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The washing machine as a reservoir for transmission of extended spectrum beta-lactamase (CTX-M-15)-producing *Klebsiella oxytoca* ST201 in newborns

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Abstract

During the time period from April 2012 to May 2013, 13 newborns (1 − 4 weeks old) and one child in a paediatric hospital ward in Germany were colonized with an extended spectrum betalactamase (ESBL) producing *Klebsiella oxytoca* (CTX-M-15).

A microbiological source-tracking analysis, including human and environmental samples, was carried out to identify the source and transmission pathways of the *K. oxytoca* clone. In addition, different hygienic intervention methods were evaluated.

Isolates of the *K. oxytoca* strains were detected in the detergent drawer and on the rubber door seal of a domestic washer-extractor machine, used in the same ward to wash laundry of the newborns as well as in two sinks. These strains were typed using PFGE and multi-locus sequence typing (MLST). The environmental findings were compared with those of the human strains and the isolates detected on the clothing. The results showed that the strains were identical using both techniques, (ST201 and pulse-field-gel-electrophoresis [PFGE] type-00531, a clone specific to this hospital and not previously isolated in Germany), emphasizing the washing machine as a reservoir and fomite for the transmission of these multidrug-resistant bacteria (MDR). After the washing machine had been taken out of use, no further colonizations were detected over a following 4-year period.

Importance

Washing machines should be further investigated as possible sites for horizontal gene transfer (ESBL-genes) and cross-contamination of clinically important Gram-negative strains. Particularly in the healthcare sector, the knowledge of possible (re-) contamination of laundry (patients' clothes, staff uniforms) with multidrug-resistant Gram-negative

bacteria could help to prevent and control nosocomial infections. This report describes an outbreak with a single strain of a multidrug-resistant bacterium (*Klebsiella oxytoca* ST201) in a neonatal intensive care unit, that was only terminated when the washing machine was removed. In addition, the study implies that changes in washing machine design and processing are required to prevent accumulation of residual water where microbial growth can occur and contaminate clothes.

Keywords

ESBL-bacteria, *Klebsiella oxytoca*, ST201, washing machine, laundry, colonization, newborns

Introduction

Water or wastewater contaminated with facultative pathogenic microorganisms provides a potential reservoir for infections (1-3). The most common bacteria causing healthcare-associated infections or permanently colonizing reservoirs linked to contaminated hospital water in the environment of patients are Gram-negative bacteria including *Pseudomonas* spp., *Enterobacter* spp., *Serratia* spp., *Stenotrophomonas* spp. and *Klebsiella* spp. (1, 2, 4). Such water reservoirs in healthcare settings include faucets, sink surfaces, bathtubs, wastewater drainage system drains, sinks, showers, and toilets (4, 5). Persisting and especially multidrug-resistant bacteria in those reservoirs pose a severe risk especially for high-risk patients, including severely immunocompromised patients, newborns (1-4 weeks old) and infants (6–8 weeks old) in neonatal intensive care units (ICU) (4, 6-10). For example, Leitner et al. (11) described an outbreak of six infections in patients with hematological malignancies with a KPC-2-producing *K. oxytoca* most commonly linked to contaminated water reservoirs. In these outbreaks, the identification of the contaminated environmental reservoir can be challenging and transmission pathways have been elucidated in only a few clinical studies (6, 12, 13).

Currently, washing machines and clothing are not assessed as potential reservoirs in outbreaks of nosocomial infections, despite evidence that a potential health risk due to contaminated laundry cannot be excluded (14-16). Recently Rehberg et al. (17), described the presence of antibiotic-resistant bacteria (ARB) and their possible transmission via washing machines.

Case description

Between April 2012 and May 2013, increased rates of colonization with *K. oxytoca* isolates, were recorded in a level 1 perinatal centre (PNC) and in several wards in the connected children's hospital (90 stationary beds, 20 ICU beds) located in western Germany. The colonizations had been noticed after the implementation of a standard screening procedure of incoming and/or patients with risk factors (newborns, children, mothers) as general control mechanism aimed at reducing the spread of multidrug-resistant bacteria. Screening included anal (newborns, children, mothers), vaginal (mothers) and in some cases additional wound (newborns, children) swabs.

Material and Methods

Investigation strategy

When the first colonizations had been detected in April 2012, an initial management strategy to analyse the cases and identify possible sources of the *K. oxytoca* strain was developed: Three major sampling events of all newborns, children and mothers at the distinct wards took place during the reported time period, following a standard screening procedure.

In March 2013 sampling of specific risk areas (water/wastewater reservoirs) was performed and ESBL-screening (anal, wounds) of all healthcare workers (physicians, nurses, and cleaning personal working in the PNC, the ICU, and the Obstetrics Department) was conducted to identify possible carriers.

In June 2013, a further sampling was conducted, using native samples (drinking and wastewater) and liquid medium swabs (eswabs).

Laboratory analyses

Microbiological cultivation

111 Human and environmental samples were collected and stored at 4 °C during transport to
112 the laboratories and processed within 48 hours.
113 All human samples (anal, vaginal and wound swabs) were streaked onto MacConkey agar
114 plates (Oxoid Deutschland GmbH, Wesel, Germany) with a 10 µg imipenem disk
115 (bioMérieux SA, Marcy-Étoile, France) and selective agar plates, e.g. CHROMagarESBL
116 (Oxoid Deutschland GmbH, Wesel, Germany).
117 All Oxoid RODAC™ contact plates (∅ 55 mm, Becton Dickinson, Heidelberg, Germany)
118 were used for environmental surface sampling and incubated at 36 ± 1 °C for 48 hours.
119 All environmental liquid medium swabs (eSwab; Copan Diagnostics, Italy) were streaked
120 on Columbia agar plates containing 5 % sheep blood (Becton Dickinson), MacConkey
121 agar plates (Oxoid Deutschland GmbH, # PO5002A), and selective agar plates, e.g.
122 CHROMagar™ ESBL (bioMérieux SA, Marcy-Étoile, France; # 43481) and incubated at
123 37 ± 1 °C for 48 hours. In addition, casein soy flour peptone broth (CASO-broth) (Merck
124 KGaA, Darmstadt, Germany) was inoculated with the same swabs and incubated at 37 ±
125 1 °C for 24 hours.
126 The disinfectant wipes, socks, and knitted hats of the newborns were put into 200 ml
127 CASO-broth with tween saponin histidine cysteine (TSHC +3% Tween Merck, # 8.22187;
128 +3 % saponin, Roth, # 5185.1; +0.1% histidine Merck, # 1.04351; +0.1 % cysteine, Merck,
129 #. 1.02839) and homogenised for 60 seconds (Stomacher 400, Seward Limited, West
130 Sussex, United Kingdom). First, 2 x 100 ml of the extract was filtered (membrane-filter
131 Millipore Hydrosol, 250 ml cup) followed by the filtration of 100 ml 0.9 % NaCl to rinse the
132 additives off the filter. The filter was then placed on CHROMagar™ ESBL and MacConkey
133 agar plates and incubated at 37 °C for 24 – 48 hours. In addition, 100 µl of each
134 concentration of a dilution series in 0.9 % NaCl was plated on CHROMagar™ ESBL and

135 MacConkey agar plates. The emulsions were treated in the same manner as the wipes,
136 except that shredding was not necessary.
137 For the water samples, approximately 100 ml were collected in a sterile polystyrene cup
138 (# 225170, Greiner, Frickenhausen, Germany) and filtered hrough a sterile nitrocellulose
139 membrane filter (pore size 0.45 ± 0.02 µm, Ø 47 mm, black grid from Millipore, # EZHAWG
140 474), according to Schulz and Hartung (18). After filtration, the membrane was placed on
141 selective CHROMagar™ ESBL and MacConkey agar plates.
142 In addition, 100 µl of each dilution of a dilution series in 0.9 % NaCl was plated on
143 CHROMagar™ ESBL and MacConkey agar.
144 *Identification and susceptibility testing of ARB*
145 All *Enterobacterales* detected on CHROMagar™ ESBL were identified via API 20 E V4.1
146 apiweb™ (bioMérieux SA, Marcy-Étoile, France) and matrix-assisted laser
147 desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS, bioMérieux SA,
148 Marcy l´Etoile, France) using Myla® software, VITEK® MS-CHCA matrix (#411 071,
149 bioMérieux) and disposable targets (# 410 893, bioMérieux).
150 Susceptibility to antibiotics was tested using the Micronaut-S MDR MRGN-Screening 3
151 system (MERLIN, Gesellschaft für mikrobiologische Diagnostika GmbH, Bornheim-
152 Hersel, Germany) and results were interpreted using EUCAST criteria.
153 *Testing of disinfectant tolerance*
154 To detect a possibly increased tolerance of the *K. oxytoca* isolates towards disinfectants,
155 surface disinfectant and the disinfecting general-purpose detergent which had been used
156 in the washing machines were both examined according to the regulations (7 and 9) of
157 the Disinfectant Commission of the Verbund für Angewandte Hygiene e.V. (VAH) (19).
158 The VAH method 7 was used to determine the minimal inhibitory concentration of the

environmental *K. oxytoca* strains in comparison to a Gram-negative reference strain *Pseudomonas aeruginosa* DSM 939 (20). The VAH method 9 respectively EN 13727 is a quantitative suspension test which was used to identify efficacy gaps, taking into account the concentration-time-relation, interfering substances and temperature of the disinfectants (21-23). The surface disinfectant used was based on quaternary ammonium compounds and alkylamine (formulation: didecyldimethylammoniumchlorid 12.5 g/100 g; bis-(aminopropyl)laurylamine 1.5 g/100 g). The laundry disinfectant was based on peracetic acid (formulation: 40 – 80 ppm peracetic acid; 2.5 – 5 g/l).

Molecular resistance characterization

Molecular characterization of ESBL-genes

Possible ESBL-production was confirmed by Polymerase Chain Reaction (PCR). Five specific primer sets were used to detect β-lactamase-encoding genes belonging to the *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ families (24-27). The PCR products were visualised by gel electrophoresis on a 1 % agarose / TBE gel and stained with Midori Green (Biozym Scientific GmbH, Hessisch Oldendorf, Germany). The resulting amplicons were purified using an innuPREP DOUBLEpure Kit (Analytik Jena AG, Jena, Germany) according to the manufacturer's recommendations. Custom-sequencing was performed by Microsynth (Göttingen, Germany). The nucleotide sequences were analyzed using Chromas 2.6.5.

Plasmid characterization (Inc-typing)

To distinguish the Inc-type of possible plasmids, two to three colonies of a fresh culture of all *K. oxytoca* isolates were resuspended in 100 µl nuclease free water, heated to 95 °C for 10 min and centrifuged at 14,000 g for 5 min. The supernatant was carefully removed and used for PCR. The PCR mixture contained 2 x OneTaq Master mix (New England Biolabs) and 0.2 µM of each primer (Table 1) (28-30).

183 The PCR conditions were set on 94 °C for 3 min, followed by 30 cycles at 94 °C for 30 s,
184 60 °C for 30 s and 68 °C for 1 min. The final extension was performed at 68 °C for 5 min.
185 The PCR products were analyzed using agarose gelelectrophoresis on a 1 %
186 agarose/TBE gel, and stained with Midori Green (Biozym Scientific GmbH, Hessisch
187 Oldendorf, Germany).

188 *Plasmid conjugation testing*

189 The possibility of plasmid transfer via conjugation was analyzed. All *K. oxytoca* as well as
190 wild type *E. coli* strains were streaked onto LB-agar containing a) 20 mg/l chloramphenicol
191 and b) 20 mg/l chloramphenicol and 4 mg/l cefotaxime. *E. coli* strains that grew on LB-
192 agar containing only chloramphenicol, but not on LB-agar containing both
193 chloramphenicol and cefotaxime, were used for the following conjugation assay. For the
194 pre-culture one colony of a fresh culture of all isolated *K. oxytoca* strains as donors and
195 different wild type *E. coli* strains (recipients) were used for inoculation of 5 ml lysogeny
196 broth (LB) and incubated at 37 °C overnight with shaking. Next, 5 ml fresh LB was
197 inoculated with 100 µl of the donor (*K. oxytoca*) and 1 ml of the recipient (*E. coli*) and
198 incubated at 37 °C for overnight with shaking. After incubation 1 ml of each culture was
199 centrifuged at 14.000 rpm for 3 min. Afterwards the supernatant was discarded and the
200 pellet was resuspended using 100 µl Milli Q. The whole suspensions, as well as 100 µl of
201 the cultures were plated onto selective LB-agar containing 20 mg/l chloramphenicol and
202 4 mg/l cefotaxime and incubated at 37 °C overnight. Grown colonies were streaked onto
203 Columbia sheep blood agar and analyzed via MALDI TOF-MS.

204 *Molecular typing*

205 All *K. oxytoca* isolated from clinical samples and environmental sources were typed by
206 the German National Reference Centre (GNRC) using PFGE according to Tenover et al.

(31) and modified according to Ribot et al. (32). In addition, MLST of all *K. oxytoca* strains was conducted. Briefly, bacteria were cultivated on Columbia blood agar plates at 35 °C overnight. After resuspension of two to three colonies in 80 µl PCR water, the samples were heated to 95 °C for 5 min and centrifuged at 14,000 g for 5 min. The supernatant was used for PCR according to the protocol described by Herzog et al. (33). The PCR products were purified by extraction from a 1 % agarose gel using the GeneJET Gel Extraction Kit (Fisher Scientific GmbH, Schwerte, Germany) according to the manufacture´s recommendations and sequenced by GATC Biotech AG (Konstanz, Germany). Assignment to the sequence type (ST) was performed via the specific MLST website (https://pubmlst.org/koxytoca/).

Results

Colonization with K. oxytoca in humans

Within 14 months, 27 children were colonized (but not infected) with *K. oxytoca* according to CDC definitions (34). The occurrence of *K. oxytoca* varied from sensitive isolates with no ESBL -activity to ESBL-producing *K. oxytoca* and the particular ESBL-producing *K. oxytoca* PFGE type-00531. Fourteen children were tested positive for the ESBL-*K. oxytoca* PFGE type-00531.

K. oxytoca was mostly identified in rectal swab screening samples. In 9 out of 24 newborns *K. oxytoca* was also detected in throat swap samples. As an exception, a 4-year-old boy in the ICU, who never had any direct contact with the PNC, also showed rectal colonization with PFGE type-00531/ST201 ESBL-*K. oxytoca* in March 2013.

All ESBL-producing *K. oxytoca* strains were resistant to piperacillin/tazobactam, with a minimum inhibitory concentration (MIC) up to > 64/4 mg/l, to cefotaxime (MIC ≥ 64 mg/l),

ceftazidime (MIC > 128 mg/l) and ciprofloxacin (ranging from MIC 2 to >2 mg/l) and can be classified as multidrug-resistant according to Magiorakos et al. (35). Figure 1 summarises the occurrence of *K. oxytoca* isolates over time in the PNC, ICU, and on four different wards.

Until October 2012, only newborns in the PNC or infants in the ICU were colonized with ESBL-*K. oxytoca,* some of which belonged to PFGE type-00531/ST201 (Figure 1). From the end of October 2012 to February 2013, *K. oxytoca* was also detected on other wards and on older paediatric patients (see above).

During an extended screening of mothers and healthcare workers, a total of 695 swabs (vaginal and rectal) from 428 persons were obtained on the Obstetrics Ward. The screening identified four mothers colonized with *K. oxytoca,* five mothers colonized with ESBL-producing *E. coli*, and one with ESBL-producing *K. pneumoniae.* None was positive for the special PFGE type-00531 ESBL-producing *K. oxytoca.* A transmission between mother and newborn was not documented.

Environmental monitoring

All environmental samples taken during the first and second on-site inspections were tested negative for *Enterobacterales* and non-fermenting organisms. During the second on-site sampling in October 2012, only low concentrations of Gram-positive skin and environmental bacteria were present. However, during the third on-site inspection in June 2013, sampling was conducted using native samples (drinking and wastewater) and liquid medium swabs (eswabs). Thus, *Enterobacterales* as well as non-fermenting bacteria were detected. All *K. oxytoca* isolates were identical ESBL-producing strains and belonged to

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PFGE type-00531 (Table 2). In the following these *K. oxytoca* strains are termed "environmental".

Water-associated bacteria such as *P. aeruginosa, Serratia* spp., *Enterobacter* spp., *K. pneumoniae,* and *S. maltophilia* were detected in the siphons of hand wash basins. Identical clones of *K. oxytoca* PFGE type-00531/ST201 were isolated from the siphons of two sinks in the healthcare workers' staff room and in the room used for cleaning and disinfection. The same clone was also isolated in high concentrations from samples of residual water in the rubber seal and one swab sample (in addition to *P. aeruginosa*) from the detergent compartment of one of the two washing machines (Table 2), located in the ground floor of the same ward.

Following identification of the washing machine as potential reservoir for this specific *K. oxytoca* clone, newborn clothing (hats and socks) that had been washed in this machine were microbiologically analysed. *K. oxytoca* PFGE type-00531/ST201 of the same specific clone was isolated with a total count of > 10^9 CFU / ml.

All ESBL-producing *K. oxytoca* PFGE type-00531/ST201 found in the residual water and environmental samples were also resistant to piperacillin/tazobactam, with a minimum inhibitory concentration (MIC) ranging from 8/4 mg/l up to > 64/4 mg/l, to cefotaxime (MIC > 2 mg/l), ceftazidime (64 mg/l) and ciprofloxacin (MIC ranging from 2 mg/l to > 2 mg/l) (Table 3).

The presence of the ESBL gene CTX-M-15 was confirmed by sequencing in all human and environmental strains. The transfer of putative plasmids that harboured these genes under laboratory conditions into wild type *E. coli* strains via conjugation was not successful. In addition, no amplificates for *inc (A/C, L, HI2, M, I1-αγ, N, FIIk, FIA, FIB, FII)*

277 genes were obtained from the strains indicating that the CTX-M genes might be
278 chromosomally located as described by Rodríguez et al. (36).
279
280 ***Epidemiological links***
281 Retrospective analysis demonstrates that only newborns who had worn clothing that had
282 been washed in the in-house washing machine were colonised with the redundant *K.*
283 *oxytoca* clone. Although the siphons of the staff sinks were also identified as a possible
284 reservoir, no staff members were identified as carriers or spreaders of ESBL-
285 *Enterobacterales* at the time of screening.
286 All clinical and environmental isolates of the *K. oxytoca* PFGE type-00531/ST201
287 displayed an identical PFGE banding pattern and were thus considered clonally identical.
288 This clone was specific for the newborns/infants and some environmental samples.
289
290 ***Evaluation of a possibly increased disinfectant tolerance of the K. oxytoca strains***
291 The environmental *K. oxytoca* isolates showed no increased tolerance to the surface
292 disinfectant based on quaternary ammonium compounds and alkylamine. The minimal
293 inhibitory alkylamine concentration on the *K. oxytoca* strains was identified as max.
294 0.003% and 0.0075% on the reference strain *Pseudomonas aeruginosa*. Furthermore, the
295 examination according to VAH method 9 showed a ≥ 5 $\log_{10}$ CFU reduction of the *K.*
296 *oxytoca* strains at a concentration of 0.5% and 15 min in the presence of interfering
297 substances (dirty conditions – 3,0 g/l bovine serum albumin + 3,0 ml/l sheep erythrocytes).
298 The VAH-recommended concentration and application time is 0.5% over 60 minutes (37).
299 *K. oxytoca* isolates from the environment showed no increased tolerance to the laundry
300 disinfectant based on peracetic acid. The minimal inhibitory concentration of the *K.*

oxytoca strains and the *P. aeruginosa* reference strain was identified as 5 g/l. The analysis according to VAH method 9 (in the presence of 2.75g/l laundry disinfectant with an exposure time of 15 min under dirty conditions at 20°, 40° and 60°C) demonstrated that the *K. oxytoca* strains were more susceptible than the Gram-negative reference strain *P. aeruginosa* DSM 939. At a temperature of 60 °C all *K. oxytoca* strains showed a ≥ 5 $\log_{10}$ reduction.

Hygienic containment measures and control management strategies

A task force was reinforced to manage the control interventions for prevention of the colonization of further patients and to identify possible sources of the suspected *K. oxytoca* strain.

The management procedures can be divided into three main phases until the colonisations were under control and the source could finally be identified. Therefore, during each phase step-by-step approaches with different strategies were necessary in parallel to sampling during the whole report period (Figure 1, Table 4).

Long-term clinical effects/concomitant infection control interventions

After the washing machine had been taken out of use, no further colonization of newborns with *K. oxytoca* has been detected to this day. All garments worn by the newborns and children were laundered by a professional external hospital laundry service after the outbreak. The two colonized sinks were replaced by sinks with specialised thermosiphon systems. As a further consequence of the prolonged cluster process, the existing infection control measures (isolating colonized patients, enforcing hand hygiene measures, and cleaning the ward, particularly the sinks and equipment) were reinforced via extra training.

The screening plan for all newborns and children, and all measures implemented within the containment program, continues to be maintained.

Discussion

Reservoirs and transmission routes of waterborne pathogens in healthcare systems

When the first five cases of colonization with *K. oxytoca* had been detected between August and October 2012, it was suggested to be a person-to-person transmission. Although this has not been reported for *K. oxytoca*, Price et al. (38) showed that, in the presence of standard infection control measures, healthcare workers (HCWs) were frequent sources of *Staphylococcus aureus* transmission. Furthermore, Heudorf et al. (39) mentioned that pathogens classified by the WHO as highest priority can be found on staff gowns, which can be the starting point of transmissions (40).

In this case the occurrence of *K. oxytoca* isolates continued over a 1-year period which indicated a reservoir in the environment rather than hospital personnel or mothers. Despite the strict implementation of control management and containment measures, newborns and children continued to acquire the cluster organism (PFGE type-00531/ST201) which had never been detected before by the GNRC. The single database strain was isolated in the Netherlands in 2018 (41).

Washing machine

In this case, the particular *K. oxytoca* strain was not only detected in two sinks but also in a domestic washing machine with integrated dryer. Even recently published articles reviewing the main water-associated reservoirs in hospitals do not consider the washing

machine as a potential hazard in a clinical environment (4, 5). However, in our report the knitted clothes of patients were washed in an in-house washing machine designed for household use and enabling a washing temperature of 65°C. It has been shown, that resistance genes as well as different microorganisms can persist in domestic washing machines (17, 42).

Recently, a study suggested the potential role of washing machines in the distribution of antibiotic-resistant Gram-negative bacteria during laundering (17); however, until today, no transmission of pathogens from a washing machine to patients could be proven. In this case, we assume that the *K. oxytoca* strain PFGE type-00531/ST201 was disseminated to clothing after the washing process via the residual water on the rubber mantle and/or via the final rinsing process that runs unheated and detergent-free water through the detergent compartment, while the water coming to washing machine met the quality requirements of the hospital's internal water controls. Consequently, we conclude that newborns were colonized by wearing hats and socks that were contaminated by the washing process.

It remains unclear how the washing machine was contaminated. However, *Enterobacterales* can survive in wet environments for an extended period of time (1), especially waterborne bacteria such as *P. aeruginosa* and *Klebsiella* strains, which have the ability to survive in a viable but not culturable state. Similarly, their environmental stability is supported by the formation of biofilms to enhance their chances of multiplication and horizontal gene transfer (43). Zhang et al. (44) discusses that biofilm detachment is promoted by disinfectants and thus affects the overall bacterial antibiotic resistance of microbes in tap water.

372 Furthermore, Rehberg et al. (17) demonstrated that ARB can survive the washing
373 process. In tests with *P. aeruginosa* outbreak strains, even the use of temperatures above
374 50 °C could not achieve a secure reduction of those strains. Even though a 65 °C washing
375 program had been used for every wash cycle, it is likely that the temperature in the area
376 of the rubber mantle or the rubber door seal would have been much lower, providing an
377 optimal humid environment and nutrient supply for the growth of Gram-negative
378 microorganisms (43, 45). Moreover, the occasional use of washing machines at low
379 temperatures supports the formation of biofilms (14).
380 It is important to emphasize that the use of regular domestic washing machines for
381 washing patients clothing in a clinical setting is not permitted according to the current
382 hygiene regulations in Germany. However, the washing machine concerned was located
383 outside the actual central laundry preparation and was only intended for washing mothers'
384 clothes and, as in this case, for washing the caps and socks and was operated exclusively
385 by the nursing staff.
386

387 **Conclusion**

388 While previous studies have implicated sinks as potential reservoirs for clusters of
389 infection caused by *K. oxytoca*, this report focuses on the washing machine. The extensive
390 time period of the outbreak shows that while facing several potential environmental
391 reservoirs, a multi-dimensional containment approach is necessary. Here, the approach
392 included reinforcement of infection control policies such as hand hygiene, contact
393 precautions, isolation, admission/routine rectal screening and clear delineation between
394 handwashing sinks and sinks used for other purposes. However, only after the washing
395 machine was removed and only disposable caps and socks were used the ongoing

colonization was terminated. The results suggest that, for prompt management of outbreaks or colonization clusters, the choice of environmental sampling points and the use of an effective method should take the ecological properties of the causative strain into account. Indeed the environmental strain had not been found before the third-on-site inspection, as the use of e-swabs allowed a better detection.

In addition, the study implies that changes in washing machine design and processing are required to prevent accumulation of residual water where microbial growth can occur and contaminate clothes. Furthermore, the use of professional washing machines and the routine check with a temperature logger is an urgent requirement.

In summary, the present study shows that, in situations where an increase in colonization is observed, non-professional washing machines, if used, and clothes should be assessed and investigated as potential reservoirs and vectors for transmission.

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Conflicts of interest

None declared.

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References

1. Rutala WA, Weber DJ. 1997. Water as a reservoir of nosocomial pathogens. Infect Control Hosp Epidemiol 18:609-16.
2. Sib E, Voigt AM, Wilbring G, Schreiber C, Faerber HA, Skutlarek D, Parcina M, Mahn R, Wolf D, Brossart P, Geiser F, Engelhart S, Exner M, Bierbaum G, Schmithausen RM. 2019. Antibiotic resistant bacteria and resistance genes in biofilms in clinical wastewater networks. Int J Hyg Environ Health 222:655-662.
3. Müller H, Sib E, Gajdiss M, Klanke U, Lenz-Plet F, Barabasch V, Albert C, Schallenberg A, Timm C, Zacharias N, Schmithausen RM, Engelhart S, Exner M, Parcina M, Schreiber C, Bierbaum G. 2018. Dissemination of multi-resistant Gram-negative bacteria into German wastewater and surface waters. FEMS Microbiol Ecol 94.
4. Kanamori H, Weber DJ, Rutala WA. 2016. Healthcare Outbreaks Associated With a Water Reservoir and Infection Prevention Strategies. Clin Infect Dis 62:1423-35.
5. Kizny Gordon AE, Mathers AJ, Cheong EYL, Gottlieb T, Kotay S, Walker AS, Peto TEA, Crook DW, Stoesser N. 2017. The Hospital Water Environment as a Reservoir for Carbapenem-Resistant Organisms Causing Hospital-Acquired Infections-A Systematic Review of the Literature. Clin Infect Dis 64:1435-1444.
6. Lowe C, Willey B, O'Shaughnessy A, Lee W, Lum M, Pike K, Larocque C, Dedier H, Dales L, Moore C, McGeer A, Team MSHIC. 2012. Outbreak of extended-spectrum β-lactamase-producing Klebsiella oxytoca infections associated with contaminated handwashing sinks(1). Emerg Infect Dis 18:1242-7.
7. Jeong SH, Kim WM, Chang CL, Kim JM, Lee K, Chong Y, Hwang HY, Baek YW, Chung HK, Woo IG, Ku JY. 2001. Neonatal intensive care unit outbreak caused by a strain of Klebsiella oxytoca resistant to aztreonam due to overproduction of chromosomal beta-lactamase. J Hosp Infect 48:281-8.

8. Schulz-Stübner S, Kniehl E. 2011. Transmission of extended-spectrum β-lactamase Klebsiella oxytoca via the breathing circuit of a transport ventilator: root cause analysis and infection control recommendations. Infect Control Hosp Epidemiol 32:828-9.

9. Decré D, Burghoffer B, Gautier V, Petit JC, Arlet G. 2004. Outbreak of multi-resistant Klebsiella oxytoca involving strains with extended-spectrum beta-lactamases and strains with extended-spectrum activity of the chromosomal beta-lactamase. J Antimicrob Chemother 54:881-8.

10. Zárate MS, Gales AC, Picão RC, Pujol GS, Lanza A, Smayevsky J. 2008. Outbreak of OXY-2-Producing Klebsiella oxytoca in a renal transplant unit. J Clin Microbiol 46:2099-101.

11. Leitner E, Zarfel G, Luxner J, Herzog K, Pekard-Amenitsch S, Hoenigl M, Valentin T, Feierl G, Grisold AJ, Högenauer C, Sill H, Krause R, Zollner-Schwetz I. 2015. Contaminated handwashing sinks as the source of a clonal outbreak of KPC-2-producing Klebsiella oxytoca on a hematology ward. Antimicrob Agents Chemother 59:714-6.

12. Gaillot O, Maruéjouls C, Abachin E, Lecuru F, Arlet G, Simonet M, Berche P. 1998. Nosocomial outbreak of Klebsiella pneumoniae producing SHV-5 extended-spectrum beta-lactamase, originating from a contaminated ultrasonography coupling gel. J Clin Microbiol 36:1357-60.

13. Gastmeier P, Groneberg K, Weist K, Rüden H. 2003. A cluster of nosocomial Klebsiella pneumoniae bloodstream infections in a neonatal intensive care department: Identification of transmission and intervention. Am J Infect Control 31:424-30.

14. Bloomfield SF, Cookson B, Falkiner F, Griffith C, Cleary V. 2007. Methicillin-resistant Staphylococcus aureus, Clostridium difficile, and extended-spectrum beta-lactamase-producing Escherichia coli in the community: assessing the problem and controlling the spread. Am J Infect Control 35:86-8.

15. Callewaert C, Van Nevel S, Kerckhof FM, Granitsiotis MS, Boon N. 2015. Bacterial Exchange in Household Washing Machines. Front Microbiol 6:1381.

16. Voskoboinik L, Amiel M, Reshef A, Gafny R, Barash M. 2017. Laundry in a washing machine as a mediator of secondary and tertiary DNA transfer. Int J Legal Med 132:373-378.

17. Rehberg L, Frontzek A, Melhus Å, Bockmühl DP. 2017. Prevalence of β-lactamase genes in domestic washing machines and dishwashers and the impact of laundering processes on antibiotic resistant bacteria. J Appl Microbiol 123:1396-1406.

18. Schulz J, Hartung J. 2009. Nachweis von MRSA in Schweinestallluft mit Impingement und nachfolgender Membranfiltration. Gefahrstoffe Reinhaltung der Luft:348–352.

19. VAH. 2015. Anforderungen und Methoden zur VAH-Zertifizierung chemischer Desinfektionsverfahren. mhp.

20. Gebel J. 2016. Bestimmung der bakteriostatischen und levurostatischen Wirksamkeit sowie geeigneter Neutralisationsmittel (VAH Methode 7). *In* e.V. D-KiVfAH (ed), Desinfektionsmittel-Kommission im Verbund für Angewandte Hygiene eV, Ed Anforderungen und Methoden zur VAH-Zertifizierung chemischer Desinfektionsverfahren. mhp, Wiesbaden.

21. Gebel J. 2016. Bestimmung der bakteriziden, levuroziden, fungiziden, tuberkuloziden bzw. mykobakteriziden Wirksamkeit im quantitativen Suspensionsversuch (VAH Methode 9). *In* e.V. D-KiVfAH (ed), Desinfektionsmittel-Kommission im Verbund für Angewandte Hygiene eV, Ed Anforderungen und Methoden zur VAH-Zertifizierung chemischer Desinfektionsverfahren. mhp, Wiesbaden.

22. VAH. 2016. Zur Äquivalenz der Desinfektionsmittel-Testung gemäß VAH-Methoden und der Testung gemäß den aktuellen europäischen Normen. Hygiene + Medizin 41:83-84.

23. DIN. 2014. DIN EN 13727:2014-03. Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Prüfung der fungiziden Wirkung chemischer Desinfektionsmittel im humanmedizinischen Bereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 1). 03. DIN, https://www.who.int/foodsafety/publications/antimicrobials-sixth/en/.

24. Paterson DL, Mulazimoglu L, Casellas JM, Ko WC, Goossens H, Von Gottberg A, Mohapatra S, Trenholme GM, Klugman KP, McCormack JG, Yu VL. 2000. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in Klebsiella pneumoniae isolates causing bacteremia. Clin Infect Dis 30:473-8.

25. Grimm V, Ezaki S, Susa M, Knabbe C, Schmid RD, Bachmann TT. 2004. Use of DNA microarrays for rapid genotyping of TEM beta-lactamases that confer resistance. J Clin Microbiol 42:3766-74.

26. Gröbner S, Linke D, Schütz W, Fladerer C, Madlung J, Autenrieth IB, Witte W, Pfeifer Y. 2009. Emergence of carbapenem-non-susceptible extended-spectrum beta-lactamase-producing Klebsiella pneumoniae isolates at the university hospital of Tübingen, Germany. J Med Microbiol 58:912-22.

27. Geser N, Stephan R, Hächler H. 2012. Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. BMC Vet Res 8:21.

28. Villa L, García-Fernández A, Fortini D, Carattoli A. 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J Antimicrob Chemother 65:2518-29.

29. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219-28.

30. Carattoli A, Seiffert SN, Schwendener S, Perreten V, Endimiani A. 2015. Differentiation of IncL and IncM Plasmids Associated with the Spread of Clinically Relevant Antimicrobial Resistance. PLoS One 10:e0123063.

31. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33:2233-9.

32. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ. 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of Escherichia coli O157:H7, Salmonella, and Shigella for PulseNet. Foodborne Pathog Dis 3:59-67.

33. Herzog KA, Schneditz G, Leitner E, Feierl G, Hoffmann KM, Zollner-Schwetz I, Krause R, Gorkiewicz G, Zechner EL, Högenauer C. 2014. Genotypes of Klebsiella oxytoca isolates from patients with nosocomial pneumonia are distinct from those of isolates from patients with antibiotic-associated hemorrhagic colitis. J Clin Microbiol 52:1607-16.

34. Sehulster L, Chinn R, Arduino M, al. e. 2004. Recommendations from CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). American Society for Healthcare Engineering/American Hospital Association, Chicago.

35. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18:268-81.

36. Rodríguez I, Thomas K, Van Essen A, Schink AK, Day M, Chattaway M, Wu G, Mevius D, Helmuth R, Guerra B, consortium S-E. 2014. Chromosomal location of blaCTX-M genes in clinical isolates of Escherichia coli from Germany, The Netherlands and the UK. Int J Antimicrob Agents 43:553-7.

37. VAH. 2019. https://vah-online.de/de/. Accessed 19.03.2019.

38. Price JR, Cole K, Bexley A, Kostiou V, Eyre DW, Golubchik T, Wilson DJ, Crook DW, Walker AS, Peto TEA, Llewelyn MJ, Paul J, group MMMi. 2017. Transmission of Staphylococcus aureus between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. Lancet Infect Dis 17:207-214.

39. Heudorf U, Gasteyer S, Müller M, Serra N, Westphal T, Reinheimer C, Kempf V. 2017. Handling of laundry in nursing homes in Frankfurt am Main, Germany, 2016 - laundry and

professional clothing as potential pathways of bacterial transfer. GMS Hyg Infect Control 12:Doc20.

40. Organization WH. 2019. Critically important antimicrobials for human medicine, 6th revision. World Health Organization.

41. Jolley KA, Bray JE, Maiden MCJ. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res 3:124.

42. Dögen A, Sav H, Gonca S, Kaplan E, Ilkit M, Novak Babic M, Gunde-Cimerman N, de Hoog GS. 2017. Candida parapsilosis in domestic laundry machines. Med Mycol 55:813-819.

43. Gattlen J, Amberg C, Zinn M, Mauclaire L. 2010. Biofilms isolated from washing machines from three continents and their tolerance to a standard detergent. Biofouling 26:873-82.

44. Zhang J, Li W, Chen J, Qi W, Wang F, Zhou Y. 2018. Impact of biofilm formation and detachment on the transmission of bacterial antibiotic resistance in drinking water distribution systems. Chemosphere 203:368-380.

45. Amberg C. 2014. Biofilm in washing machines and how it is assessed. Hygiene requirements and measurements in dishwashingBonn: Swissatest testmaterials AG.

565 **TABLE CAPTIONS**

566

567 **TABLE 1** Primers used for Inc-typing.

568

569 **TABLE 2** Occurrence of Gram-negative *Enterobacterales*, non-fermenting organisms,
570 and ESBL-producing *K. oxytoca* PFGE type-00531 isolates in environmental samples
571 obtained during on-site inspection of risk areas.

572

573 **TABLE 3** Occurrence of Gram-negative *Enterobacterales*, non-fermenting organisms,
574 and ESBL-producing *K. oxytoca* PFGE type-00531 isolates in environmental samples
575 obtained during on-site inspection of risk areas.

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577 **TABLE 4** Hygienic measures and control management procedures.

578 **TABLES**

579 **TABLE 1** Primers used for Inc-typing.

IncHI2_fw	TTT CTC CTG AGT CAC CTG TTA ACA C	Carattoli et al. (29)
IncHI2_rev	GGC TCA CTA CCG TTG TCA TCC T	Carattoli et al. (29)
IncA/C_fw	GAG AAC CAA AGA CAA AGA CCT GGA	Carattoli et al. (29)
IncA/C_rev	ACG ACA AAC CTG AAT TGC CTC CTT	Carattoli et al. (29)
IncL_fw	CGG AAC CGA CAT GTG CCT ACT	Carattoli et al. (30)
IncM_fw	GGA TGA AAA CTA TCA GCA TCT GAA G	Carattoli et al. (30)
IncL/M_rev	GAA CTC CGG CGA AAG ACC TTC	Carattoli et al. (30)
IncI1-αγ_fw	CGA AAG CCG GAC GGC AGA A	Carattoli et al. (29)
IncI1-αγ_rev	TCG TCG TTC CGC CAA GTT CGT	Carattoli et al. (29)
IncN_fw	GTC TAA CGA GCT TAC CGA AG	Carattoli et al. (29)
IncN_rev	GTT TCA ACT CTG CCA AGT TC	Carattoli et al. (29)
IncFIIk_fw	TCT TCT TCA ATC TTG GCG GA	Villa et al. (28)
IncFIIk_rev	GCT TAT GTT GCA CRG AAG GA	Villa et al. (28)
IncFIA_fw	CCA TGC TGG TTC TAG AGA AGG TG	Carattoli et al. (29)
IncFIA_rev	GTA TAT CCT TAC TGG CTT CCG CAG	Carattoli et al. (29)
IncFIB_fw	TCT GTT TAT TCT TTT ACT GTC CAC	Villa et al. (28)
IncFIB_rev	CTC CCG TCG CTT CAG GGC ATT	Villa et al. (28)
IncFII_fw	CTG ATC GTT TAA GGA ATT TT	Villa et al. (28)
IncFII_rev	CAC ACC ATC CTG CAC TTA	Villa et al. (28)

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582 **TABLE 2** Occurrence of Gram-negative *Enterobacterales*, non-fermenting organisms,
583 and ESBL-producing *K. oxytoca* PFGE type-00531 isolates in environmental samples
584 obtained during on-site inspection of risk areas.

Room	**Sampling point**	**CFU / ml or total Gram-negative bacteria count**	**Microbiological differentiation**	**CTX-M**	**K. oxytoca PFGE type-00531 / ST201**
Nursing care room, storage rooms	Working basin, siphon	2,1 x 10^5	*S. marcescens*	-	
	Hand wash basin, siphon water	1.6 x 10^6	***K. oxytoca*** *R. terrigena* *E. cloacae* complex	**positive** negative negative	yes
	Hand wash basin drainage hole cover	scattered/swab (no CFU/ml)	*E. cloacae* complex *K. pneumoniae*	negative negative	
Staff toilet	Hand wash basin, fresh water	not evaluable/100 ml	Gram-negative non-fermenting rods	-	
	Hand wash basin, siphon water	1.5 x 10^4	*P. aeruginosa* *S. maltophilia*	-	
	Toilet, water	1.0	*S. maltophilia*	-	
Staff room	Water from the kitchen sink siphon	3.0 x 10^5	***K. oxytoca*** *P. aeruginosa*	**positive** negative	yes
	Water reservoir tank, espresso machine	10.0 CFU/50 ml	*S. maltophilia*	-	
Hygiene sluice, PNC	Hand wash basin, siphon water	1.3 x 10^4	*P. aeruginosa,* Gram-negative non-fermenting rods	-	
Basement, laundry room	Tumble dryer, water tray	not evaluable/50 ml	Gram-negative non-fermenting rods	-	
	Washing machine 1, residual water, rubber mantle	>10^6 ESBL 3.9 x 10^2	***K. oxytoca***	**positive**	yes
	Washing machine 1, detergent compartment	not evaluable/swab	***K. oxytoca*** *P. aeruginosa*	**positive** negative	yes
	Washing machine 2, detergent compartment	not evaluable/swab	*P. aeruginosa*	-	
Laundry	Hat 1	> 10^9	***K. oxytoca***	**positive**	yes
	Hat 2	> 10^9	***K. oxytoca***	**positive**	yes

	Hat 3	> 10^9	***K. oxytoca***	**positive**	yes
	Hat 4	> 10^9	***K. oxytoca***	**positive**	yes
	Socks 1	> 10^9	***K. oxytoca***	**positive**	yes
	Socks 2	> 10^9	***K. oxytoca***	**positive**	yes

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588 **TABLE 3** Occurrence of Gram-negative *Enterobacterales*, non-fermenting organisms,

589 and ESBL-producing *K. oxytoca* PFGE type-00531 isolates in environmental samples

590 obtained during on-site inspection of risk areas.

no	ID	Sample Type	Pipera-cillin (MIC)	Piperacillin/ Tazobactam (MIC)	Cefo-taxim (MIC)	Cef-tazidim (MIC)	Imi-penem (MIC)	Mero-penem (MIC)	Ami-kacin (MIC)	Cipro-floxacin (MIC)
1	*K. oxytoca*	washing machine	R (>16)	I (16/4)	R (>2)	R (32)	S (≤1)	S (≤0,125)	S (≤4)	R (2)
2	*K. oxytoca*	washing machine	R (>16)	R (>64/4)	R (>2)	R (32)	S (≤1)	S (≤0,125)	S (8)	R (2)
3	*K. oxytoca*	washing machine	R (>16)	R (64/4)	R (>2)	R (32)	S (≤1)	S (≤0,125)	S (8)	R (2)
4	*K. oxytoca*	siphon washbasin staff lobby	R (>16)	R (32/4)	R (>2)	R (32)	S (≤1)	S (≤0,125)	S (8)	R (>2)
5	*K. oxytoca*	siphon washbasin staff lobby	R (>16)	S (8/4)	R (>2)	R (32)	S (≤1)	S (≤0,125)	S (≤4)	R (2)
6	*K. oxytoca*	siphon washbasin staff lobby	R (>16)	I (16/4)	R (>2)	R (64)	S (≤1)	S (≤0,125)	S (≤4)	R (2)
7	*K. oxytoca*	human	R (>16)	R (>64/4)	R (>2)	R (>128)	S (≤1)	S (≤0,125)	I (16)	R (>2)
8	*K. oxytoca*	human	R (>16)	R (>64/4)	R (>2)	R (>128)	S (≤1)	S (≤0,125)	S (8)	R (>2)
9	*K. oxytoca*	human	R (>16)	R (>64/4)	R (>2)	R (>128)	S (≤1)	S (≤0,125)	S (8)	R (>2)
10	*K. oxytoca*	human	R (>16)	R (>64/4)	R (>2)	R (>128)	S (≤1)	S (≤0,125)	S (≤4)	R (>2)
11	*K. oxytoca*	human	R (>16)	R (>64/4)	R (>2)	R (>128)	S (≤1)	S (≤0,125)	S (8)	R (>2)
12	*K. oxytoca*	human	R (>16)	R (>64/4)	R (>2)	R (>128)	S (≤1)	S (≤0,125)	S (≤4)	R (>2)
13	*K. oxytoca*	human	R (>16)	R (>64/4)	R (>2)	R (>128)	S (≤1)	S (≤0,125)	S (≤4)	R (2)
14	*K. oxytoca*	human	R (>16)	R (>64/4)	R (>2)	R (>128)	S (≤1)	S (≤0,125)	S (≤4)	R (>2)
15	*K. oxytoca*	human	R (>16)	R (>64/4)	R (>2)	R (>128)	S (≤1)	S (≤0,125)	S (8)	R (>2)
16	*K. oxytoca*	human	R (>16)	R (>64/4)	R (>2)	R (>128)	S (≤1)	S (≤0,125)	S (≤4)	R (>2)

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593 **TABLE 4** Hygienic measures and control management procedures.

Hygienic measures and control management strategies	
1) 04 - 08 / 2012	- Environmental monitoring including sampling of siphons, sinks, and showers via contact plates - Continuation of admission screening and weekly routine screening, implementation of a second newborn-screening 48 hours post-partum - Screening of all mothers on admission and on discharge - Monitoring of pathways of newborns from birth to admission in the perinatal centre - Maintenance of incubator isolation until two-time negative screening results - Advanced training of health care workers with special focus on toilet and hand hygiene - Microbiological analyses of vaginal ultrasound probes and heated towels used for the primary care of the newborns - Renovation/decontamination of ward rooms, removal of unused sinks - Installation of new wall-mounted disinfection dispensers - Clinical procedure screening for any cross-contamination possibilities - Feeding of newborns with only precooked single nurture packages - Use of sterile water for bathing of newborns
2) 08 - 10/2012	- Screening of all health care workers working in the perinatal centre, intensive care unit and the obstetrics department
3) 10/2012 - 06/2013	- Extended environmental sampling from different areas on the ward and functionally linked areas in the hospital. Identification and sampling of specific risk factors (e.g. washing machine) - Training of staff in the correct handling of the disinfectant wipes - Training of health care workers in correct disinfection and cleaning of incubators - Preparation of disinfectant wipes by a newly validated/approved preparation machine - Construction measures to provide work space for the hygienic processing of incubators and ensure sufficient storage capacities - Marking of all thermometers (rectal and commercially available thermometers) as well as incubators for traceability - Intensive audit of all procedures running on ward during routine operations

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596 **FIGURE CAPTIONS**

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598 **FIG 1** Course of the outbreak with different *K. oxytoca* strains within one year and their

599 distribution on different wards (PNC: perinatal centre, ICU: intensive care unit).