Diagnostic Microbiology and Infectious Disease 88 (2017) 82–87

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

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

Improvement of infection control management by routine molecular evaluation of pathogen clusters

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ARTICLE INFO

Article history:
Received 23 November 2016
Received in revised form 20 January 2017
Accepted 23 January 2017
Available online 26 January 2017

Keywords:
Multidrug-resistance
Infection control
Hospital hygiene
Healthcare-associated infections
Molecular typing

ABSTRACT

Background: Undetected pathogen clusters can often be a source of spreading in-hospital infections. Unfortunately, detection of clusters can be problematic because epidemiological connection is not always easily established. Infection prevention and control (IPC) measures, however, are most effective when applied at the earliest possible stage.

Aim: The goal of our study was to evaluate the benefits of routine use of molecular typing techniques for IPC management in a large University teaching hospital.

Methods: We implemented daily routine molecular typing of pathogen clusters using cost-effective standard methods such as random amplified polymorphic DNA PCR, multiple-locus variable number tandem repeat analysis, and *spa*-typing over a 4-year study period (2012–2015).

Findings: Four pathogen clusters were evaluated: (I) 14 cases of *Clostridium difficile* in a peripheral ward, (II) 17 cases of methicillin-resistant *Staphylococcus aureus* (MRSA) in two intensive-care units (ICUs), (III) 21 cases of multidrug-resistant *Klebsiella pneumoniae* within one department, and (IV) 6 cases of vancomycin-resistant *Enterococcus faecium* in an interdisciplinary ICU. Typing revealed that cluster I was not caused by an outbreak strain but was likely due to different endogenous infections. Clusters III and IV showed a classical space–time clustering of point source outbreaks. Cluster II represented a prolonged temporal cluster, which would have gone undetected without molecular typing because of large intercase intervals.

Conclusion: Implementing daily routine molecular typing is effective for detecting and analyzing pathogen clusters. Falsely suspected outbreaks can be quickly resolved, whereas actual outbreaks can be identified faster, so that targeted IPC measures can be applied earlier.

1. Introduction

Healthcare-associated infections (HAI) and multidrug-resistant organisms (MDRO) are a major cause of excess patient morbidity and mortality, which cause significant added expenses for hospitals (Chen et al., 2005; De Angelis et al., 2010; Kubler et al., 2012; Sydnor and Perl, 2011). Undetected pathogen clusters may lead to outbreaks that are difficult to contain. However, clusters often remain undetected because the epidemiological connection cannot be easily established owing to many reasons, including frequent intra-hospital transfer of patients or failure of laboratory surveillance. In the latter case, microbiological detection of phenotypically similar pathogens in different patients may not be considered epidemiologically connected because samples are sent from different wards or specialties. Infection prevention and control (IPC) measures, however, are most cost-effective when applied at the earliest possible stage. Timely use of IPC measures minimizes pathogen transmission and ensures adequate care and patient safety despite limited resources. Only early identification of potential clusters enables the IPC team to apply timely intervention strategies aimed at preventing or containing nosocomial infections and transmission of pathogens. Integrating molecular typing of pathogens into the everyday laboratory routine provides a tool for early cluster detection and, therefore, allows quick, targeted interventions. As a result, mortality and morbidity due to HAI may be considerably reduced. Additionally, molecular typing can help to prevent erroneous outbreak detections and, therefore, avoid cost-intensive IPC measures. More advanced methods such as whole-genome sequencing (WGS) can also be used to detect outbreak strains (Zhou et al., 2016). Those methods, however, are currently cost- and labor-intensive, as well as more time-consuming. Thus, their implementation is not possible in all settings owing to financial issues. In addition, such methods often are not considered as first-choice because of long waiting times for results.

It is of major importance to know the endemic situation in your hospital to be able to detect variations in incidences or a sudden emergence of an uncommon lineage. We therefore implemented daily molecular typing of pathogens in our laboratory using cost-effective standard methods such as random amplified polymorphic DNA PCR (RAPD PCR), multiple-locus variable number tandem repeat analysis (MLVA), and *spa*-typing. However, identification of transmission by using only

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http://dx.doi.org/10.1016/j.diagmicrobio.2017.01.013
0732-8893/

one typing method for specific microorganisms is insufficient to truly assess transmission events (van den Broek et al., 2009). We therefore used a second downstream method with high discriminatory power, i.e. pulsed-field gel electrophoresis (PFGE), as confirmation. We applied the typing system to all suspected pathogen clusters in our hospital and *spa*-typed every methicillin-resistant *Staphylococcus aureus* (MRSA) strain, which was detected either during the admission screening or in clinical materials. The goal of our study was to evaluate the benefits and costs of molecular typing available on a daily basis for IPC management in a large University teaching hospital.

Four major pathogen clusters have been detected and evaluated within a 4-year study period (2012–2015). The four clusters comprised (I) 14 cases of *Clostridium difficile* (Cd) in a peripheral ward, (II) 17 cases of MRSA in two intensive care units (ICUs), (III) 21 cases of multidrug-resistant (MDR) *Klebsiella pneumoniae* (Kp) in two different wards, and (IV) 6 cases of vancomycin-resistant *Enterococcus faecium* (VRE) in an interdisciplinary ICU.

2. Material and methods

2.1. Study site and population

The study was conducted at the Heidelberg University Hospital (HUH), a 2200-bed tertiary care University teaching hospital and one of the largest and most renowned hospitals in Germany. HUH provides a full range of medical and surgical services, including active programs for solid organ and bone marrow transplantation. Laboratory surveillance data such as microbiological screening reports and results of diagnostic samples were routinely collected. If a pathogen cluster was suspected, strains were recovered and used for typing. Using a laboratory information system (Swisslab, Roche Diagnostic IT solutions, Berlin, Germany) based algorithm, a possible pathogen cluster was automatically reported when ≥2 isolates were detected in screening or diagnostic samples within the same clinical department (i.e. department of internal medicine, department of surgery etc.) at least 48 h after admission of the patients. The IPC team assessed the alert reports on a daily basis and isolates were typed if an epidemiological connection was suspected (i.e. temporal overlap of the patients on the same ward or procedure room etc.). Additionally, for the assessment of the endemic MRSA background at our hospital, every newly detected MRSA isolate in screening or diagnostic samples during the study period was typed.

2.2. Microbiological methods

Screening swab samples and diagnostic samples were inoculated on suitable media and incubated under aerobic or anaerobic conditions, as appropriate, for 48 h (aerobic microorganisms) or 72 h (anaerobic microorganisms) at 36 °C. If growth on plates was detected, identification of microorganisms was performed by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany) as described elsewhere (Eigner et al., 2009). Susceptibility testing was performed using VITEK2 (Biomérieux, Nuertingen, Germany) or MIC test strips (Liofilchem, Piane Romano, Italy), respectively, and the results were interpreted according to the EUCAST clinical breakpoints.

2.3. Molecular methods

Every newly detected MRSA isolate in screening or diagnostic samples during the study period underwent *spa* typing as described elsewhere (Harmsen et al., 2003). Additionally, for cluster evaluations, *spa* typing was confirmed by PFGE following treatment with the restriction enzyme Sma1. *Cd* isolates were typed by RAPD PCR with the primers AP4 and AP5 as described elsewhere (Barbut et al., 1993), and the results were confirmed by standard ribotyping. VRE isolates were typed by MLVA as described elsewhere (MLVA for *Enterococcus faecium*, UMC Utrecht. Website: http://www.umcutrecht.nl/en/Research/Miscellaneous/MLVA-typing). PFGE performed with the restriction enzyme SmaI was used for confirmation of VRE typing results. Kp isolates were typed using RAPD-PCR with the primers 208 and 272 as described elsewhere (Mahenthiralingam et al., 1996). PFGE performed after the digestion of chromosomal DNA by SpeI was used for the confirmation of Kp typing results. PFGE results were analyzed according to the criteria defined by Tenover et al. (1995).

2.4. Statistics

For descriptive purposes, arithmetic mean value, standard deviation, median, interquartile range, and cumulative frequencies were calculated as appropriate. All statistical comparisons were conducted with a significance level of 0.05. Statistical analysis was performed using the SPSS statistical package (SPSS v. 21.0, Chicago, IL).

2.5. Ethical considerations

All isolated strains were collected during routine sampling. The current study only described a collection of bacteria that comprised strains obtained from patients and additional environmental isolates. Data collected from patients were anonymized and restricted to the information about possible clinical symptoms of infection and length of stay at HUH. Ethical approval was therefore not required.

3. Results

During one year of the study period, 192 MRSA, 204 MDR *K. pneumoniae* and 705 VRE isolates were detected in screening or diagnostic samples. In total, 270 isolates were typed; details are depicted in Table 1. The costs for typing totaled €5439, €1297 and €1959 per year for MRSA, MDR *K. pneumonia*, VRE, respectively (Table 1).

3.1. Cluster I: C. difficile (space–time clustering)

Fifteen Cd isolates from 15 different patients of a peripheral ward were collected between May and July 2014 (Fig. 1). The rate of Cd infections (CDI) in that ward was much higher than the average rate for the previous months, which suggested a Cd outbreak. The mean time interval between detection of infections in individual patients was 3 days (standard deviation [SD] ± 3.9) with a minimum detection interval of 0 days and a maximum interval of 12 days. All cases were primarily diagnosed by symptomatic diarrhea and presence of Cd toxin DNA in stool samples. Each diagnosis was verified by additional culturing of toxigenic Cd isolates obtained from the stool sample. Infection control interventions were implemented immediately after detection of the accumulation in May 2014. The primary intervention comprised the mandatory use of specific sporicidal surface disinfectants, enforcement of hygiene practices aimed at prevention of spore-forming pathogen transmission, and stronger thermal disinfection of bedpans. Nevertheless, new CDI cases were detected even after these infection control measures had been implemented.

Thus, the Cd isolates were rapidly typed by RAPD-PCR, which revealed two clusters consisting of two and three isolates, respectively, as well as ten isolates with unique RAPD-profiles. These typing results were confirmed by ribotyping, which revealed that the accumulation was not caused by a single strain (Fig. 1). These results suggested that those cases occurred as unique endogenous Cd infection episodes rather than because of the initially suspected spread by patient-to-patient transmission. In this context, the perioperative antibiotic regimes of the department were reviewed, and it was revealed that patients were administered an incorrectly prolonged (2–3 days) post-operative prophylactic antibiosis with Ceftriaxon intravenously, which probably caused the accumulation. After the antibiotic stewardship team had