Journal of Hospital Infection 103 (2019) 27–34

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Molecular epidemiology study of a nosocomial *Moraxella catarrhalis* outbreak in a neurological rehabilitation unit

P. Warnke [a,*], T. Köller [a], B. Kreikemeyer [a], I. Barrantes [b], H. Mach [c], A. Podbielski [a]

[a] Institute of Medical Microbiology, Virology & Hygiene, University Medicine Rostock, Germany
[b] Institute for Biostatistics and Informatics in Medicine and Ageing Research, University Medicine Rostock, Germany
[c] Center for Medical Rehabilitation, Waldeck-Schwaan, Germany

ARTICLE INFO

Article history:
Received 14 February 2019
Accepted 25 April 2019
Available online 2 May 2019

Keywords:
Moraxella catarrhalis
Outbreak
Molecular epidemiology

SUMMARY

Background: *Moraxella catarrhalis* is a common agent causing upper and lower respiratory tract infections, particularly of ventilated patients. The bacteria are transmitted between humans by direct and indirect contacts. However, reports of nosocomial outbreaks by this pathogen are scarce.

Aim: To analyse *M. catarrhalis* strains isolated during an outbreak in a medical rehabilitation centre to reveal their clonal relationship and to elucidate potential transmission routes.

Methods: Extensive environmental and medical staff sampling was performed. Phenotypic and genotypic analyses of 15 isolates were executed, including repetitive element palindromic polymerase chain reaction (repPCR) and whole-genome sequencing. Furthermore, an intensified hygiene regimen was installed.

Findings: The clonal nature of nine patient isolates and a simultaneous presence of separate entities including a strain isolated from a physician during staff screening was confirmed. Although neither asymptomatic carriers among the staff persons nor outbreak strain-contaminated fomites were identified for a specific intervention, the outbreak ceased due to maximum general and specific hygiene precautions. Retrospective analysis showed the increasing prevalence of *M. catarrhalis* strains over a period of two years before the incidence. Since then and after returning to the regular hygiene regimen, only one patient with a phenotypically diverse *M. catarrhalis* isolate has been documented.

Conclusion: The first *M. catarrhalis* outbreak involving nine patients of a neurological and trauma rehabilitation centre was reported. Potential transmission pathways were discussed. Comprehensive outbreak analyses insinuated the extension of routine laboratory storage time for defined species.

* Corresponding author. Address: Institute of Medical Microbiology, Virology & Hygiene, University Medicine Rostock, Schillingallee 70, D–18057 Rostock, Germany. Tel.: +49 381 494 5930; fax: +49 381 494 5902.
E-mail address: philipp.warnke@med.uni-rostock.de (P. Warnke).

https://doi.org/10.1016/j.jhin.2019.04.019

Introduction

Moraxella catarrhalis are Gram-negative diplococci that physiologically colonise the healthy mucosal tissues of the human upper respiratory tract [1,2]. Due to expression of several defined virulence factors and, potentially, influences of a dysbalanced surface microbiome, *M. catarrhalis* has the potential to act as a pathogen [3,4]. In this role, it causes otitis media in children, and it is involved in exacerbations of chronic obstructive pulmonary disease (COPD). Additionally, *M. catarrhalis* is a causative agent in conjunctivitis and rhinosinusitis [5—8].

The bacteria are transmitted exclusively between humans by direct and indirect contacts. Airborne transmission is plausible although thus far it has not been documented by molecular methods. Transmission has been demonstrated among family members, household contacts and in medical institutions — including both endemic and epidemic settings. Risk factors include bacterial load, crowding, winter season, day-care attendance, length of stay in a medical institution, and respiratory therapy [9—16].

Over the last three decades, varying methods have been employed to analyse the clonal nature or the genetic variability of *M. catarrhalis* strains predominantly collected in medical institutions, i.e. sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), esterase electrophoresis, restriction endonuclease analysis, DNA-typing, pulsed-field gel electrophoresis, multilocus sequence-typing, and whole-genome sequencing (WGS) [14,17—23]. Depending on the utilized method, individual *M. catarrhalis* strains displayed different phenotypic or genetic distances. Of note, these differences were less pronounced when assessing WGS determined gene content and chromosomal organization data from clinical isolates as compared to results from the older phenotypic tests applied on all types of *M. catarrhalis* strains [17,18,24]. Also, WGS did not recognize specific genes that could explain pathogenic differences between *M. catarrhalis* strains, which prompted speculations of differing promoter strengths/activities in such strains [25]. Overall, the extent of genetic variation across the species *M. catarrhalis* is not finally resolved.

Potential nosocomial *M. catarrhalis* outbreaks have been examined by prospective or retrospective analysis of long-term strain collections [10,12,22,26]. In other cases, the suspected outbreak was confirmed by phenotypic analysis of strains accumulated during a study period [14,27,28]. In a subset of cases, possible transmission events between patients and staff members were observed [14,22]. However, so far WGS has not been employed as a specific technique in such analyses.

In this study, a nosocomial outbreak of *M. catarrhalis* was analysed that took place in spring 2018 in a neurological and trauma rehabilitation centre and potentially involved 14 patients and one physician. It was shown that WGS and traditional molecular approaches came to identical results, but only WGS unambiguously demonstrated that a subset of just nine isolates were part of the infection chain.

Material & methods

Setting

Early rehabilitation patients transferred from hospitals throughout Germany are randomly assigned to three early rehabilitation wards. Specialized care on these wards includes ventilation, weaning measures and early mobilization of patients. Medical care is delivered by nurses constantly assigned to only one of three wards and by physicians and physiotherapists who move between the three specialized wards and beyond. Patients do not have direct contact with staff members working for the kitchen, cleaning, housekeeping and transport services or for administration.

Proper hygiene measures with these patients are ensured by a local hygiene team consisting of two specifically trained physicians and one specialized nurse. Since 2013, hygiene specialists of the Rostock University Hospital supervised this team on a regular basis.

Each patient on the specialized wards was screened for epidemiologically or therapeutically problematic bacteria immediately after transfer to the rehabilitation centre, and thereafter on a weekly basis throughout their stay or until internally transferred to the regular rehabilitation wards. Screening sites include nose, throat, groin and if applicable, chronic wounds. Screening is performed by specifically trained staff members [29] utilizing FLOQSwabs and modified Amies medium (Copan, Brescia, Italy).

Environmental sampling and screening of staff members

Neither hospital environment nor personnel was screened on a routine basis. Only after the outbreak were suspected environmental samples were collected by FLOQSwabs moistened with a drop a sterile 0.9% NaCl solution when applied on dry surfaces and transported in modified Amies medium according to the instructions of the manufacturer (Copan). Environmental sampling sites are specified in the Results section.

All staff members of the rehabilitation centre present in this period were screened for an *M. catarrhalis* carrier status, irrespective of their functions and contacts with the specialized wards. Screening sites were the throat and the volar sides of both forearms.

All samples were marked with pseudonyms and thereafter processed this way. The resolution of pseudonyms was known only to the Medical Director of the rehabilitation centre and was only used if the person gave its written consent at the time of sampling. The procedure was communicated in advance to the responsible State Institution (LAGuS) and was confirmed by a written statement.

Microbiological diagnostics

Samples were processed to the rules of the fully DAkkS-accredited Microbiology Laboratory according to DIN EN ISO 15189 and 17025. For details see the supplementary material.

Initial epidemiological analysis

In the first step, isolates were typed by the DiversiLab technique (bioMerieux) employing the procedures of the manufacturer. For details, see the supplementary material.

DNA sequencing and bioinformatics

The moraxella strains were subjected to next-generation sequencing (NGS)-based assessment of whole genomes in two individual 600-cycle sequencing runs (six strains and one negative control per run) on a MiSeq system. For details, see the supplementary material.

Results

Outbreak characteristics

Indicators for a nosocomial *M. catarrhalis* outbreak became apparent on 18th April 2018, by routine screening procedures because of two positive patient samples taken on 16th April on the same ward (Ward B). The local hygiene team was informed by the diagnostic laboratory on 18th April 2018. However, starting from that day, 10 patients from Ward B and one patient from Ward A were potentially affected by the outbreak. The incidence lasted until 14th May 2018 (Figure 1). In a retrospective analysis, in 2018 three additional patients harbouring *M. catarrhalis* were identified residing in this ward before an outbreak situation was suspected.

In eight of the overall 14 patients, no signs of pending infections were found during daily routine examinations, indicating a commensal status of the *M. catarrhalis* strain. Among the other six patients, one presented clinical signs and laboratory data consistent with pneumonia, two with a probable pneumonia, and the three others showed signs consistent with lower-airway infection (Supplementary Table S1). In three of the symptomatic patients, no other agent typically associated with airway infections was found when applying culture-based as well molecular diagnostics (FTD respiratory pathogens 21 plus kit, Fast Track Diagnostics, Esch-sur-Alzette, Luxembourg) indicating the causative nature of the *M. catarrhalis* strain.

The affected patients of Ward B were treated in six diverse rooms within the ward, with the majority in at least two different rooms during their extended therapy periods. There was no common room to which every patient was allocated at least for a short period during their stay, although seven out of 13 *M. catarrhalis* patients of Ward B stayed in one specific room (fictional no. 7) at some time in their therapy. Neither stay at the ward's lounge nor contact with physiotherapists or ear,

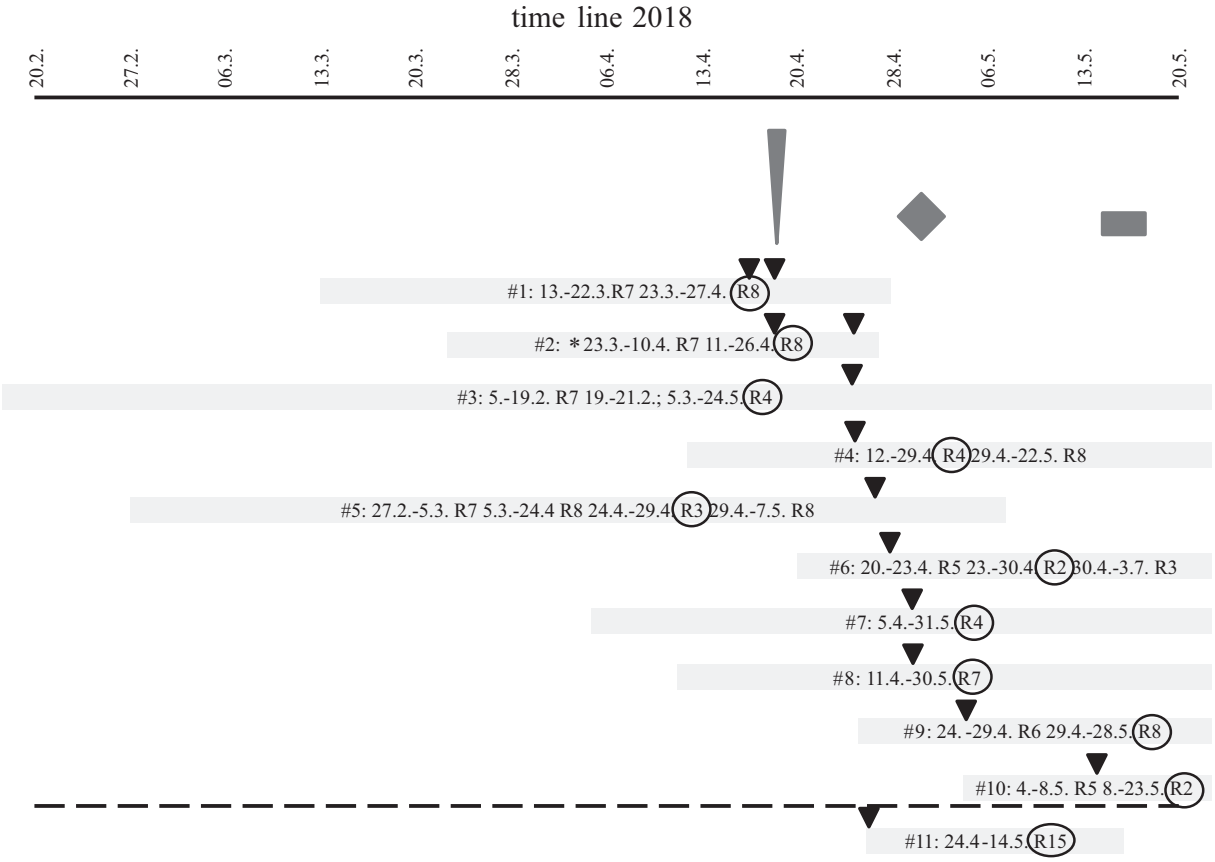

Figure 1. Line list of patients potentially associated with the *Moraxella catarrhalis* outbreak. A timeline indicates the period before and during the *M. catarrhalis* outbreak in the year 2018. All dates in this figure are denoted as dd/mm/yy. All room numbers are fictional. The 10 patients shown above the dashed line resided on Ward B, the one patient shown below the dashed line on Ward A. Their isolates were included in the molecular analyses. The small triangles indicate the days when positive *M. catarrhalis* samples were taken, the long triangle the day when the outbreak was first suspected. The diamond demarks the day of environmental sampling, the rectangle the period of staff sampling. For each patient from Ward B, encircled room numbers denote the place in which a positive *M. catarrhalis* sample was gained. The asterisk marks the *M. catarrhalis* outbreak index patient.

nose and throat (ENT) specialists including the swallowing test were in common for all *M. catarrhalis* patients. Besides, during the outbreak, patients of the other specialized wards were examined and treated by the same physiotherapists and ENT specialists without further demonstration of the outbreak strain.

Hygiene measures for outbreak counteraction

A potential outbreak was formally stated on April 26 when four patients of Ward B were found to harbour *M. catarrhalis*. On that day, after informing the local health authority, the members of the local hygiene team met with the physicians and nurses of this ward to discuss procedures and/or devices potentially involved in the transmission chain. Hygienically correct airway suction therapy as well as correct usage of hand hygiene and protective gloves and gowns were intensely questioned and trained. A line list was started on the next day. Because two other patients demonstrated the presence of *M. catarrhalis* in the next two days, in spite of the intensified hygiene measures, a microbiological examination of the patients' environment was performed on 30th April in two rooms of Ward B (Supplementary Table S2). Overall, in 32 items tested for bacterial load, *M. catarrhalis* was not present in any of them.

Although repeated education on all relevant hygiene measures took place on Ward B, four additional *M. catarrhalis* patients were identified in the following days (Figure 1). Even though medical chart reviews for all affected patients did not reveal one or several staff members in contact with all affected patients, the outbreak team decided to perform a screening of all present employees for an *M. catarrhalis* carrier status. The screening was executed between May 14 and May 16 and comprised all 95 staff members working at least temporarily on Ward B or having contact with at least one patient of Ward B at that time. Among the screened staff members, one person was found to carry an *M. catarrhalis* strain in his throat which displayed the antibiotic resistance profile of the outbreak strain. It was a physician predominantly working on Ward A, i.e. one the specialized wards not affected by the outbreak. He reported that his one-year-old child was currently suffering from a middle-ear infection for several weeks. The physician was instructed to wear a surgical mask in addition to employing careful hand hygiene measures when coming into close physical contact with patients until further notice.

Of note, on 14th May, the last sample positive for the *M. catarrhalis* outbreak strain was taken from one of the patients of Ward B.

Additional activities for outbreak confirmation and source identification

A retrospective analysis of all patient data of the year 2018 identified three additional Ward B patients with *M. catarrhalis* strains colonising their airways. These strains were isolated between 27th February and 27th March and displayed the antibiotic resistance profile of the outbreak strain. Since isolates were generally stored for two weeks before disposal, none could be integrated into the following molecular analysis.

An analysis of the years before the incidence is complicated by the fact that the microbial laboratory in charge changed at the beginning of 2018. Given the uncertainty of consistent diagnostic procedures in a foreign laboratory over several years, the overall isolation rates for *M. catarrhalis* in the complete rehabilitation centre was 0 between 2010 and 2015, and nine and 31 in 2016 and 2017, respectively, indicating an increasing problem with these bacteria in the past.

In order to confirm a true outbreak, the *M. catarrhalis* strains of the 11 patients identified after stating a potential outbreak situation as well as the strain isolated from the staff member were subjected to two different molecular analysis techniques.

The initially performed analysis based upon the DiversiLab technology classified nine strains of the patients from Ward B as potentially identical, clearly supporting the outbreak hypothesis. However, the patient strain first sampled during the outbreak analysis evidently displayed a different banding pattern, putting it far from the other nine isolates. Thus, according to this result, not patient no. 1 but patient no. 2 had to be regarded as the outbreak index patient (Figure 2). The strain from Ward A patient no. 11 as well as the strain from the staff member were not part of the outbreak cluster.

Since no *M. catarrhalis* was detected in any of the overall 32 environmental samples (Supplementary Table S2), no additional information was gained concerning potential environmental intermittent sources or the route of transmission.

Results from the WGS analysis

The available 11 patient and one staff strains were subjected to WGS (Supplementary Table S4).

The novel genomes were annotated for candidate antibiotic resistance genes. In this regard, the resistance of the patient isolates to ampicillin was demonstrated to result from carriage of the β-lactamase gene *bla*BRO-1_1 (Supplementary Table S6).

To have a broader view of the relationship between the isolates, a pangenome was built, entailing the protein encoded sequences from the novel isolates, together with those from the *M. catarrhalis* reference genomes (Supplementary Table S3). The obtained pangenome entails 1274 genes present all strains (core genes), 1052 genes present in two to 18 strains and 658 genes only present in single strains (both grouped as accessory genes). These analyses were extended by comparing the single nucleotide polymorphisms between the 12 novel genome sequences and the *M. catarrhalis* reference strain CCRI-195ME (Figure 3). In all cases the cladograms assigned the nine outbreak patient isolates to one homogenous group, and the three other strains isolated from the first examined patient no. 1 from Ward B, the simultaneously present patient no. 11 from Ward A and the staff member, on to different branches, thereby confirming the result from the DiversiLab analysis.

Discussion

M. catarrhalis outbreaks have occasionally been described in the nosocomial setting [14,19,22,27]. Before 1988, lacking methodological armentarium for bacterial typing only permitted the assumption of a nosocomial spread [30]. Since then, methods with restricted discriminatory power have been

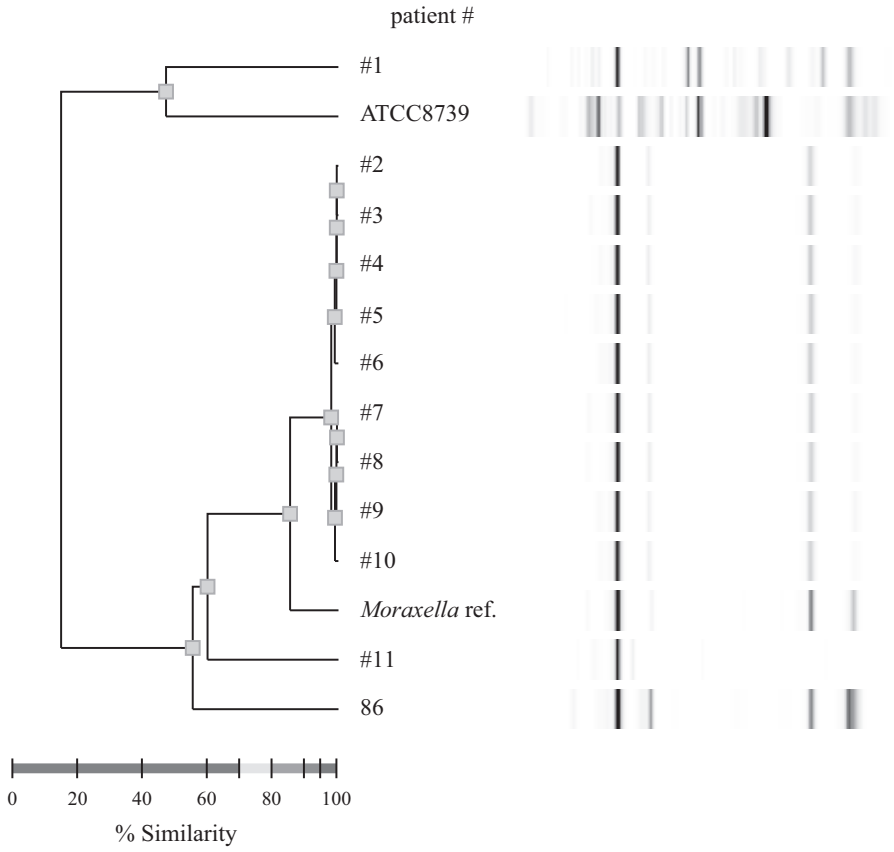

Figure 2. Similarity assessment of the *Moraxella catarrhalis* strains by the DiversiLab technique. The band pattern of random primed polymerase chain reaction fragments separated by gel electrophoresis and a dendrogram of similarity is shown. The percentage of similarity is defined by the DiversiLab software. Similarities above 95% are regarded as indicative for a clonal identity. The patient isolates are demarked by numbers: '86' marks the *M. catarrhalis* strain isolated from a staff member, and 'MC16652' a clinical isolate from the institute's strain collection with no association with the outbreak. As an external reference, the DNA from the *Escherichia coli* ATCC 8739 strain was included in the analysis.

employed to investigate the clonality of the involved isolates. Nevertheless, *M. catarrhalis* outbreaks were thought to be commonplace among paediatric and even adult respiratory patients [10,14,20,21,26,28].

Currently, the introduction of WGS into bacterial typing and the accumulating genome data due to this method allows for a re-evaluation of the statements on nosocomial *M. catarrhalis* incidents. This is even more important when looking at patients such as neurological and traumatological rehabilitation patients with a long uninterrupted history of intense hospital treatment and thus a high cumulative risk of collecting nosocomial infectious agents. In addition, a refined typing method could also help to clarify the role of medical staff as colonised carriers and transmitters for these bacteria.

In this study, both repetitive element palindromic polymerase chain reaction (repPCR) and WGS were employed to analyse the clonal relationship of the *M. catarrhalis* patient and staff isolates. Both methods came up with the same result, i.e. nine patients were colonised or infected by one strain (further addressed as the outbreak strain), while all other persons carried different isolates.

All patients carrying the outbreak strain were located on Ward B. Interestingly, the isolate from patient no. 1 that stimulated the initial warning about a potential hygiene problem turned out to be of different origin.

Yet the true index patient may not have been identified, because in 2018 there were three *M. catarrhalis*-positive patients on Ward B before strains were collected for the molecular analysis. Also, there were more of them in the years 2016 and 2017.

When trying to elucidate the route of transmission, direct contacts between mobile patients and/or healthcare workers have to be considered. In addition, *M. catarrhalis* displays some degree of tenacity, allowing for indirect contact transmission via contaminated fomites. When using settle plates for environmental sampling, Ikram et al. could demonstrate *M. catarrhalis* in the vicinity of 20% of their patients [10].

The medical chart analysis of our patients positive for the *M. catarrhalis* outbreak strain did not identify a specific room, person, device or therapy common to all of them, ruling out a single responsible factor as the cause for transmission. Although every staff member with at least occasional contact with these patients could be contacted and consented to a screening test, no asymptomatic upper respiratory tract or skin carrier of the outbreak strain could be identified among them.

Yet, contact transmission by staff members could not be formally ruled out, because hand surfaces were not part of the screening program. Instead this study focused on the forearms, because hands are frequently disinfected while forearms are not, but may be contaminated during the same procedures that

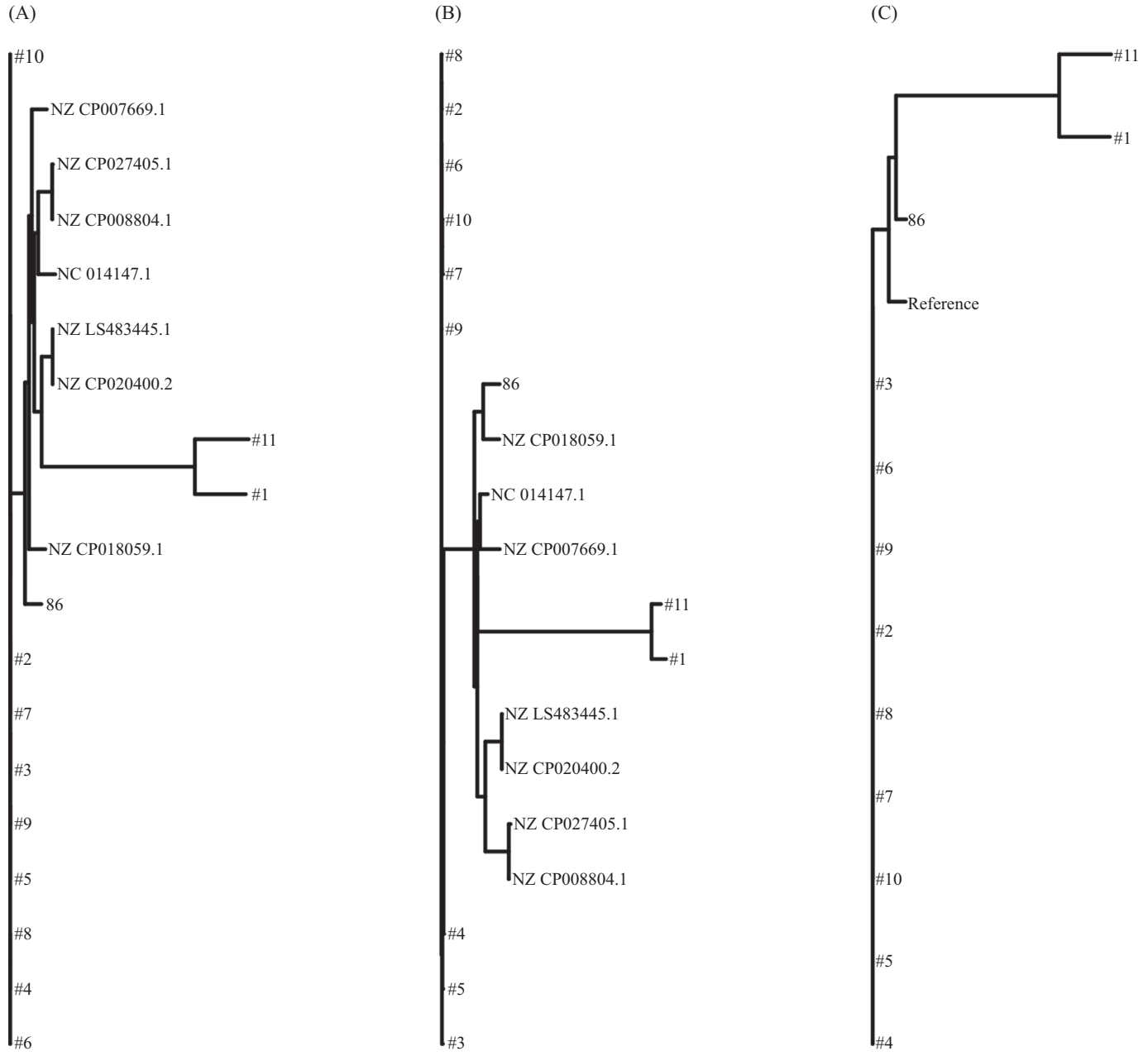

Figure 3. Similarity assessment of the *M. catarrhalis* strains by whole-genome sequencing. Cladograms of the core genes (a) and accessory genes (b) in the obtained *M. catarrhalis* pangenome and in addition from single nucleotide polymorphisms (c) between the novel genomes and the *M. catarrhalis* strain CCRI-195ME (NCBI accession CP18095) are shown. The patient isolates are marked by numbers: '86' indicates the *M. catarrhalis* strain isolated from a staff member. The reference genome sequences denoted by the initials NZ and NC are listed in Supplementary Table S3.

contaminate hands. Overall, the present study cannot contribute to former data on a potential role of healthcare workers in the transmission chain [14,22].

Contrary to the findings of Ikram et al., no surface or device in the patients' environment was contaminated with *M. catarrhalis* [10]. This discrepancy could be due to the different methods used, i.e. air sampling vs. swabs.

Although acute rehabilitation patients were randomly assigned to one of the three specific wards, all patients of the outbreak cohort were cared for in Ward B. Therefore, an obvious explanation points towards individual failures in hygiene management of staff members exclusively working in that ward. Potentially, the presence of the majority of patients in the ward-specific lounge, their at least occasionally unattended movements in this room, and their usage of the TV remote control (in spite of the environmental sampling results) could have contributed to the bacteria's distribution once they were established in some patients.

The bundle of measures finally installed was sufficient to overcome the outbreak. Because the intensified screening of patients and disinfection of surfaces was terminated after the outbreak ceased, and the specific *M. catarrhalis* outbreak

strain was not isolated in the following year, the repeated teaching of hygiene measures plus the psychological impression of the outbreak situation apparently has a lasting effect on the hygienic discipline of all involved staff members.

In conclusion, the present study describes an *M. catarrhalis* outbreak in a specific entity of high-risk patients in front of an increasing prevalence of this species in the specialized rehabilitation centre. The molecular analysis reveals a more complex situation than the simple phenotypic identification and resistance testing of *M. catarrhalis* would have suggested. In spite of combining detailed epidemiological, environmental sampling and molecular analyses, neither the index patient nor the route of transmission could clearly be identified. Yet, the outbreak was successfully ended. As a consequence, *M. catarrhalis* isolates will now be stored for 12 months in our lab to enable a successful workup of potential future outbreak situations.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Jana Normann and the support in sample and data collection of the hygiene specialists Angela Stassewski and Jana Stoll. Purchase of the Illumina MiSeq was kindly supported by the EU-EFRE (European Funds for Regional Development, grant no UHROM 10) programme and funds from the University Medicine Rostock awarded to B.K.

Author contributions

P.W. initiated the study, organized the microbiological and epidemiological evaluation and contributed to the manuscript, T.K. performed the epidemiological analysis, B.K. the WGS, I.B. the bioinformatics analysis, H.M. the patient and staff diagnostics and evaluation of clinical data, and A.P. the environmental sampling. In addition, A.P. he supervised the outbreak management and wrote the manuscript.

Conflict of interest statement
None.

Funding sources
None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2019.04.019.

References

[1] Aebi C. Moraxella catarrhalis — pathogen or commensal? Adv Exp Med Biol 2011;697:107—16.
[2] De Boeck I, Wittouck S, Wuyts S, Oerlemans EFM, van den Broek MFL, Vandenheuvel D, et al. Comparing the healthy nose and nasopharynx microbiota reveals continuity as well as niche-specificity. Front Microbiol 2017;8:2372.
[3] Blakeway LV, Tan A, Peak IRA. Seib KL Virulence determinants of *Moraxella catarrhalis*: distribution and considerations for vaccine development. Microbiology 2017;163:1371—84.
[4] Paulsson M, Riesbeck K. How bacteria hack the matrix and dodge the bullets of immunity. Eur Respir Rev 2018;27.
[5] Ariza-Prota MA, Pando-Sandoval A, Garcia-Clemente M, Fole-Vazquez D, Casan P. Community-acquired moraxella catarrhalis bacteremic pneumonia: two case reports and review of the literature. Case Rep Pulmonol 2016;2016:5134969.
[6] Leung AKC, Hon KL, Wong AHC. Wong AS Bacterial conjunctivitis in childhood: etiology, clinical manifestations, diagnosis, and management. Recent Pat Inflamm Allergy Drug Discov 2018; 12:120—7.
[7] Ngo CC, Massa HM, Thornton RB, Cripps AW. Predominant bacteria detected from the middle ear fluid of children experiencing otitis media: a systematic review. PLoS One 2016;11:e0150949.
[8] Sethi S. Murphy TF Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med 2008; 359:2355—65.
[9] Ejlertsen T, Thisted E, Ebbesen F, Olesen B, Renneberg J. *Branhamella catarrhalis* in children and adults. A study of prevalence, time of colonisation, and association with upper and lower respiratory tract infections. J Infect 1994;29:23—31.
[10] Ikram RB, Nixon M, Aitken J, Wells E. A prospective study of isolation of *Moraxella catarrhalis* in a hospital during the winter months. J Hosp Infect 1993;25:7—14.
[11] Jacoby P, Carville KS, Hall G, Riley TV, Bowman J, Leach AJ, et al. Crowding and other strong predictors of upper respiratory tract carriage of otitis media-related bacteria in Australian Aboriginal and non-Aboriginal children. Pediatr Infect Dis J 2011;30:480—5.
[12] Levy F, Leman SC, Sarubbi FA. Walker ES Nosocomial transmission clusters and risk factors in Moraxella catarrhalis. Epidemiol Infect 2009;137:581—90.
[13] Masaki H, Qin L, Zhou Z, Onizuka T, Watanabe K, Hu B, et al. A prospective study of intrafamilial transmission and antimicrobial susceptibility of Moraxella catarrhalis. Microbiol Immunol 2011;55:599—604.
[14] Patterson TF, Patterson JE, Masecar BL, Barden GE, Hierholzer Jr WJ. Zervos MJ A nosocomial outbreak of Branhamella catarrhalis confirmed by restriction endonuclease analysis. J Infect Dis 1988;157:996—1001.
[15] Thors V, Morales-Aza B, Pidwill G, Vipond I, Muir P. Finn A Population density profiles of nasopharyngeal carriage of 5 bacterial species in pre-school children measured using quantitative PCR offer potential insights into the dynamics of transmission. Hum Vaccin Immunother 2016;12:375—82.
[16] Watanabe H, Hoshino K, Sugita R, Asoh N, Guio H, Qin L, et al. Molecular analysis of intrafamiliar transmission of Moraxella catarrhalis. Int J Med Microbiol 2005;295:187—91.
[17] Bootsma HJ, van der Heide HG, van de Pas S, Schouls LM. Mooi FR Analysis of Moraxella catarrhalis by DNA typing: evidence for a distinct subpopulation associated with virulence traits. J Infect Dis 2000;181:1376—87.
[18] Davie JJ, Earl J, de Vries SP, Ahmed A, Hu FZ, Bootsma HJ, et al. Comparative analysis and supragenome modeling of twelve Moraxella catarrhalis clinical isolates. BMC Genomics 2011;12:70.
[19] Masaki H, Asoh N, Kawazoe K, Watanabe K, Onizuka T, Shimogama S, et al. Possible relationship of PFGE patterns of Moraxella catarrhalis between hospital- and community-acquired respiratory infections in a community hospital. Microbiol Immunol 2003;47:379—85.
[20] McKenzie H, Morgan MG, Jordens JZ, Enright MC, Bain M. Characterisation of hospital isolates of *Moraxella (Branhamella) catarrhalis* by SDS-PAGE of whole-cell proteins, immunoblotting and restriction-endonuclease analysis. J Med Microbiol 1992; 37:70—6.
[21] Picard B, Goullet P, Denamur E, Suermondt G. Esterase electrophoresis: a molecular tool for studying the epidemiology of *Branhamella catarrhalis* nosocomial infection. Epidemiol Infect 1989;103:547—54.
[22] Qin L, Masaki H, Gotoh K, Furumoto A, Terada M, Watanabe K, et al. Molecular epidemiological study of *Moraxella catarrhalis*

isolated from nosocomial respiratory infection patients in a community hospital in Japan. Intern Med 2009;48:797—803.

[23] Roberts MC, Pang YJ, Spencer RC, Winstanley TG, Brown BA, Wallace Jr RJ. Tetracycline resistance in *Moraxella (Branhamella) catarrhalis*: demonstration of two clonal outbreaks by using pulsed-field gel electrophoresis. Antimicrob Agents Chemother 1991;35:2453—5.

[24] Verhaegh SJ, Streefland A, Dewnarain JK, Farrell DJ, van Belkum A. Hays JP Age-related genotypic and phenotypic differences in *Moraxella catarrhalis* isolates from children and adults presenting with respiratory disease in 2001-2002. Microbiology 2008;154:1178—84.

[25] Tan A, Blakeway LV, Bakaletz LO, Boitano M, Clark TA, Korlach J, et al. Complete genome sequence of *Moraxella catarrhalis* Strain CCRI-195ME, isolated from the middle ear. Genome Announc 2017;5.

[26] Matlow AG, Low DE, Paret G, Jarrett S, Bohn D, Barker G, et al. Molecular epidemiological characterization of respiratory isolates of *Moraxella catarrhalis* in a pediatric intensive care unit. Can J Infect Dis 1992;3:189—92.

[27] Morgan MG, McKenzie H, Enright MC, Bain M, Emmanuel FX. Use of molecular methods to characterize *Moraxella catarrhalis* strains in a suspected outbreak of nosocomial infection. Eur J Clin Microbiol Infect Dis 1992;11:305—12.

[28] Richards SJ, Greening AP, Enright MC, Morgan MG, McKenzie H. Outbreak of *Moraxella catarrhalis* in a respiratory unit. Thorax 1993;48:91—2.

[29] Warnke P, Harnack T, Ottl P, Kundt G. Podbielski A Nasal screening for Staphylococcus aureus—daily routine with improvement potentials. PLoS One 2014;9:e89667.

[30] Ahmad F, McLeod DT, Power JT, Calder MA. *Branhamella catarrhalis* prevalence in a hospital population. J Hosp Infect 1985;6:71—4.