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A flexible bronchoscope as a source of an outbreak with OXA-48 carbapenemase producing *Klebsiella pneumoniae*

Summary

Background: Carbapenem resistant *Klebsiella pneumonia* (CRKP) is an emerging pathogen. We report an outbreak including eight patients with *K. pneumoniae*-OXA-48 in one intensive care unit.

Methods: The outbreak was analyzed by epidemiologic investigation and genotyping of the pathogen.

Results: Originating from one colonized patient four other patients were infected or colonized with the genotypically identical pathogen via contaminated bronchoscopes. Two of those patients died of pneumonia. The outbreak was terminated when the two endoscopes were sent to the manufacturer. Even after repair, one of the two endoscopes continued to show microbial contamination in the final rinse water. The cleaning and disinfection process was critically evaluated by an independent contractor. The cleaning solution recommended by the manufacturer, containing peracetic acid was replaced with an enzyme-based solution. Subsequent samples obtained monthly revealed no further contamination.

Conclusion: The incidence of outbreaks associated with endoscopic procedures is probably underestimated. The reports involving multi-resistant pathogens may only represent the tip of the iceberg. Vigilance and active surveillance may help to detect the accumulation of phenotypically identical pathogens in respiratory secretions at an early stage. Subsequently, application and reprocessing protocols for endoscopes should be critically evaluated. Shorter sampling intervals and active surveillance of endoscopes are recommended. In order to prevent biofilm formation, design, material and workmanship of the endoscopes as well as the properties of cleaning solutions should be optimal. The use of an enzyme cleaner has proven itself in the described outbreak.

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Introduction

The spread of carbapenem-resistant Enterobacteriaceae (CRE) witnessed since the start of the new millennium is a global threat [1]. These pathogens can cause serious healthcare-associated (nosocomial) infections, in particular in immunocomprised, long-term hospitalized patients who had previously received antibiotic treatment. The increased mortality risk in blood stream infections with CRE of between 24 and 70 % is associated with advanced age, the severity of the underlying disease (Apache score) and inadequate antibiotic treatment [2–7]. Effective calculated antibiotic therapy is limited since, in addition to resistance to beta-lactam antibiotics, many CREs have also developed resistance to several other classes of antibiotics, such as fluoroquinolones, tetracycline, aminoglycosides and, even already, to colistin [1].

In particular, *Klebsiella pneumoniae* (KP) can produce clinically relevant carbapenemases, such as class A KPC-type enzymes, zinc-dependent class B metallo-

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beta-lactamases VIM, IMP and NDM as well as the plasmid-mediated class D OXA-48-type carbapenemases. Plasmid-mediated resistance is largely responsible for the spread of OXA-48 to other Enterobacteriaceae species, such as *Escherichia coli* and *Citrobacter freundii* [1]. OXA-48-producing *K. pneumoniae* was first sporadically detected in 2001 in Turkey, and was soon followed by a major outbreak in Istanbul [8, 9]. At the same time, isolates were identified in the Middle East and in North African countries [10]. The emergence of OXA-48 in Western Europe (United Kingdom, Belgium, France, Netherlands and Germany) has been imputed to the transfer of colonized patients from the aforementioned countries of origin, as documented by the discovery of genetically identical strains in a major outbreak in a Dutch hospital [11].

In the middle of 2008 the Robert Koch Institute (RKI) issued its first report on a cluster of KPC-2 producing *K. pneumoniae* (CPKP) in Baden-Wurttemberg, and that was followed by the first outbreak in 2010 in a regional university hospital [12, 13]. Reports were published in 2011 about a further outbreak in that region, with isolation of a KPC-2 and VIM-1 producing CPKP [14].

Since first detected in July 2010, KPC-2 producing *K. pneumoniae* was isolated during a prolonged outbreak in a university hospital in central Germany from 98 out of more than 140,000 inpatients [15]. These bacteria were last detected at the beginning of April 2013. The majority of patients were merely carriers and did not experience any adverse health effects.

Follow-up examination of discharged patients from among that population revealed that, while 65 % were decolonized within six months, there was evidence of ongoing colonization over more than two years [16]. Between 1 October 2013 and 30 September 2014, KPC-2 producing Enterobacteriaceae were isolated from 132 patients at a hospital in the south of the federal state of Hesse. Spread on plasmids the genes bla_{KPC-2} and bla_{TEM} have been found in several species. They are thought to have been transmitted in foodstuffs prepared in the hospital kitchen. It is also believed that the kitchen waste water was contaminated by a spiral drain cleaner, which had been used to unblock drains in both the kitchen and in patient rooms [17].

Apart from oral-faecal transmission routes, there are also increasing reports of iatrogenic transmission, e.g. linked to endoscopic procedures [18–24].

In this paper we now report on an outbreak of OXA-48 producing *K. pneumoniae* in an intensive care unit of a medium-sized Berlin hospital [25].

Method

Structures and screening

The hospital Paulinenkrankenhaus (PKH) is an institution specializing in cardiac follow-up care of cardiac patients. The department of internal medicine provides an internal medicine department: an intensive care unit with 21 ventilation beds; an intermediary ward with nine beds; and five standard wards with 118 telemetry-monitored beds.

The intensive care unit (ICU) has four single rooms, four two-bed rooms and three three-bed rooms. The scheduled staffing ratio is 1:2.1 patients. Of the some 1,000 patients treated each year in the ICU, around three-quarters are transferred directly from the operating rooms or from the ICUs of the cardiac surgery referring hospitals. The remaining cases are internal referrals from the standard and intermediate wards. The mean ICU stay for the outbreak year was 9.2 days.

In the ICU screening for methicillin-resistant *Staphylococcus aureus* (MRSA) on admission was established since 2004. Additionally, risk-adapted screening for multiresistant Gram-negative bacteria (MRGN) and vancomycin-resistant enterococci (VRE) was introduced since 2011 and applied to almost one out of every three patients.

There is close collaboration with the primary referring hospital, based on staffing and institutional structures, with regard to infection control management.

A standardized infection control and isolation policy for dealing with MRGN and VRE, stratified in terms of risk areas, has been in place for several years now and is updated in accordance with the current guidelines and local conditions at regular meetings of the infection control team.

Microbiological methods

Swabs from patients were inoculated onto selective agar plates containing cefpodoxime (ChromID ESBL, bioMerieux) and incubated for one day at 37 °C. Furthermore, the samples were transferred to a MacConkey agar plate on which an antibiotic disk containing 10 µg ertapenem, 10 µg meropenem and 10 µg imipenem was placed, in order to assess for culture growth in the carbapeneme inhibition zones after 16-24 hour incubation at 37 °C.

Identification and susceptibility testing were carried out in VITEK2® (bioMerieux, France) for all cultures exhibiting growth on ChromID ESBL agar plates /or additionally cultures on MacConkey agar plates were classified as non-susceptible to carbapenem and oxidase-negative as per EUCAST criteria. For enterobacteria with intermediate susceptibility or resistance to at least one carbapenem (ertapenem, imipenem or meropenem) in VITEK2® (bioMerieux, France), multiplex PCR was performed to identify acquired carbapenemase genes as described by Poirel et al. [26].

The swabs taken for environmental tests were enriched in 10 ml tryptic soya broth (TSB) for two days and then cultured at 37 °C on Columbia blood agar and MacConkey agar plates.

The working channels of bronchoscopes were rinsed out in 20 ml sterile isotonic saline. 10 ml aliquots of the flushing solution was collected, neutralized and passed through a 0.2 µm cellulose membrane. The membrane was then transferred to a Columbia blood agar plate and cultured for two days at 37 °C. 100 µl aliquots of the flushing solution were plated onto Columbia-blood agar and MacConkey agar plates and incubated for two days at 37 °C. Besides, a swab was taken from the proximal and distal end of the bronchoscope and processed using the same procedure as for the environmental tests. Species identification and susceptibility tests were carried out in VITEK2® (bioMerieux, France).

Results

Within a four-day period starting on 23 May 2013, carbapenem-resistant *K. pneumoniae* (CRKP) was isolated from lower respiratory tract secretions collected during bronchoscopy from four ICU patients.

In order to a suspected cluster of healthcare-associated infections, a meeting of the outbreak team was convened and the outbreak reported to the competent health authority in compliance with Section 6(3) of the German Protection against Infection Act (IfSG) [27].

The infected patients were isolated in cohorts from the remaining patients and

cared for in accordance with the provisions governing infection control measures for patients colonized or infected with multi-resistant Gram-negative bacteria (MRGN), as set out in the Recommendations by the Committee for Hospital Hygiene and Infection Prevention (KRINKO) at the RKI [28, 29]. Wherever possible, the staffing ratio was increased.

As part of the outbreak management strategy, immediate microbiological screening was initiated (rectal swab, urine and tracheal secretion samples) for all ICU patients, and was repeated for contact patients at weekly intervals for three weeks.

Epidemiological investigation

All patients with microbiological proof of OXA-48-CRKP were recorded and their medical files scrutinized in terms of any stay and contacts in an external hospital, admission date, transfers within the present hospital, contact with internal patients and application of medical procedures.

Three of the four patients had undergone bronchoscopy, the endoscopes and flushing solution were subjected to microbiology testing.

Patient A had been admitted from an external hospital, where he had shared a room with the CRKP carrier (index patient). Screening of MRGN from rectal swab, urine and tracheal secretions, performed before transfer on 10 May and on admission to our hospital on 19 May, was negative.

The index patient was directly admitted to the external hospital from a region with high prevalence of CRE and had been screened preoperatively in accordance with the RKI recommendations for multi-resistant enterococci (MRE). Since the screening results were negative, no additional infection control measures were applied firstly. By chance, screening was repeated on postoperative day 2 after the patient had been transferred to a different ward, and this led to isolation of CRE from the rectal swab after two days (7 May).

Patient A underwent bronchoscopy on admission day, 19 May, using bronchoscope 1. After processing in accordance with the local standard operating procedure (SOP), the same bronchoscope was used in Patient B on the following morning. The third patient, Patient C, was sharing a room with Patient B and underwent bronchoscopy on 20 May with bronchoscope 2. Besides, Patient C had been a contact patient in an external hospital to a second contact patient of the index case who subsequently was also tested positive for CRE.

On 22 and 23 May, carbapenem-resistant *K. pneumoniae* with OXA-48 carbapenemase production was isolated from lower respiratory tract secretions from all three patients.

On 26 May, OXA-48-CRKP was isolated from the bronchoalveolar lavage (BAL) collected from bronchoscope 1 used for a fourth patient (Patient D) (Figure 1).

Microbiology testing of bronchoscopes 1 and 2 was conducted on 5 June 2013.

On 9 June both endoscopes showed high concentrations of bacterial contamination (> 1,000 cfu/ml). OXA-48-CRKP was isolated from the flushing solution from bronchoscope 1, and *Enterobacter cloacae*, *Klebsiella oxytoca* and *Stenotrophomonas maltophilia* were isolated from that of bronchoscope 2. Patients who had been investigated with the contaminated endoscopes were identified on the basis of a 'look back' procedure.

A further two patients (Patients E and F) underwent examination with bronchoscope 1 (30 May and 1 June) before it was subjected to microbiology testing. Thanks to weekly screening, it was possible to identify CRKP colonization in Patient E on 11 June and in Patient F on 9 June.

The rectal swabs from two other contact patients (Patients G and H) of Patient F tested positive for CRKP on 10 June and 24 June, respectively.

The outbreak ended on sending both endoscopes to the manufacturer for inspection.

Genotyping

Diagnostic workup for carbapenemases was performed with multiplex PCR (primers: bla_{OXA-48}, bla_{BIC}, bla_{NDM} and bla_{KPC}) [21]. Gene bla_{OXA-48} was identified in the isolates from all eight patients and from bronchoscope 1. Strains were typed using the amplified fragment-length polymorphism (AFLP) method

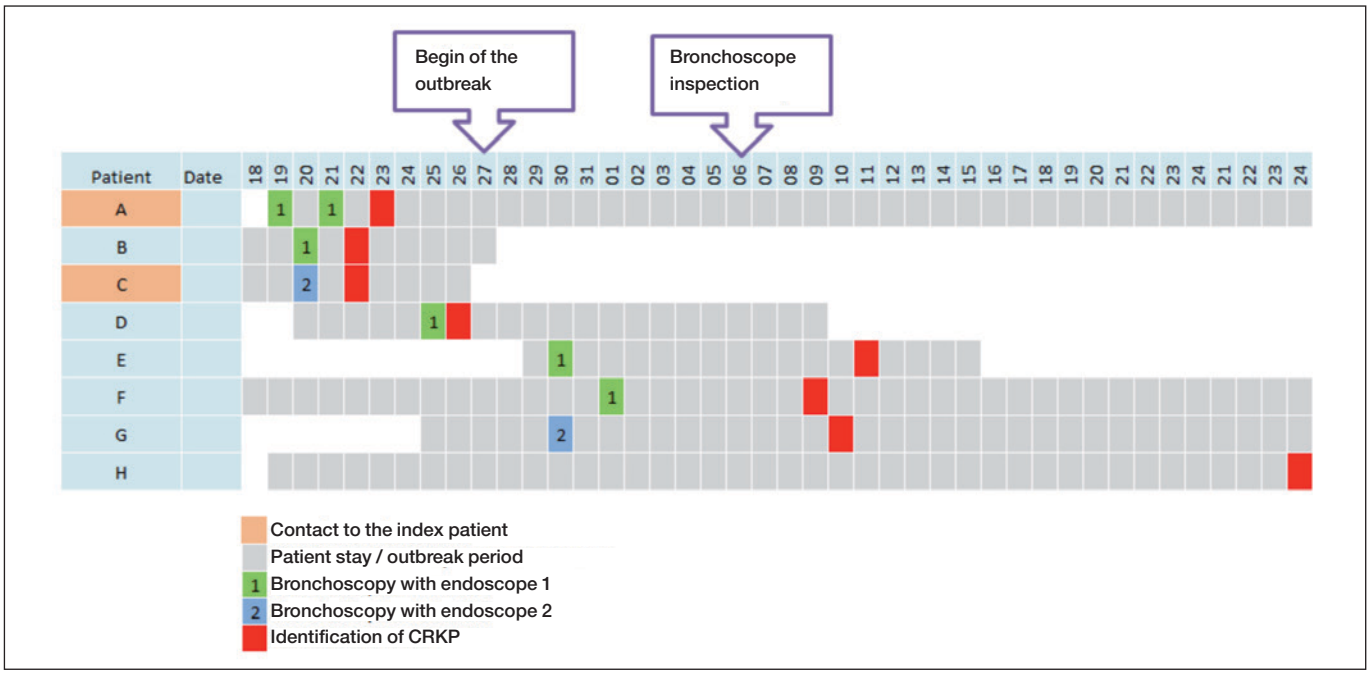

Figure 1: Outbreak with carbapenem-resistant *Klebsiella pneumoniae*.

and showed more than 99 % concordance (Pearsson coefficient, unweighted pair group method (UPGMA)) [30].

Clinical course

Three patients (B, C, D) died immediately from severe infection with OXA-48-*K. pneumoniae*.

Patient A who had had direct contact with the external index patient was the first person to be examined with bronchoscope 1, and was presumably the source of the endoscope contamination. He died six months, after implantation of a left ventricular assist system (LVAD), from recurrent OXA48-CRKP-infections (respiratory tract infection, urinary tract infection, mediastinitis).

Patients B and D, with septic pneumonia, had had direct contact with bronchoscope 1, which was contaminated with OXA-48-CRKP.

Patient C had had indirect contact with the external index patient and had shared a room with Patient A, but had been examined with bronchoscope 2, which was tested negative for OXA-48-CRKP. In this patient OXA-48-CRKP was first isolated from bronchial secretions, urine as well as the rectal swab, and a few days later from intraoperatively collected gallbladder secretions and in a blood culture. Retrospectively viewed, that primary intra-abdominal focus of infection was unlikely to have been linked to endoscopy.

Patients E and F contracted OXA-48-CRKP from direct contact with bronchoscope 1. Patient E was discharged without infection, Patient F died two months later from comorbidities.

Patient G who had had contact with bronchoscope 2 and Patient H, who did not undergo bronchoscopy, had been colonized with OXA-48-CRKP, but were later discharged in a clinically cured state.

In summary, starting with Patient A the pathogen was spread via bronchoscope 1 to four other patients, two of whom later died from septic pneumonia.

Inspection of equipment and reprocessing process

Neither the environmental nor endoscope washer-disinfector (EWD) tests produced any evidence of CRKP. The standardized reprocessing process was evaluated in collaboration with experts from the infection control department and the endoscope manufacturer, and no quality defects were noted [31]. The flow monitor of the EWD (Olympus ETD3 PAA) was inspected regularly and the results documented in the reprocessing log book. The software settings for the flow monitor were newly configured on 25 June to ensure that an acoustic alarm would be triggered in the event of malfunctioning and the process aborted.

At the time of safety inspection, worn components were replaced for both bronchoscopes and surface defects in the working channel repaired (bronchoscope 1: flaked areas of angulation rubber; bronchoscope 2: perforated angulation rubber) and the angulation pulleys were resoldered and adjusted.

Once repaired, the endoscopes were used again on 12 July. The previous six-monthly microbiology testing intervals were now shortened to monthly intervals.

Bacterial contamination was identified in the flushing solution collected from bronchoscope 1 on 4 September (> 200 cfu/ml coagulase-negative staphylococci and micrococci), but no CPKP or other pathogens were isolated. Following bronchoscopy of a patient with a confirmed diagnosis of *S. maltophilia* from respiratory tract secretions, phenotypically identical bacteria were isolated from the flushing fluid collected from bronchoscope 1, following reprocessing, at the time of monthly inspection on 6 October. *S. maltophilia* was isolated once again from the flushing solution on 10 October even though the bronchoscope had not been used for clinical purposes in the meantime and had been reprocessed.

Following this, bronchoscope 1 was re-inspected by the manufacturer.

The first report of an incident involving a medical device was submitted to the German Federal Institute for Drugs and Medical Devices (BfArM), which on final evaluation did not see any need for further action.

Bronchoscope inspection for bacteria embedded in biofilm

Using an endoscope tongs, a sterile sponge was pulled through the endoscope working channel under sterile conditions. Next, histology sections of the sponge material were investigated using fluorescence in situ hybridization (FISH) molecular biology method at the Biofilm Centre of the German Heart Centre Berlin (DHZB) [32]. No microorganisms or biofilm were detected after inspecting several sections and sectional planes.

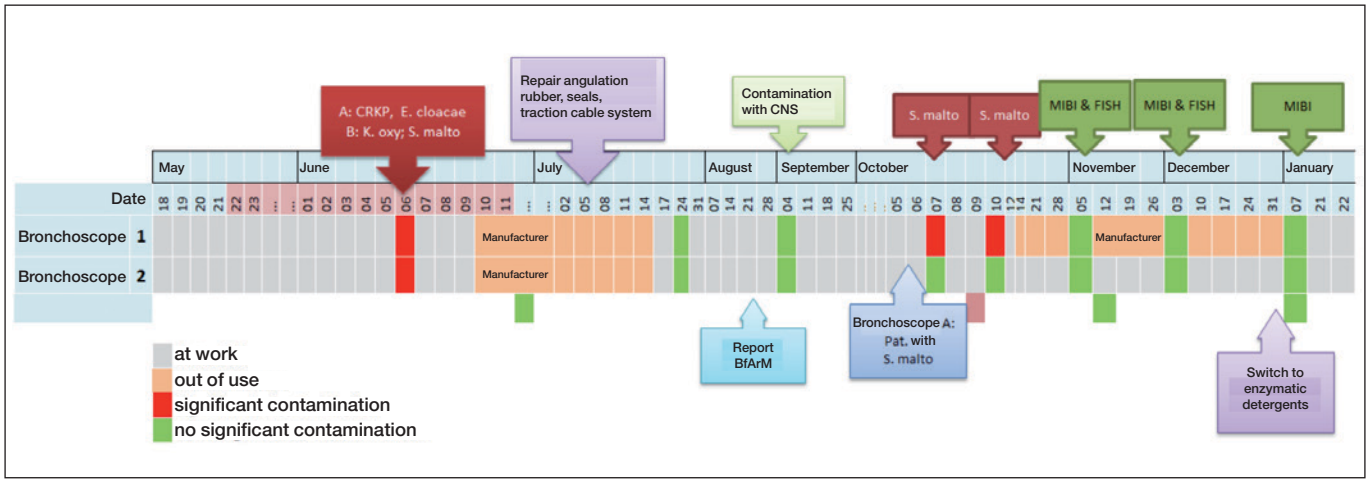

Figure 2: Inspection of equipment and reprocessing process. Abkürzungen: CRKP=Carbapenem-resistant *Klebsiella pneumoniae*; S. malto, *Stenotrophomonas maltophilia*; K oxytoca=*Klebsiella oxytoca*; KNS=coagulase-negative staphylococci; MIBI=Microbiological inspection; FISH=Fluorescence in situ hybridization.

Findings of outbreak investigation and measures taken

In summary, it was possible to rule out environmental contamination of the treatment rooms in the intensive care unit and the reprocessing rooms in the endoscopy department. Both endoscopes were in need of repair because of worn parts. Independent experts were unable to identify any quality defects in the standardized endoscope reprocessing process. In that respect, the main focus was on the cleaning process.

Surfactant solutions of non-foaming substances (tensides), enzymatic detergents or combined detergent and disinfectant solutions are used to clean flexible endoscopes. Since peracetic acid and aldehydes can result in protein fixation they are not recommended by KRINKO. Alkaline cleaning is very effective in dissolving protein and fat residues and is also endowed with microbicidal activity. But it can adversely affect materials. The manufacturer's specifications regarding material compatibility should be observed. While the various detergents differ in terms of efficacy, to date no particular substances have been found to be markedly superior to others [31]. However, in the manufacturer's product information on a peracetic acid-based product, particular attention is drawn to the product's non-protein fixing and pH-neutral properties for their high cleaning efficacy.

Despite observance of the manufacturer's recommendations, there was a discrepancy to the KRINKO recommendations [31]. In our hospital the standard operating procedure for reprocessing endoscopes was amended. Since then, to remove residues the bronchoscope was thoroughly rinsed after use with an enzymatic detergent and, following a 15-minute exposure time, thoroughly rinsed with sterile filtered water. Henceforth only disposable brushes were used for mechanical cleaning of the endoscope channels.

The monthly microbiology tests conducted over the following months did not reveal any significant contamination of the cleaning water from either of the two bronchoscopes.

Discussion

Already in the past, there have been detailed reports of numerous outbreaks with different pathogens caused by contaminated flexible endoscopes [23].

To date, in the literature there are six reports [18–22, 24] of transmission of multiresistant *Klebsiella* spp linked to endoscopic procedures, including one resulting from contamination of a cleaning brush [18]. Orsi et al. identified endoscopy as being an independent risk factor for acquisition of CRKP [33].

In the outbreak reported on in this paper it has been possible, thanks to genotyping, to identify one bronchoscope as the source of transmission [25].

It remains unclear whether a similar link to an outbreak of infection caused by non-resistant pathogenic microorganisms could have been identified and eliminated equally quickly. There are very few endoscopy departments that engage in close-knit surveillance in addition to microbiology testing every six months for quality assurance purposes.

By shortening to monthly sampling intervals it was possible to promptly detect *S. maltophilia* in a reprocessed bronchoscope and in the respiratory tract secretions of a patient after the CRKP outbreak had ended.

An unusual cluster of healthcare-associated infections with phenotypically identical pathogens should, in principle, initiate an evaluation of clinical processes in respect of any potential transmission risks.

That calls for increased vigilance and implementation of close-knit surveillance of pathogens and infections at ward level.

It is important to maintain ideally electronic documentation with assignment of endoscope serial numbers to specific cases so that contact persons can be quickly identified on the basis of a structured 'look-back' procedure.

Our report also underlines the need for critical review of the putatively safe endoscopic reprocessing process, manufacturer's instructions and the official recommendation for endoscope cleaning if something is amiss.

The manufacturer of the endoscope washer-disinfectors (EWDs) queried the wisdom of using a peracetic-acid based cleaning solution for the manual pre-cleaning step.

Staff from the infection control department did not detect any shortcomings in the reprocessing process, or any discrepancies in the EWD validation protocols.

No single cause could be identified for this outbreak. Presumably several factors were implicated, which in the complex interactions between human resources, materials and processes had led to contamination of an endoscope and, in turn, to CRKP transmission in the four corroborated cases.

This is borne out in a report published in 2013 where, despite valid guidelines and recommendations, mistakes in endoscope reprocessing processes constitute a widespread problem [32]. The most commonly implicated causes were, in addition to deviation from standard reprocessing procedures, biofilm formation and problems within endoscopes which precluded their proper cleaning.

Ebstein et al. reported in 2014 on an outbreak with NDM-producing *E. coli*, where the pathogen had been transmitted in 39 cases during duodenoscopy [34]. Endoscope contamination persisted for several months. It was only after switching the endoscope reprocessing method from automated disinfection with ortho-phthalaldehyde to ethylene oxide gas sterilization that no further cases occurred.

Biofilm formation at macroscopically unidentifiable damaged sites within the working channel or in other parts of the endoscope may also have contributed to survival of CRKP and *S. maltophilia* as described here. That possibility cannot be ruled out even if, after passage with a sponge, fluorescence in situ hybridization (FISH), albeit not validated for such an application, failed to find any evidence of that.

It has not been possible to ascertain what role contributory factors might have played in biofilm formation, e.g. prolonged periods, as seen outside core hours, between bronchoscope use in the intensive care unit and reprocessing start.

But what was clear is that no further contamination was detected in the monthly tests after switching to disposable materials for mechanical endoscope pre-cleaning and also changing to an enzymatic cleaning solution for endoscope cleaning.

Besides, it will, no doubt, be in the interest of the manufacturers of endoscopes and EWDs if future trends in materials, improvements in the cleaning process and better detergent solutions can reduce biofilm formation and, as such, enhance patient safety.

Conclusion

– The outbreak incidence linked to flexible endoscopes is thought to be greatly underestimated.

– Outbreaks with 4MRGN present a particular challenge to treatment and infection control management.
– Cognizance of these risks should be translated into greater vigilance and surveillance of healthcare-case associated infections.
– In the event of clusters of phenotypically identifiable pathogens in respiratory tract secretions, the endoscopy practices and endoscope cleaning processes must be critically appraised.
– Shortening of test intervals and active surveillance of endoscopes is recommended.
– For such measures a reasonable cost-benefit relationship should be assured and the measures regularly updated in accordance with quality circle recommendations.
– The endoscope design, materials and workmanship as well as the detergent solution properties should be optimized to prevent biofilm formation.
– Enzymatic detergents have proven their worth in the outbreak described here.

Conflict of interest

The authors declare that they have no conflict of interests as understood by the guidelines of the International Committee of Medical Journal Editors.

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