 45 minutes of distaining, 100 micro liter of destining solution was transferred to a new well and destining solution was measured with ELISA reader (Labile ER 2007 Micro plate washer, DIAGNOVA) at 595 nm.

Scanning Electron Microscopy

SEM was performed following the method of Hawser *et al* [18]. Briefly, *P. aeruginosa* biofilms that were formed on bronchoscope external channel surface were scratched with bistury blade and transferred on pieces of PVC (1.0 cm²) and were fixed with 2.5% (v/v) glutaraldehyde in PBS for 5 h at room temperature. The samples were then washed with PBS and dehydrated in an ethanol series (30, 50, 70, 90, and 100%). All of the samples were subsequently dried overnight, gold coated and viewed under SEM (Zeiss EVO MA 5).

Results

In epidemiological investigation and management results from the surveillance culture, one Colistin-resistant *Pseudomonas aeruginosa* isolate was identified from the external channel of the most frequently used bronchoscope. Although the infection control interventions described above were implemented,

Seven additional cases of bronchial infection caused by Colistin-resistant *P. aeruginosa* were still identified subsequently. To clarify the source of the contamination, on 29th November 2013, the second surveillance culture was performed on the frequently used bronchoscope. Of the six samplings obtained, one Colistin-resistant *Pseudomonas aeruginosa* isolate was again identified from the external channel of the contaminated bronchoscope. During this period, the infection control practitioners also identified that this specific bronchoscope had been used on all the infected patients before they developed the bronchial infection. Therefore, on 2nd December 2013, the bronchoscope was suspended from use. In addition, because the original disinfection procedure was apparently unable to decontaminate the Colistin-resistant *P. aeruginosa* from bronchoscope, the whole protocol was revised as follows: opening of all joints of bronchoscope, washing with tap-water, irrigating the external channel of bronchoscope with diluted

enzyme (newly added), bronchoscope immersed in the diluted enzyme for 15 min, irrigating external channel of bronchoscopes with 0.55% OPA (freshly made daily), and then immersion of the bronchoscope in 0.55% OPA for 15 min (extended for 15 min), followed by thoroughly rinsing with sterile distilled water. On 3rd December 2013, the third surveillance culture of the contaminated bronchoscope was performed.

Again, Colistin-resistant *P. aeruginosa* was isolated from the external channel of the bronchoscope. Then, weekly ethylene oxide (ETO) sterilization was added to the disinfection protocol for bronchoscopes [15]. On 8th December 2013, the fourth surveillance culture of the contaminated bronchoscope was done, and no microorganism was identified this time. Since then, the bronchoscope has been re-used again, and no more cases of bronchoscopy-related bronchial infection have been found. Above results were confirmed by our biofilm evaluating tests. Biofilm embedded living bacteria was existed till third infection control interventional stage (Figures 1-3 and Table 3).



Figure 3. External surface of bronchoscope with biofilm and without biofilm.

Table 3. Biofilm detecting test results in 3 infection control stages.

	14 November First infection control stage	29 November Second infection control stage	3 December Third infection control stage
ATP consumption test	Positive (+)	Positive (+)	Negative (-)
Crystal Violet Test	Positive (+)	Positive (+)	Negative (-)
Scanning Electron Microscopy	Positive (+)	Positive (+)	Negative (-)

Results of antibiotic susceptibility testing are shown in Table 2. According to the disc diffusion method, most isolates were resistant to routinely used antibiotics (94%). According to the E-test, most isolates were resistant to gentamicin and cephalosporins, and all isolates were sensitive to aztreonam.

PFGE results revealed that all 15 isolates from the patients and three isolates from the bronchoscope shared a common pattern but several subtypes were identified, whereas the two non-related isolates showed different patterns (Figure 4).

MIC of Colistin resistance was determined 8 mg/l, this MIC level has been defined colistin resistant by all known standard systems (Table 4).

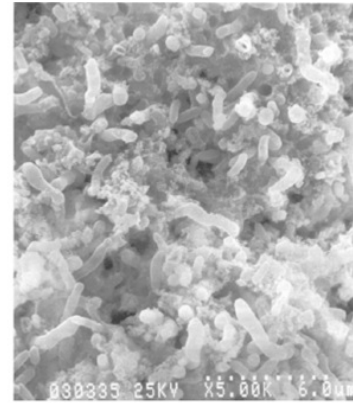


Figure 4. Electron Microscopy scan of *Pseudomonas aeruginosa* in Biofilm of bronchoscope.

Results of ATP consumption and Biofilm formation detection by Scanning Electron Microscopy and Crystal Violet testing are shown in Table 3.

According to the above methods Biofilm embedded microorganisms had remained “living” form until using ETO. After ETO application (third interventional stage “E18”) above test results became negative. These results show, FDA-CDC confirmed methods are merely effective on planktonic forms of bacteria and they are non-effective against biofilm embedded forms. According to above tests, ATP consumption remains positive till (E18).

Discussion

To our knowledge, this is the first report of an outbreak of pan drug-resistant *P. aeruginosa* due to a contaminated bronchoscope in turkey. All the 15 cases of bronchial infection in this outbreak occurred at the outpatient department where the patients received bronchoscopy. Even though two-thirds of the patients were hospitalized for treatment,

None of them was categorized as having bronchial infection initially. The outbreak therefore elucidated the infection control surveillance system. In addition, the attending bronchoscopy, pulmonary doctors were not alert enough to recognize, or even suspect on the cluster of these cases and probability of non-efficiency of CDC-FDA recommended sterilization procedure on biofilm embedded bacteria. Hence, the outbreak was not identified until the microbiology staff informed the infection control practitioners. Again, it cannot be overemphasized that the responsibility of infection control is not limited to certain personnel but ascribed to all the personnel working in the hospital. All HCWs in the hospital should receive continuous medical education on infection control issues regularly. All 15 clinical isolates were identified as resistant to Colistin by the disc diffusion test initially and MIC later on. 8 mg/l was MIC of all isolated *Pseudomonas aeruginosa*, which has been defined

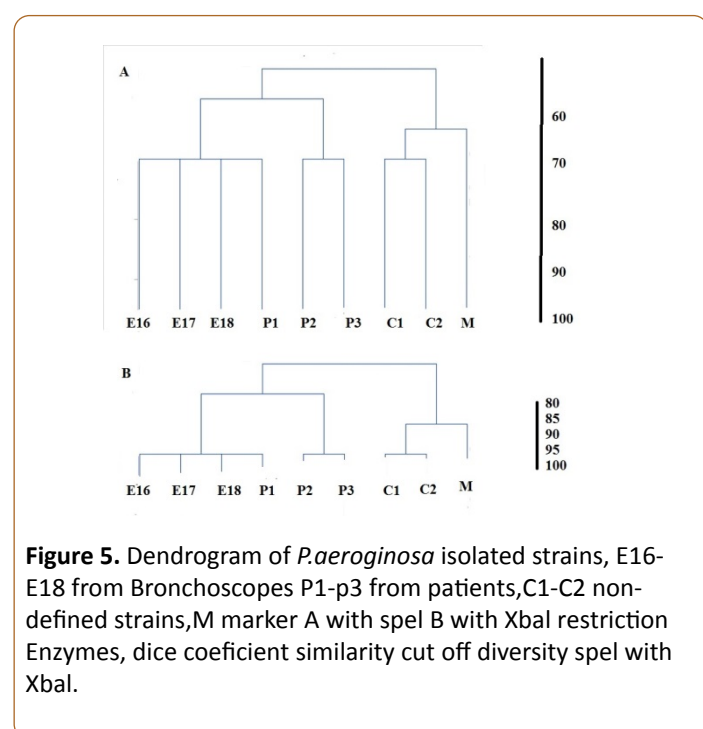
resistant by all known standard systems (Table 4). However, all 18 isolates (15 from the patients, 3 from the bronchoscope) involved in the outbreak shared a common PFGE pattern, Although several subtypes were also identified Moreover, the contaminated bronchoscope had been used on all 15 patients before they developed the bronchial infection, while no new case of bronchial infection caused by Colistin-resistant *P. aeruginosa* was further identified after suspension of the contaminated bronchoscope. These results suggest that this event was an outbreak of Colistin-resistant *P. aeruginosa* bronchial infection, and the contaminated bronchoscope was the cause of the outbreak.

A recent report from a nearby university-affiliated hospital indicated that the prevalence rate of Colistin resistance among clinical samples of *P. aeruginosa* isolates was 7% [19].

Table 4. Minimum inhibitory concentration (MIC) amount of colistin resistant bacteria in different standard system (mg/L).

	<i>Pseudomonas aeruginosa</i>			<i>Acinetobacter baumannii</i>		Enterobacteriaceae	
	S	I	R	S	R	S	R
EUCAST	≥ 4	-	> 4	≥ 2	> 2	≥ 2	> 2
CLSI	≥ 2	≥ 4	≥ 8	≥ 2	≥ 4	-	-
BSAC	≥ 2	-	≥ 8	-	-	≥ 4	≥ 8
SFM	≥ 2	-	> 2	≥ 2	> 2	≥ 2	> 2

EUCAST: European Committee on Antimicrobial Susceptibility Testing; **CLSI:** Clinical and Laboratory Standard Institute (USA); **BSAC:** British Society for Antimicrobial Chemotherapy; **SFM:** Societe de Francaise de Microbiologie



Isolates of Colistin-resistant *P. aeruginosa* identified in this outbreak might be from clonal spread rather than new mutants. Resistance patterns of *P. aeruginosa* isolates should be continuously monitored, and further studies should be performed to evaluate whether the clone had spread in this area. During the intervention of this outbreak, we did not uncover the reason why the OPA disinfection protocol could not thoroughly eradicate *P. aeruginosa* until ETO sterilization was added. We speculate that this should be due to biofilm formation around bacteria in bronchoscope that prevents the penetration of the disinfectant material from biofilm barrier and penetration of ETO from that barrier. Addition of ETO apparently broke this barrier. Our biofilm detecting methods confirmed existing biofilm and living bacteria till using ETO for their sterilizations (Table 4). Olympus Bronchoscope experts confirmed our idea as they had similar experience with the similar equipment. Their electron microscopic study also confirmed biofilm existence in our bronchoscope (Figure 3-5). Previous studies indicated that the most usual factors for disinfection failure, which also led to endoscopy-related outbreaks, included equipment malfunction, inadequate disinfection practice, and contamination of washer, accessories, water or other solutions used [6,7] to elucidate whether these factors were associated with the outbreak presented here, the following points have been examined or improved during the investigation. The concentration and the immersion time of the 3M diluted enzyme used were both above the highest requirements recommended by the manufacturer. In addition, the immersion time of 0.55% OPA was changed from 10 to 15 min that is longer than the 12 min recommended by the US Center for disease control and US Food and Drug Administration. Other factors, such as contaminated water or solutions, were not identified by surveillance cultures. However, the OPA disinfection, with either the initial or the revised protocols, indeed failed to decontaminate bronchoscope. Two possibilities may explain this disinfection failure:

First, the outbreak isolates might have generated biofilms in the bronchoscope, leading to the disinfection failure [17,20]. OPA is unable to destroy the microorganisms within biofilms, and, worst of all, may facilitate the accumulation of biofilms [18,21]. A heat-sterilization procedure, such as autoclave, is more effective in eliminating the microbial biofilms. However, because some elements in the endoscopes may be vulnerable to high temperatures, ETO is suggested as one of the alternative methods for endoscope disinfection [19,22]. Indeed, by adopting the ETO procedure, we successfully decontaminated bronchoscope.

Second, a gene mutation might have been occurred, resulting in reduced susceptibility or even full resistance of the microorganism to OPA [23-25]. It has been shown that decreased porin expression played a major role in the resistance to aldehyde-based disinfectants [23,26]. A recent report from an Asian hospital indicated that decreased expression of outer membrane proteins (porins) is one of the important factors contributing to the aldehyde resistance of Gram negative rods [16,27].

Although we did not examine the OPA resistance among our outbreak isolates, these bacteria may also possess the same characteristics that contribute to the Colistin resistance and therefore to the OPA resistance. Further studies are needed to elucidate these issues.

Retrospectively, we recognized that one mistake had been made in managing this outbreak. When *P. aeruginosa* was identified from the bronchoscope on the first surveillance, we did not suspend the usage of this contaminated bronchoscope immediately, though we had presumed that the contaminated bronchoscope might not be the cause of this outbreak. Continuing usage of the contaminated bronchoscope led to further cases of infection.

This clearly taught us that, once identified, a contaminated instrument should be suspended and not used until the potential pathogen has been eradicated or proved otherwise. Again, to prevent endoscopy-related infections, strict implementation of the disinfection protocol of endoscopes cannot be overemphasized [7,24,25,28].

In conclusion, the outbreak of colistin-resistant *P. aeruginosa* bronchial infection was due to the disinfection failure of a contaminated bronchoscope, which had been colonized by the resistant clone probably after being used on the case 1 patient. Infection after bronchoscopy or other endoscopic procedures should be closely monitored. If two consecutive or more patients develop infections after the procedure, especially if the same clinically relevant microbial species are involved, the existence of an outbreak of infection should be highly suspected and a prompt investigation should be initiated. Furthermore, if scopes are found to be contaminated or colonized by a resistant pathogen that may possess characteristics

Similar to the Colistin-resistant *P. aeruginosa* described herein, the deficiency of OPA disinfection should be considered and more effective decontamination strategies should be considered. Before the sterility can be ensured, further use of the contaminated scopes should be prevented.

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There is not any conflict of interest between co-authors of this manuscript.

Ethical Issues

There is not any Ethical issue in this research.

Conflict of Interest

There is not any conflict of interest between co-authors of this manuscript.

Study Highlights

What is current knowledge?

1- The most standard and recommended disinfection protocol for bronchoscope in the hospital was FDA-CDC guideline method; it is effective on non-biofilm embedded forms of bacteria. The routine disinfection protocol for bronchoscope in the hospital was cleaning surface of bronchoscope tube with sterile gauze soaked in 70% ethanol or immersion of the scope in detergent/disinfectant solution.

2- XbaI and SpeI restriction enzymes has been used for Pulse Field Gel Electrophoresis based on epidemiological studies for *Pseudomonas aeruginosa* outbreaks in health centers, they were fulfilled without evaluating their cut of value (Coefficient similarity) and their priority and efficiency.

What is new here?

1-These results show, FDA-CDC confirmed methods are merely effective on planktonic forms of bacteria and they are non-effective against biofilm embedded bacteria, this guideline must be updated.

2- XbaI restriction enzyme turned out better than SpeI in interpreting bacterial pulse types with BioNumerics 6.0. The most suitable cut off value for SpeI was above 75% Dice similarity while for XbaI above 95% Dice similarity with BioNumerics 6.0, So Using XbaI is recommended, PFGE relevant protocol must be updated.

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