THE INVASIVE STRATEGIES OF ENTEROCOCCUS FAECALIS IN LUTS

Hypothesis / aims of study
Anderson et al. (1) reported intracellular bacterial colonies in a murine model of acute UTI infected with E. coli. Subsequently, the phenomenon was described in humans suffering from acute UTI (2). Although E. coli is thought to be the foremost invasive uropathogen, some other bacteria have also been found to invade the urothelium (2). E. faecalis accounts for a significant proportion of acute and chronic bladder infections worldwide (3), although the invasive capabilities of this bacteria has yet to be reported. Previous studies have relied on microbiological techniques for proof of intracellular colonisation. These methods are indirect, so confocal microscopy and digital analyses were deployed to test the hypothesis that E. faecalis exhibits host cell invasion as part of a uropathogenic lifestyle.

Study design, materials and methods
Five strains of cryo-stored E. faecalis were previously isolated from five LUTS patients exhibiting less than $10^5$ CFU ml$^{-1}$ of growth on routine MSU culture. A bladder transitional cell carcinoma cell line (T24) was grown to 80% confluency on pre-coated chamber slides before infecting with E. faecalis at an A600 of ~0.5. 3.5 hrs post infection, the cells were incubated for a further 24 hrs in a combination of membrane-impermeable antibiotics to kill extracellular bacteria. In preparation for imaging, cells were fixed in 4% formaldehyde and fluorescently labeled with DAPI (DNA), wheat germ agglutinin (cell membrane) and phalloidin (F-actin). High resolution, 3-channel Z-stacks comprising at least 100 slices were taken of each cell using a confocal microscope before extensive digital analysis.

Results
3-dimensional analyses of T24 cells challenged with each of the five strains of E. faecalis show considerable intracellular colonization (Fig 1). Exploration of these cells using Z-axis profile plots, whereby the average light intensity of DAPI (bacteria) and phalloidin (cytoskeletal actin) is calculated at each slice through the Z-stack, show further definitive proof of the invasive ability of E. faecalis (Fig 2).

Interpretation of results
This is the first time that E. faecalis has been reported to definitively invade a cell. Considering that these strains were isolated from routine-culture negative LUTS patients, it is quite possible that invasive E. faecalis may be responsible for some LUTS.

Concluding message
These data suggest that some LUTS may be generated by low-grade intracellular infection of the bladder by E. faecalis. These results therefore may have far-reaching implications for our diagnosis, treatment and understanding of the aetiology of LUTS.

Figure 1. 3-D model of an entire DAPI labelled T24 cell infected with E. faecalis. A large cluster of intracellular E. faecalis (white arrow) can be seen in close proximity to the T24 nucleus (broken white arrow).
Figure 2. (A) 3-D volume through entire Z-stack showing extensive intracellular colonization by DAPI (blue) labelled *E. faecalis*. (B) Z-axis profile plot presents the average pixel intensity of a given channel moving through the Z-stack. It can be clearly seen that actin filaments (red) surround the invading *E. faecalis* (blue).

References

Disclosures
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