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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**Running Head: The washing machine as a reservoir for ESBL-bacteria**

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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**
Human and environmental samples were collected and stored at 4 °C during transport to the laboratories and processed within 48 hours.

All human samples (anal, vaginal and wound swabs) were streaked onto MacConkey agar plates (Oxoid Deutschland GmbH, Wesel, Germany) with a 10 µg imipenem disk (bioMérieux SA, Marcy-Étoile, France) and selective agar plates, e.g. CHROMagarESBL (Oxoid Deutschland GmbH, Wesel, Germany).

All Oxoid RODAC™ contact plates (Ø 55 mm, Becton Dickinson, Heidelberg, Germany) were used for environmental surface sampling and incubated at 36 ± 1 °C for 48 hours.

All environmental liquid medium swabs (eSwab; Copan Diagnostics, Italy) were streaked on Columbia agar plates containing 5 % sheep blood (Becton Dickinson), MacConkey agar plates (Oxoid Deutschland GmbH, # PO5002A), and selective agar plates, e.g. CHROMagar™ ESBL (bioMérieux SA, Marcy-Étoile, France; # 43481) and incubated at 37 ± 1 °C for 48 hours. In addition, casein soy flour peptone broth (CASO-broth) (Merck KGaA, Darmstadt, Germany) was inoculated with the same swabs and incubated at 37 ± 1 °C for 24 hours.

The disinfectant wipes, socks, and knitted hats of the newborns were put into 200 ml CASO-broth with tween saponin histidine cysteine (TSHC +3% Tween Merck, # 8.22187; +3 % saponin, Roth, # 5185.1; +0.1% histidine Merck, # 1.04351; +0.1 % cysteine, Merck, # 1.02839) and homogenised for 60 seconds (Stomacher 400, Seward Limited, West Sussex, United Kingdom). First, 2 x 100 ml of the extract was filtered (membrane-filter Millipore Hydrosol, 250 ml cup) followed by the filtration of 100 ml 0.9 % NaCl to rinse the additives off the filter. The filter was then placed on CHROMagar™ ESBL and MacConkey agar plates and incubated at 37 °C for 24 – 48 hours. In addition, 100 µl of each concentration of a dilution series in 0.9 % NaCl was plated on CHROMagar™ ESBL and
MacConkey agar plates. The emulsions were treated in the same manner as the wipes, except that shredding was not necessary.

For the water samples, approximately 100 ml were collected in a sterile polystyrene cup (# 225170, Greiner, Frickenhausen, Germany) and filtered through a sterile nitrocellulose membrane filter (pore size 0.45 ± 0.02 µm, Ø 47 mm, black grid from Millipore, # EZHAWG 474), according to Schulz and Hartung (18). After filtration, the membrane was placed on selective CHROMagar™ ESBL and MacConkey agar plates.

In addition, 100 µl of each dilution of a dilution series in 0.9 % NaCl was plated on CHROMagar™ ESBL and MacConkey agar.

Identification and susceptibility testing of ARB

All *Enterobacterales* detected on CHROMagar™ ESBL were identified via API 20 E V4.1 apiweb™ (bioMérieux SA, Marcy-Étoile, France) and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS, bioMérieux SA, Marcy l’Étoile, France) using Myla® software, VITEK® MS-CHCA matrix (#411 071, bioMérieux) and disposable targets (# 410 893, bioMérieux).

Susceptibility to antibiotics was tested using the Micronaut-S MDR MRGN-Screening 3 system (MERLIN, Gesellschaft für mikrobiologische Diagnostika GmbH, Bornheim-Hersel, Germany) and results were interpreted using EUCAST criteria.

Testing of disinfectant tolerance

To detect a possibly increased tolerance of the *K. oxytoca* isolates towards disinfectants, surface disinfectant and the disinfecting general-purpose detergent which had been used in the washing machines were both examined according to the regulations (7 and 9) of the Disinfectant Commission of the Verbund für Angewandte Hygiene e.V. (VAH) (19).

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 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*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub> 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).
The PCR conditions were set on 94 °C for 3 min, followed by 30 cycles at 94 °C for 30 s, 60 °C for 30 s and 68 °C for 1 min. The final extension was performed at 68 °C for 5 min. The PCR products were analyzed using agarose gelelectrophoresis on a 1 % agarose/TBE gel, and stained with Midori Green (Biozym Scientific GmbH, Hessisch Oldendorf, Germany).

Plasmid conjugation testing

The possibility of plasmid transfer via conjugation was analyzed. All *K. oxytoca* as well as wild type *E. coli* strains were streaked onto LB-agar containing a) 20 mg/l chloramphenicol and b) 20 mg/l chloramphenicol and 4 mg/l cefotaxime. *E. coli* strains that grew on LB-agar containing only chloramphenicol, but not on LB-agar containing both chloramphenicol and cefotaxime, were used for the following conjugation assay. For the pre-culture one colony of a fresh culture of all isolated *K. oxytoca* strains as donors and different wild type *E. coli* strains (recipients) were used for inoculation of 5 ml lysogeny broth (LB) and incubated at 37 °C overnight with shaking. Next, 5 ml fresh LB was inoculated with 100 µl of the donor (*K. oxytoca*) and 1 ml of the recipient (*E. coli*) and incubated at 37 °C for overnight with shaking. After incubation 1 ml of each culture was centrifuged at 14,000 rpm for 3 min. Afterwards the supernatant was discarded and the pellet was resuspended using 100 µl Milli Q. The whole suspensions, as well as 100 µl of the cultures were plated onto selective LB-agar containing 20 mg/l chloramphenicol and 4 mg/l cefotaxime and incubated at 37 °C overnight. Grown colonies were streaked onto Columbia sheep blood agar and analyzed via MALDI TOF-MS.

Molecular typing

All *K. oxytoca* isolated from clinical samples and environmental sources were typed by the German National Reference Centre (GNRC) using PFGE according to Tenover et al.
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
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 > 10⁹ 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)
genes were obtained from the strains indicating that the CTX-M genes might be chromosomally located as described by Rodríguez et al. (36).

**Epidemiological links**

Retrospective analysis demonstrates that only newborns who had worn clothing that had been washed in the in-house washing machine were colonised with the redundant *K. oxytoca* clone. Although the siphons of the staff sinks were also identified as a possible reservoir, no staff members were identified as carriers or spreaders of ESBL-*Enterobacterales* at the time of screening.

All clinical and environmental isolates of the *K. oxytoca* PFGE type-00531/ST201 displayed an identical PFGE banding pattern and were thus considered clonally identical. This clone was specific for the newborns/infants and some environmental samples.

**Evaluation of a possibly increased disinfectant tolerance of the *K. oxytoca* strains**

The environmental *K. oxytoca* isolates showed no increased tolerance to the surface disinfectant based on quaternary ammonium compounds and alkylamine. The minimal inhibitory alkylamine concentration on the *K. oxytoca* strains was identified as max. 0.003% and 0.0075% on the reference strain *Pseudomonas aeruginosa*. Furthermore, the examination according to VAH method 9 showed a ≥ 5 log$_{10}$ CFU reduction of the *K. oxytoca* strains at a concentration of 0.5% and 15 min in the presence of interfering substances (dirty conditions – 3.0 g/l bovine serum albumin + 3.0 ml/l sheep erythrocytes). The VAH-recommended concentration and application time is 0.5% over 60 minutes (37). *K. oxytoca* isolates from the environment showed no increased tolerance to the laundry 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 log₁₀ 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 \textit{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, \textit{Enterobacterales} can survive in wet environments for an extended period of time (1), especially waterborne bacteria such as \textit{P. aeruginosa} and \textit{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.
Furthermore, Rehberg et al. (17) demonstrated that ARB can survive the washing process. In tests with *P. aeruginosa* outbreak strains, even the use of temperatures above 50 °C could not achieve a secure reduction of those strains. Even though a 65 °C washing program had been used for every wash cycle, it is likely that the temperature in the area of the rubber mantle or the rubber door seal would have been much lower, providing an optimal humid environment and nutrient supply for the growth of Gram-negative microorganisms (43, 45). Moreover, the occasional use of washing machines at low temperatures supports the formation of biofilms (14).

It is important to emphasize that the use of regular domestic washing machines for washing patients clothing in a clinical setting is not permitted according to the current hygiene regulations in Germany. However, the washing machine concerned was located outside the actual central laundry preparation and was only intended for washing mothers’ clothes and, as in this case, for washing the caps and socks and was operated exclusively by the nursing staff.

**Conclusion**

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

Acknowledgements

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Conflicts of interest

None declared.

Funding source

None declared.
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TABLE CAPTIONS

TABLE 1 Primers used for Inc-typing.

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

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

TABLE 4 Hygienic measures and control management procedures.
### TABLE 1

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

<table>
<thead>
<tr>
<th>Room</th>
<th>Sampling point</th>
<th>CFU / ml or total Gram-negative bacteria count</th>
<th>Microbiological differentiation</th>
<th>CTX-M</th>
<th>K. oxytoca PFGE type-00531 / ST201</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursing care room, storage rooms</td>
<td>Working basin, siphon</td>
<td>2.1 x 10^5</td>
<td><em>S. marcescens</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand wash basin, siphon water</td>
<td></td>
<td>1.6 x 10^6</td>
<td><em>K. oxytoca</em>, <em>R. terrigena</em>, <em>E. cloacae</em> complex</td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>Hand wash basin drainage hole cover</td>
<td>scattered/swab (no CFU/ml)</td>
<td></td>
<td><em>E. cloacae</em> complex <em>K. pneumoniae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staff toilet</td>
<td>Hand wash basin, fresh water</td>
<td>not evaluable/100 ml</td>
<td>Gram-negative non-fermenting rods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand wash basin, siphon water</td>
<td>1.5 x 10^4</td>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toilet, water</td>
<td>1.0</td>
<td><em>S. maltophilia</em></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staff room</td>
<td>Water from the kitchen sink siphon</td>
<td>3.0 x 10^5</td>
<td><em>K. oxytoca</em>, <em>P. aeruginosa</em></td>
<td>positive</td>
<td>yes</td>
</tr>
<tr>
<td>Water reservoir tank, espresso machine</td>
<td>10.0 CFU/50 ml</td>
<td><em>S. maltophilia</em></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hygiene sluice, PNC</td>
<td>Hand wash basin, siphon water</td>
<td>1.3 x 10^4</td>
<td><em>P. aeruginosa</em>, Gram-negative non-fermenting rods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basement, laundry room</td>
<td>Tumble dryer, water tray</td>
<td>not evaluable/50 ml</td>
<td>Gram-negative non-fermenting rods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washing machine 1, residual water, rubber mantle</td>
<td>&gt;10^6 ESBL 3.9 x 10^2</td>
<td><em>K. oxytoca</em></td>
<td>positive</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Washing machine 1, detergent compartment</td>
<td>not evaluable/swab</td>
<td><em>K. oxytoca</em>, <em>P. aeruginosa</em></td>
<td>positive</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Washing machine 2, detergent compartment</td>
<td>not evaluable/swab</td>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laundry</td>
<td>Hat 1</td>
<td>&gt; 10^9</td>
<td><em>K. oxytoca</em></td>
<td>positive</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>Hat 2</td>
<td>&gt; 10^9</td>
<td><em>K. oxytoca</em></td>
<td>positive</td>
<td>yes</td>
</tr>
</tbody>
</table>
TABLE 3 Occurrence of Gram-negative Enterobacterales, non-fermenting organisms, and ESBL-producing *K. oxytoca* PFGE type-00531 isolates in environmental samples obtained during on-site inspection of risk areas.

<table>
<thead>
<tr>
<th>no</th>
<th>ID</th>
<th>Sample Type</th>
<th>Pipera-cillin (MIC)</th>
<th>Pipercillin/Tazobactam (MIC)</th>
<th>Cefotaxim (MIC)</th>
<th>Cefotaxidim (MIC)</th>
<th>Imipenem (MIC)</th>
<th>Meropenem (MIC)</th>
<th>Amikacin (MIC)</th>
<th>Ciprofloxacin (MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K. oxytoca</td>
<td>washing machine</td>
<td>R (&gt;16)</td>
<td>I (16/4)</td>
<td>R (&gt;2)</td>
<td>R (32)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (2)</td>
</tr>
<tr>
<td>2</td>
<td>K. oxytoca</td>
<td>washing machine</td>
<td>R (&gt;16)</td>
<td>R (&gt;64/4)</td>
<td>R (&gt;2)</td>
<td>R (32)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (2)</td>
</tr>
<tr>
<td>3</td>
<td>K. oxytoca</td>
<td>washing machine</td>
<td>R (&gt;16)</td>
<td>R (64)</td>
<td>R (&gt;2)</td>
<td>R (32)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (2)</td>
</tr>
<tr>
<td>4</td>
<td>K. oxytoca</td>
<td>washing machine</td>
<td>R (&gt;16)</td>
<td>R (32/4)</td>
<td>R (&gt;2)</td>
<td>R (32)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (&gt;2)</td>
</tr>
<tr>
<td>5</td>
<td>K. oxytoca</td>
<td>siphon washbasin</td>
<td>R (&gt;16)</td>
<td>S (8/4)</td>
<td>R (&gt;2)</td>
<td>R (32)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (2)</td>
</tr>
<tr>
<td>6</td>
<td>K. oxytoca</td>
<td>siphon washbasin</td>
<td>R (&gt;16)</td>
<td>I (16/4)</td>
<td>R (&gt;2)</td>
<td>R (64)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (2)</td>
</tr>
<tr>
<td>7</td>
<td>K. oxytoca</td>
<td>human</td>
<td>R (&gt;16)</td>
<td>R (&gt;64/4)</td>
<td>R (&gt;2)</td>
<td>R (&gt;128)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>I (16)</td>
<td>R (&gt;2)</td>
</tr>
<tr>
<td>8</td>
<td>K. oxytoca</td>
<td>human</td>
<td>R (&gt;16)</td>
<td>R (64/4)</td>
<td>R (&gt;2)</td>
<td>R (&gt;128)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (&gt;2)</td>
</tr>
<tr>
<td>9</td>
<td>K. oxytoca</td>
<td>human</td>
<td>R (&gt;16)</td>
<td>R (64/4)</td>
<td>R (&gt;2)</td>
<td>R (&gt;128)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (&gt;2)</td>
</tr>
<tr>
<td>10</td>
<td>K. oxytoca</td>
<td>human</td>
<td>R (&gt;16)</td>
<td>R (64/4)</td>
<td>R (&gt;2)</td>
<td>R (&gt;128)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (&gt;2)</td>
</tr>
<tr>
<td>11</td>
<td>K. oxytoca</td>
<td>human</td>
<td>R (&gt;16)</td>
<td>R (64/4)</td>
<td>R (&gt;2)</td>
<td>R (&gt;128)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (&gt;2)</td>
</tr>
<tr>
<td>12</td>
<td>K. oxytoca</td>
<td>human</td>
<td>R (&gt;16)</td>
<td>R (64/4)</td>
<td>R (&gt;2)</td>
<td>R (&gt;128)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (&gt;2)</td>
</tr>
<tr>
<td>13</td>
<td>K. oxytoca</td>
<td>human</td>
<td>R (&gt;16)</td>
<td>R (64/4)</td>
<td>R (&gt;2)</td>
<td>R (&gt;128)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (&gt;2)</td>
</tr>
<tr>
<td>14</td>
<td>K. oxytoca</td>
<td>human</td>
<td>R (&gt;16)</td>
<td>R (64/4)</td>
<td>R (&gt;2)</td>
<td>R (&gt;128)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (&gt;2)</td>
</tr>
<tr>
<td>15</td>
<td>K. oxytoca</td>
<td>human</td>
<td>R (&gt;16)</td>
<td>R (64/4)</td>
<td>R (&gt;2)</td>
<td>R (&gt;128)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (&gt;2)</td>
</tr>
<tr>
<td>16</td>
<td>K. oxytoca</td>
<td>human</td>
<td>R (&gt;16)</td>
<td>R (64/4)</td>
<td>R (&gt;2)</td>
<td>R (&gt;128)</td>
<td>S (≤1)</td>
<td>S (≤0,125)</td>
<td>S (≤4)</td>
<td>R (&gt;2)</td>
</tr>
</tbody>
</table>
TABLE 4 Hygienic measures and control management procedures.

<table>
<thead>
<tr>
<th>Hygienic measures and control management strategies</th>
<th></th>
</tr>
</thead>
</table>
| **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 |
FIG 1 Course of the outbreak with different *K. oxytoca* strains within one year and their distribution on different wards (PNC: perinatal centre, ICU: intensive care unit).