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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

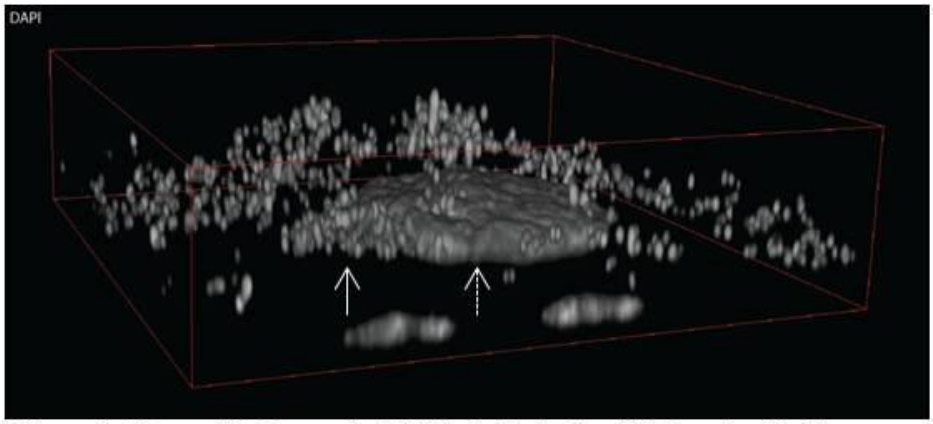

Figure 1. 3-D model of an entire DAPI labelled T24 cell infected with *E. faecalis*. A large cluster of intracellular *E. faecalis* (white arrow) can be seen in close proximity to the T24 nucleus (broken white arrow).

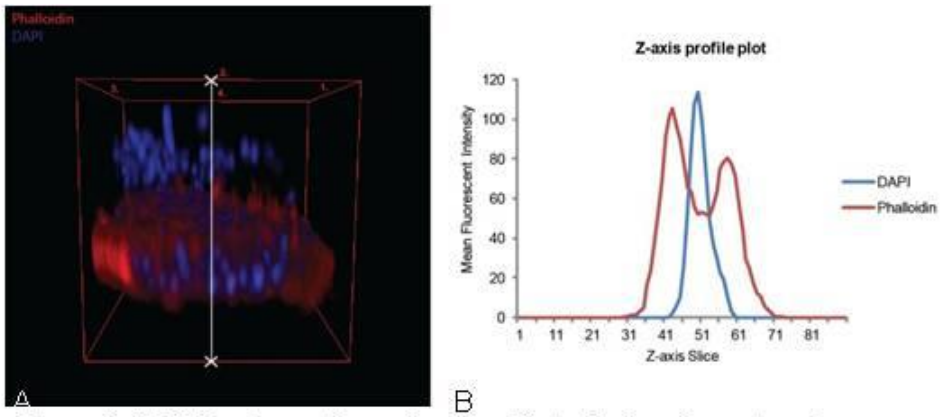

Figure 2. (A) 3-D volume through entire Z-stack showing extensive intracellular colonisation by DAPI (blue) labelled *E. faecalis*. (B) Z-axis profile plot presents the average pixel intensity of a given channel moving through the Z-stack. It can be clearly seen that actin filaments (red) surround the invading *E. faecalis* (blue).

References

1. Science 2003 Jul 4;301(5629):105-7.
2. Annu Rev Microbiol 2010;64:203.
3. BMC Infectious Diseases 2012;12(1):320.

Disclosures

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