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CITATION

Judge, Christopher; Galvin, Sandra; Burke, Liam; Thomas, Toney; Humphreys, Hilary; Fitzgerald-Hughes, Deirdre (2013): Search and you will find: detecting extended-spectrum β-lactamase-producing Klebsiella pneumoniae from a patient's immediate environment.. Royal College of Surgeons in Ireland. Journal contribution. https://hdl.handle.net/10779/rcsi.10768832.v1

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Contamination of the immediate environment of a patient with an ESBL-producing *Klebsiella pneumoniae.*

Running Title: Recovery of *K. pneumoniae* from surfaces

Word Count : 890

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Contamination of inanimate surfaces contribute to the transmission of healthcare-associated infection which is well documented for methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci VRE (3, 5, 10). The high rate of skin colonisation with these bacteria among healthcare workers increases the risk of cross-contamination of high-touch surfaces (6). Since Gram-negative bacteria survive poorly on surfaces, their role in transmission of infection has not been as widely investigated. Extended spectrum beta-lactamase-producing enterobacteriaciae (ESBL-PE) are now widespread and endemic in nosocomial settings (2, 4) and given the increasing prevalence of infections involving ESBL-PE, the role of the environment in ESBL-PE transmission should be explored. This study reports the evaluation of two ESBL-PE recovery methods from typical hospital surface materials and their application for recovery of ESBL-PE adjacent to an ESBL-positive patient.

Recovery methods were optimized and evaluated first in the laboratory by determining the limit of detection (LoD) when serially diluted suspensions of *E. coli*; ATCC 35218, *K. pneumoniae*; ATCC 700603 or NCTC 13465 were applied to representative hospital environment surfaces (i.e. mattress section, polished steel, formica). Recovery was achieved using contact plates of Brilliance UTI (Oxoid, UK) supplemented with 1 μg/ml cefotaxime. Plates were incubated at 37°C for 16-20h and bacteria were presumptively identified based on colony colour on Brilliance UTI (e.g. *E. coli*, pink, Klebsiella, dark blue). This method demonstrated the recovery of all strains tested, up to 1.5h (steel) and 2h (mattress and formica), following contamination but no recovery at 2.5h. The LoD, defined as the lowest number of colony forming units (cfu) applied per cm^2 that allowed recovery of viable ESBL-PE, was 5.6 cfu/cm^2 (mattress and formica) and 44 cfu/ml (steel). Recovery rates, based on the approximate surface area screened,

were low and highly variable (2.1, 4.2, 5.5 % for each surface respectively). A swab method was evaluated for mattress sections only. Swabs (Copan SRK, Brescia, Italy) were pre-moistened in recovery diluent before sampling the contaminated surface and returned to the sample diluent for 20 min. The swabs were sub-cultured to Brilliance ESBL agar plates (Oxoid, UK) and incubated as for contact plates. The LoD for this method was 5.6 cfu/ml.

Following laboratory evaluation of the recovery methods, they were applied in the hospital environment. Four high-touch surfaces adjacent to three ESBL-positive patients (bed handrail (steel), mattress cover, bedside locker (formica) and bedside light switch) and two sites in shared bathrooms (sink faucets and shower handrails (steel)) were sampled. Environmental screening adjacent to one of three ESBL patients yielded ESBL-producing *K. pneumoniae* from four out of six sites sampled, which was confirmed using the BD Phoenix automated system for identification and antimicrobial susceptibility testing (Becton Dickinson, NJ, USA). *K. pneumoniae* was also recovered from the patient's urine. All environmental isolates and the patient isolate were resistant to: cefepime, ceftazidime, ceftriaxone, cefuroxime and a combination of amoxicillin and clavulanic acid. ESBL production was confirmed by ESBL disk diffusion phenotypic confirmatory tests using MASTDISC™ (Cefepime-Cefepime/Clavulanic Acid ESBL ID Disc Sets, Mast Diagnostics, UK) performed and interpreted using Clinical Laboratory Standards Institute (CLSI) guidelines (1). Pulsed field gel electrophoresis (PFGE) was performed on *Xba1*-digested genomic DNA from environmental isolates and the patient isolate using the Pulsenet standardized laboratory protocol for *E. coli* (9). Analysis of banding patterns using Bionumerics software (Ver. 6.5, Applied Maths NV, Belgium) indicated that the isolates were within 90-100% genetically related

Both methods were effective for the recovery of ESBL-PE from high-touch surfaces adjacent to one of three patients with confirmed ESBL-PE infection. While the contact plate

method was useful for flat surfaces, the Eswab method can be used for irregular surfaces (e.g. sink faucets). Four of 18 sites sampled (22 %) were positive for ESBL-producing *K. pneumoniae*. All four sites were either adjacent to a single patient with a confirmed ESBL-*K. pneumoniae* urinary tract infection or in the communal bathroom which in this case was also adjacent to the patient. Given the low detection limit, low recovery rates and short survival times (1.5-2h), determined from laboratory testing, the recovery of even small numbers of ESBL-PE from surfaces suggests a relatively high initial ESBL-PE burden and that the contamination occurred within a short time prior to sampling. This was despite routine ward cleaning which took place less than 3h before sampling. Furthermore, the recovered isolates were indistinguishable from the isolate causing the urinary tract infection. This suggests patient contamination of the environment or *vice-versa*. Although relatively few studies have confirmed environmental contamination with ESBL-PE, one recent study carried out over a nine month period showed recovery of ESBL-PE from 48/370 (4 %) sites, the majority of which were *K. pneumonia*e (89 %)(7). Although environmental contamination with ESBL-PE is not believed to be as common or extensive as for MRSA and VRE (8), the present findings suggest that frequently hand-touched surfaces adjacent to ESBL-PE-positive patients and communal bathrooms, may be an overlooked reservoir for transmission. The poor recovery rates found with the methods described here, suggest that although these organisms are viable, they may be either non-culturable or difficult to culture from the environmental setting and more sophisticated methods are required to recover them. Unlike the recommendations for MRSA/VRE-positive patients, strict isolation policies are not generally enforced for infections involving ESBL-PE but as this study reveals, there may be a case for reviewing hygiene measures pertinent to some ESBL-positive patients.

Acknowledgements.

The authors wish to thank Dr. Celine Herra, Dublin Institute of Technology and Joan Moore and the staff of the Microbiology Laboratory, Beaumont Hospital, for their assistance. This work was partly funded by the Health Research Board of Ireland under Grant No. PHD/2007/11.

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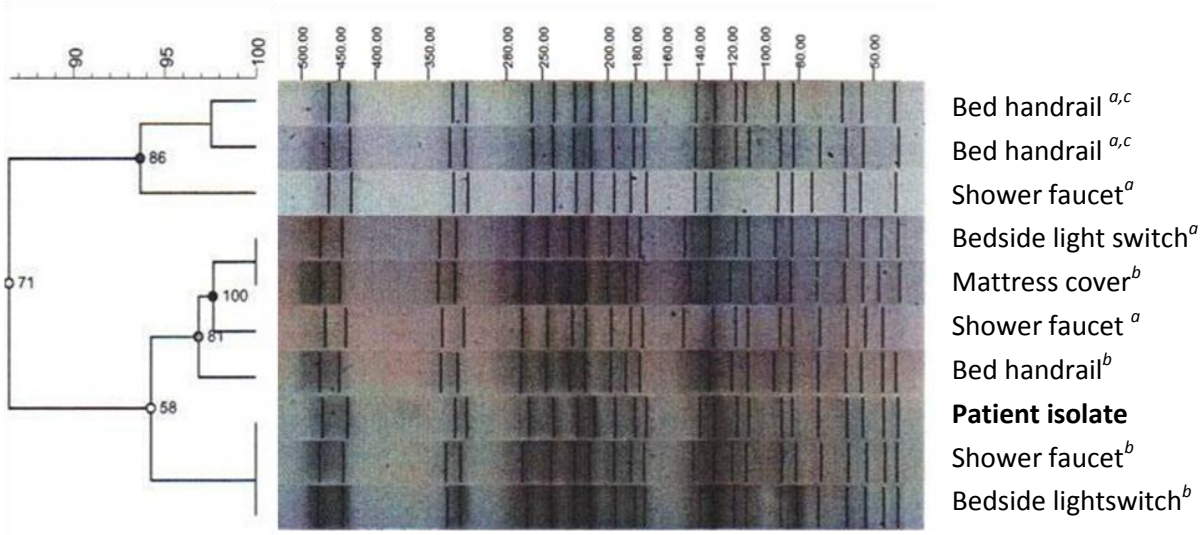

Figure 1. Dendrogram showing PFGE profiles for isolates recovered from sites adjacent to an ESBL-positive patient and the patient isolate causing infection. Pair-wise cluster analysis was performed using the Dice coefficient with an optimisation of 1% and a band matching tolerance of 1%. [a] isolate recovered using UTI-CTX contact plate, [b] isolate recovered using Eswab, [c] isolates were from two separate colonies from the original contact plate.