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

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

36 Recovery methods were optimized and evaluated first in the laboratory by determining
37 the limit of detection (LoD) when serially diluted suspensions of *E. coli*; ATCC 35218, *K.*
38 *pneumoniae*; ATCC 700603 or NCTC 13465 were applied to representative hospital
39 environment surfaces (i.e. mattress section, polished steel, formica). Recovery was achieved using
40 contact plates of Brilliance UTI (Oxoid, UK) supplemented with 1 µg/ml cefotaxime. Plates
41 were incubated at 37°C for 16-20h and bacteria were presumptively identified based on colony
42 colour on Brilliance UTI (e.g. *E. coli*, pink, *Klebsiella*, dark blue). This method demonstrated the
43 recovery of all strains tested, up to 1.5h (steel) and 2h (mattress and formica), following
44 contamination but no recovery at 2.5h. The LoD, defined as the lowest number of colony forming
45 units (cfu) applied per cm² that allowed recovery of viable ESBL-PE, was 5.6 cfu/cm² (mattress
46 and formica) and 44 cfu/ml (steel). Recovery rates, based on the approximate surface area screened,

47 were low and highly variable (2.1, 4.2, 5.5 % for each surface respectively). A swab method was
48 evaluated for mattress sections only. Swabs (Copan SRK, Brescia, Italy) were pre-moistened in
49 recovery diluent before sampling the contaminated surface and returned to the sample diluent for
50 20 min. The swabs were sub-cultured to Brilliance ESBL agar plates (Oxoid, UK) and incubated
51 as for contact plates. The LoD for this method was 5.6 cfu/ml.

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

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

71 method was useful for flat surfaces, the Eswab method can be used for irregular surfaces (e.g.
72 sink faucets). Four of 18 sites sampled (22 %) were positive for ESBL-producing *K.*
73 *pneumoniae*. All four sites were either adjacent to a single patient with a confirmed ESBL-*K.*
74 *pneumoniae* urinary tract infection or in the communal bathroom which in this case was also
75 adjacent to the patient. Given the low detection limit, low recovery rates and short survival times
76 (1.5-2h), determined from laboratory testing, the recovery of even small numbers of ESBL-PE
77 from surfaces suggests a relatively high initial ESBL-PE burden and that the contamination
78 occurred within a short time prior to sampling. This was despite routine ward cleaning which
79 took place less than 3h before sampling. Furthermore, the recovered isolates were
80 indistinguishable from the isolate causing the urinary tract infection. This suggests patient
81 contamination of the environment or *vice-versa*. Although relatively few studies have confirmed
82 environmental contamination with ESBL-PE, one recent study carried out over a nine month
83 period showed recovery of ESBL-PE from 48/370 (4 %) sites, the majority of which were *K.*
84 *pneumoniae* (89 %)(7). Although environmental contamination with ESBL-PE is not believed to
85 be as common or extensive as for MRSA and VRE (8), the present findings suggest that
86 frequently hand-touched surfaces adjacent to ESBL-PE-positive patients and communal
87 bathrooms, may be an overlooked reservoir for transmission. The poor recovery rates found with
88 the methods described here, suggest that although these organisms are viable, they may be either
89 non-culturable or difficult to culture from the environmental setting and more sophisticated
90 methods are required to recover them. Unlike the recommendations for MRSA/VRE-positive
91 patients, strict isolation policies are not generally enforced for infections involving ESBL-PE but
92 as this study reveals, there may be a case for reviewing hygiene measures pertinent to some
93 ESBL-positive patients.

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103 antimicrobial susceptibility testing. 20th informational supplement. Wayne, PA.
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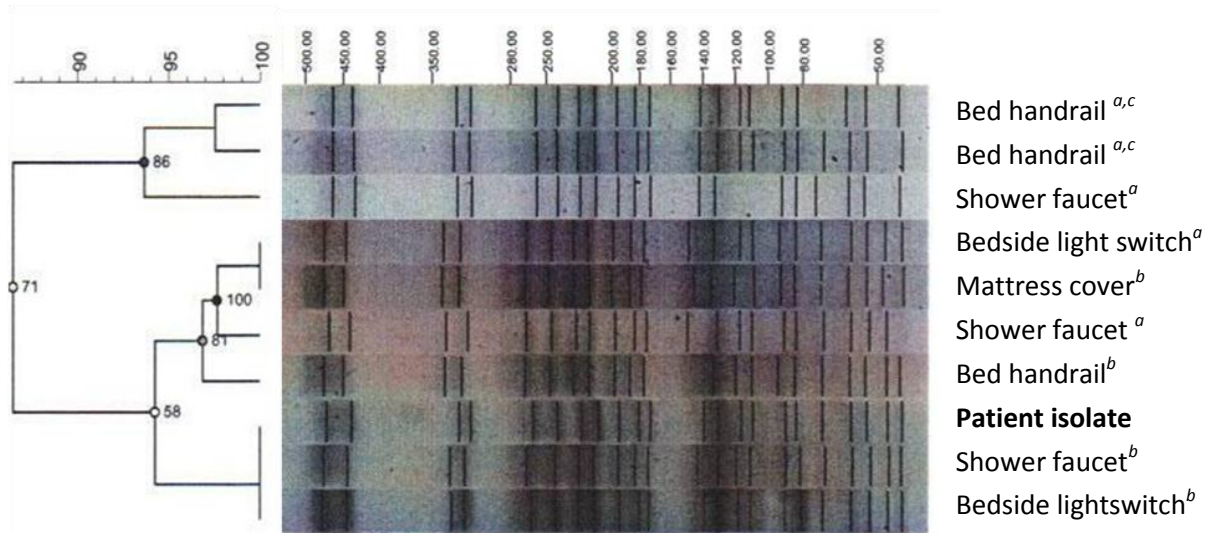
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146 **Figure 1.** Dendrogram showing PFGE profiles for isolates recovered from sites adjacent to an
 147 ESBL-positive patient and the patient isolate causing infection. Pair-wise cluster analysis was
 148 performed using the Dice coefficient with an optimisation of 1% and a band matching tolerance
 149 of 1%. ^a isolate recovered using UTI-CTX contact plate, ^b isolate recovered using Eswab, ^c
 150 isolates were from two separate colonies from the original contact plate.

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