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Bathroom contamination by antibiotic-resistant Enterobacterales (ESBLPE and CPE): an experimental study

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SUMMARY

Background: Extended-spectrum β-lactamase-producing Enterobacterales (ESBLPE) and carbapenemase-producing Enterobacterales (CPE) cause serious infections. Their presence in urine may lead to environmental contamination potentially responsible for cross-transmission.

Aim: To evaluate the level of spraying and contamination after emptying urine in the toilet and rinsing in the sink, a common practice in the healthcare setting.

Methods: For each test, the procedure was similar: seat raised, emptying urinal bottle into the toilet at the height of the bowl, rinsing in the sink and flushing. To study splash-drops, water and fluorescein were mixed in the urinal bottle. In each area, the splash-drops frequency and level were assessed with UV. To study contamination, three ESBLPE and one CPE were diluted in saline, 10^6/mL. Contamination was assessed by sampling before, immediately after and 3 h after the test. The swabs were cultured and the colonies counted and identified.

Findings: The areas at the highest risk of spraying were the toilet bowl contour ($N = 36/36$), the underside of the toilet seat ($N = 34$) and the inside of the sink ($N = 34$). Except for gloves ($N = 14$), there was low clothing contamination. The most frequently contaminated areas were inside the sink (40/48), where the highest levels of contamination were found (14/48).

Conclusion: Emptying the urinal bottles in the toilet followed by sink rinsing is associated with a significant risk of projection and contamination, depending on the area (highest risk at the sink), but the bacteria did not survive beyond 3 h. This practice, which carries a risk of cross-transmission, should be reviewed.

Introduction

Commensal bacteria of the digestive tract are responsible for many healthcare-associated infections. Extended-

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spectrum β-lactamase-producing Enterobacterales (ESBLPE) and carbapenemase-producing Enterobacterales (CPE) are among the main multidrug-resistant pathogens found in hospitals. In France, the incidence of ESBLPE increased from 0.48 to 0.67 clinical isolates per 1000 patient-days between 2010 and 2017 [1]. In 2017, the proportion of third-generation cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* in bacteraemia due to *E. coli* and *K. pneumoniae* was 10.2% and 28.8%, respectively [2]. The proportion of carbapenem-resistant *K. pneumoniae* bacteraemia, however, remained stable and very low, at ~0.5% [2].

Stools and urine colonized by Enterobacterales may be a source of spread of these bacteria. For ESBLPE and CPE, the role of environmental contamination is unclear. Studies have shown that aerosolization caused by toilet flushing can contaminate the environment and professional attire [3–6]. Furthermore, the distal points of water systems can be a reservoir of CPE [7,8]. Sink traps located near toilets are also at greater risk of being colonized by micro-organisms carrying the bla_{kpc} gene [8].

To understand the role of the environment in the spread of bacteria, it is important to assess their survival time in the environment. Experimental studies have found survival times for Enterobacterales, ESBL or not, to be highly variable, ranging from a few hours to more than one week, depending on the experimental conditions [9–11]. In the hospital environment, some ESBLPE species are able to survive longer in the environment, such as *K. pneumoniae* and *Serratia marcesens* [12–16]. They could also be associated with an increased risk of transmission.

Disabled patients frequently use a bedpan, or a urinal bottle for men. Good practices recommend that urine in urinal bottles be emptied in the bedpan washer or toilet [17]. To limit the risk of the spread of micro-organisms from faeces, it has been recommended to remove hand sprayers used to rinse bedpans and urinal bottles, and instead to use a bedpan washer, thus forcing caregivers to change their practices [18]. A survey conducted in our hospital showed that almost all caregivers rinse urinal bottles in the patient's bathroom sink. The bedpan washer is only used at the patient's discharge.

Colonized urine constitutes a potential risk of environmental contamination, which has never been assessed to date. To our knowledge, no publication has assessed the infectious risk associated with handling urinal bottles. This study aimed to assess the frequency of splashes and the level and duration of environmental contamination by ESBLPE and CPE when emptying and washing urinal bottles in the patient's bathroom.

Methods

Architecture

Bichat–Claude Bernard Hospital is an 850-bed university hospital. This study was carried out in patients' bathrooms located in an unoccupied hospital ward. The equipment included wall-hung toilets with a seat, a flush button with water storage integrated in the wall, a sink and a wall tap with a manual mixer (Appendix A). Equipment types could differ from one room to another. The distance between the toilets and the sinks was <1 m.

Protocol

To mimic patient urine, 250 mL of liquid mixture, representative of the average volume of urination in an adult, was poured into the urinal bottle. For the preparation of the liquid, 20 drops of fluorescein were diluted in 250 mL water.

Experimentation

Before each test, an ultraviolet (UV) exposure test was performed to check the absence of fluorescence in the urinal bottle, in the environment, and on professional attire. UV exposure was detected using a flashlight (Pearl, Sélestat, France).

To comply with professional practices, different successive stages of emptying and rinsing were performed with similar procedure for each test: (i) the toilet seat was up; (ii) the urinal bottle was emptied with the right hand into the toilet at a defined height; (iii) the emptied urinal bottle was rinsed twice in the sink and the rinsing water was poured into toilet. The tap was activated by the left hand; (iv) the flush button was activated with the right hand.

The operator was wearing single-use clothing, which was changed at each test, including a mask, head covering, overcoat, apron, and gloves.

Determination of the optimal discharge height by studying the projection levels

All the tests were performed in six different bathrooms. The number of splash-drops was counted in the bathroom and on professional attire in 14 areas, including the toilet bowl contour, the underside and top of the toilet seat, the space between the toilet bowl and the wall, the flush button, the wall behind the toilet, the sink contour and the inside, the wall behind the sink, the tap and mixer tap, apron, gloves, and sleeves of the cover blouse. Splash-drops in each area were counted after exposure to UV after each test. A marker or stickers were used to mark the projection areas. A splash-drop could correspond to a minimal spot or a large stain. The number of splash-drops was considered uncountable beyond 200.

The projection frequency according to the height of urinal bottle emptying was evaluated in order to determine the height with the lowest number of projections. The emptying height was defined as the distance between the urinal bottle at the time of emptying and the top of the toilet bowl. Once the height was set, 36 tests were performed by different operators.

Bacteriological contamination

Only one operator performed all the tests for bacteriological contamination in the bathroom. The significant presence of limescale in the sink and toilets matched actual conditions. The sinks are directly connected to a building drain line without a siphon. The temperature and humidity were systematically recorded at the beginning of each test [9].

Four Enterobacterales were tested: ESBL-producing *Escherichia coli* clone TN03 (strain B2 ST131 O25b:H4 TN03); ESBL-producing *S. marcescens* and *K. pneumoniae* from clinical samples; and co-expression of OXA-48 carbapenemase and ESBL-producing *Citrobacter freundii*. The *C. freundii* isolate was found in toilet limescale, with this reservoir considered as responsible for a long-term CPE outbreak in a haematology

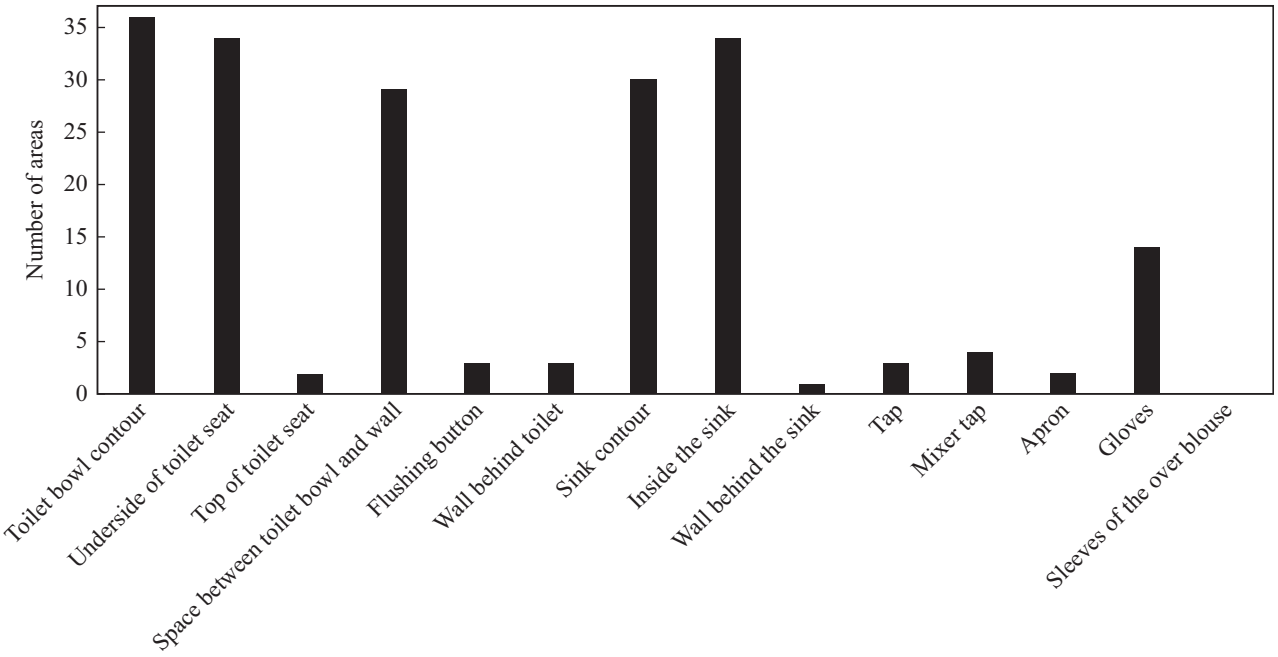

Figure 1. Number of fluorescing splash-drop areas after emptying and rinsing of urinals containing water and fluorescein ($N = 36$).

unit. All strains used were grown on Chromid®BLSE (bioMérieux®, Marcy l'Etoile, France) selective agar.

For each micro-organism, a series of 12 tests was performed. A 250 mL suspension at a concentration of 10^6 colony-forming units (cfu)/mL was made from a 0.5 McFarland bacterial suspension diluted in 0.9% NaCl. In half of the tests, 20 drops of fluorescein were added to the liquid to ensure that the sampling method would not influence the results. This suspension was then poured into the urinal bottle. Before each test and for each area, surface cleaning with 70° alcohol was carried out followed by a check of the efficacy of disinfection. The use of alcohol rather than a disinfectant was justified because disinfectant can display a residual activity over 12 h, unlike 70° alcohol. The control consisted in swabbing the whole surface of each area to ensure that there was no micro-organism (TC0). To monitor contamination immediately after (T0) and 3 h after (T3) the test, a sample was taken at T0 and T3, by swabbing the first half of each area at T0 and the second half at T3 (Appendix B). Six high-level contamination areas potentially in contact with the caregivers' hands were selected, including the inside of the sink, the sink contour, the mixer tap, the flush button, the top of the toilet seat, and the underside of the toilet seat. For the *C. freundii* isolate, an additional sample was collected in the building drain line by inserting a swab into the grid sink drain. The smallest area studied was the flush button (24 cm^2) and the largest was the inside of the sink (2751 cm^2). A standard swab (Deltalab, Rubí, Spain) was used to obtain a microbial surface sample. For each test, the surface of each area studied was the same. The surface swabbing technique depended on the presence of fluorescein in the liquid. If fluorescein was used, the direction of swabbing began from the least fluorescent areas to the most fluorescent areas when exposed to UV radiation. The direction of swabbing was random if no fluorescein was used. At the end of each test, cleaning was done with 70° alcohol.

Selective agars are directly inoculated by the swabs in order to obtain microbiological cultures. The plates were cultured at 37°C between 48 h to five days. Colonies were counted and identification was carried out with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Brucker Daltonics, Bremen, Germany). The bacterial count was considered uncountable beyond 100 cfu on the agar plate.

The data were collected using an Excel® spreadsheet and analysed with R software (Foundation for Statistical Computing, Vienna, Austria).

Results

Projections into the environment

Optimal urinal bottle emptying height

Six tests were performed at emptying heights between 30 cm and 40 cm, three between 50 cm and 60 cm, and three at the level of the toilet bowl. The number of splash-drops varied from one test and from one area to another (data not shown).

Regardless of the emptying height, the area most frequently exposed to splash-drops was the contour of the toilet bowl. For the tests with emptying at the bowl level, the median number of splash-drops was 52 whereas it was >100 for the two other emptying heights tested.

Unlike other heights, the median number of splash-drops was never >100 for the emptying tests performed at the toilet bowl level, which was selected for all the following tests.

Assessment of projection levels by area

Seven healthcare workers performed 36 tests by emptying the urinal bottle at the level of the toilet bowl. Projection levels were heterogeneous across areas and tests (Figure 1). Splash-drops were observed at the toilet bowl contour ($N = 36$), on the underside of the toilet seat ($N = 34$), inside the

sink ($N = 34$), the sink contour ($N = 30$), on the space between the toilet bowl and the wall ($N = 29$), and on the gloves ($N = 14$). The other points had few or no splash-drops.

The highest-level projection areas were the contour of the toilet bowl (median: 66.5; interquartile range: 55.5–102.5), inside the sink (5; 4–9), the underside of the toilet seat (2; 1–5), the sink contour (2; 1–3), and the space between the toilet bowl contour and the wall (1.5; 1–3).

Environmental contamination

Since the results of the first part showed a low level of contamination of the clothing, the study focused on the environment, on six areas at risk with frequent hand contact: inside the sink, sink contour, tap, flush button, underside of the toilet seat, and top of the toilet seat. For the tests performed with the OXA-48 carbapenem-resistant *C. freundii*, an additional sampling into a building drain line was performed.

For each micro-organism, 12 tests were performed three times, before, immediately after and 3 h after the challenge; thus 216 samples per micro-organism (252 for *C. freundii*) were taken, for a total of 900 samples. All 48 control samples before emptying colonized urine were negative. After emptying, 28% (84/300) of samples were positive. For all micro-organisms, the level of contamination in each area varied from one test to another (Figure 2). The most contaminated areas were inside the sink (40 positive tests) and the sink contour (22 positive tests). The less contaminated areas were the top of the toilet seat (two positive tests) and the flush button (four positive tests). The most contaminated areas (>100 cfu) were inside the sink ($N = 14$) and the contour of the sink ($N = 4$). In all 48 tests performed at 3 h, none of the 300 surface samples was positive.

Discussion

This experimental study showed that emptying the urinal bottle at the toilet bowl resulted in the fewest splash-drops. The area with the highest risk of projections was the contour of the toilet bowl. Except for gloves, professional attire was rarely contaminated. Of the six areas with the highest risk of hand contamination, the most frequently contaminated areas were inside the sink, the sink contour, the tap, and the underside of the toilet seat. The surfaces with the most bacterial contamination were the contour of the sink and the flush button. No samples taken 3 h after the test showed persistence of micro-organisms.

To our knowledge, no studies have assessed the risk of bacterial transmission associated with handling urinal bottles in hospital. Our results showed that emptying colonized urine into the toilet and rinsing the urinal bottle in the bathroom presents a potential risk of transmission to the wardmate, since contaminated environmental areas may come into contact with hands.

We used identical experimental conditions, using a single toilet cabinet in order to limit the variability associated with their layout. Consequently, the results did not take into account the variability related to the equipment, such as flushing rate, layout, limescale level, etc. Our results suggest that under these experimental conditions, with a low risk of projection, emptying and rinsing urinal bottles in the bathroom represents a potential risk of transmission to other patients through the environment.

The contamination of the sinks was considerable whereas the urinal bottles were exclusively emptied into the toilet. Sink contamination has likely occurred during the urinal bottle filling with sink water and then rinsing into the toilets. During this step, tap water may spray and aerosolize the contaminated liquid remaining at the bottom of the urinal bottle, and contaminated splash-drops can settle on the sink's surface. Contamination of toilets likely occurs with splashing when emptying the urinal bottle.

Splash-drops could be distant from the emptying and rinsing points, including flush button. Flush button was not contaminated by a contaminated gloved hand, since flush button was activated with the other hand than that used for rinsing

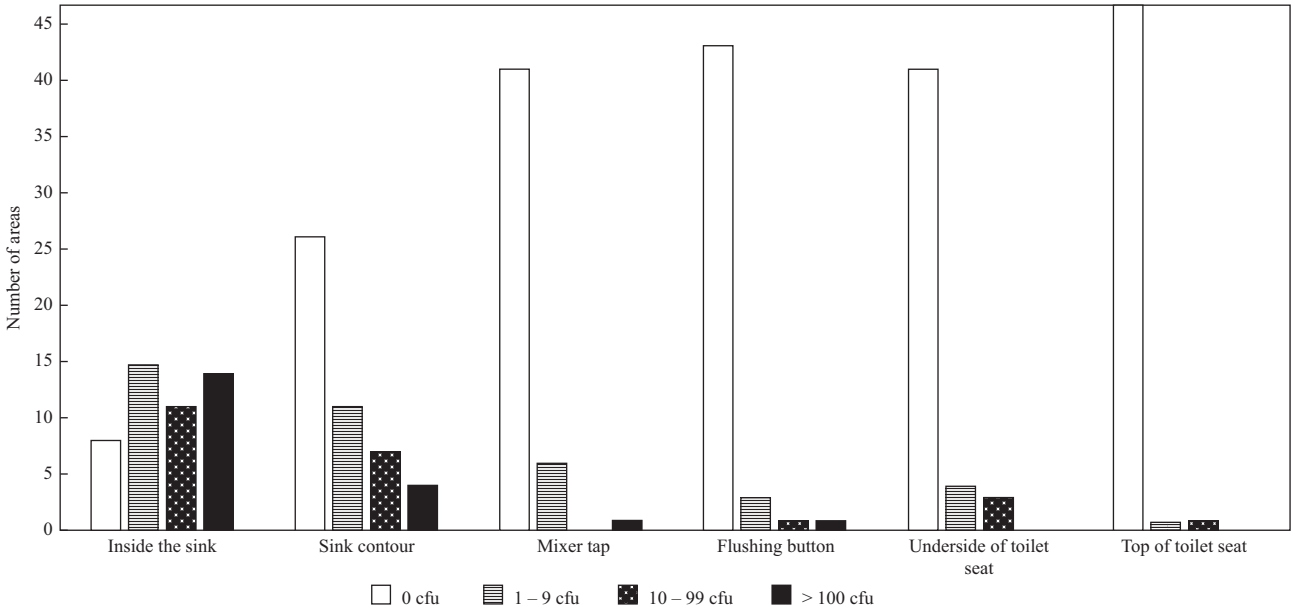

Figure 2. Number of positive samples by colony-forming unit (cfu) class according to areas for all four micro-organisms ($N = 48$).

and emptying the urinal bottle. This contamination may be the result of droplet long-distance splash-drops.

Samples at 3 h were negative, which can be explained by the lack of patient use of the toilet cabinet during the tests, with rapidly drying surfaces. A higher bacterial load may also play a role in bacterial persistence in the environment, but the choice of an initial concentration of 10^6 cfu/mL corresponded to standard colonized urine. The organic matter, present in patient secretions, may play a role by protecting Entero-bacterales from dying when dried. The absence of organic matter in the tests could hinder the survival of bacteria. To persist in the environment, Enterobacterales participate in the formation and colonization of biofilm. Biofilm development depends on the bacterial species and can take several hours or days [19,20]. In the hospital setting, surface cleaning is usually performed once a day. In our study, disinfection of the surfaces with 70% alcohol was systematically performed before and after each test, possibly preventing the formation of biofilm and making it difficult for bacteria to survive.

Contamination of professional attire was not evaluated, but splash-drops were found, particularly on gloves, which may cause cross-transmission if standard precautions are not followed. The contamination study only involved areas that could come into contact with hands. Contamination of the toilet bowl was assessed, but this area is at high risk of projection and may be an environmental niche.

The contamination risk associated with the management and storage of urinal bottles after emptying and flushing was not assessed. If not washed in a bedpan washer, urinal bottles stored in the patient's room may be a reservoir for patient, staff, and environment contamination.

Unlike contamination of distal water points or the patient's room environment, the environmental risk of transmission associated with excreta has rarely been described in hospital [7,16,21]. Our study focused on a very common practice and confirms the need to ban rinsing of urinal bottles in the sink, and to use bedpan and urinal washers. The risk associated with urine colonized at a concentration of 10^6 cfu/mL is transient, but it can be very different for stools with a bacterial burden of up to 10^{10} cfu/g. Apart from hand transmission, other modes of transmission related to excreta management still need to be evaluated [6,22].

In conclusion, emptying urinal bottles in the toilet followed by rinsing of the urinal bottle in a sink is a common practice in healthcare settings, and should be banned. If urine is colonized by resistant Enterobacterales there is a risk of projections and environmental contamination, but this risk was transient in experimental conditions. When using reusable urinal bottles, it is important to remind caregivers to empty and wash them at bedpan washers to limit environmental contamination. The availability of a limited number of bedpan washers, sometimes away from the point of care, may lead to deviations in practices with a potential for cross-transmission.

Conflict of interest statement
None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2020.07.033.

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