Increased Human Pathogenic Potential of *Escherichia coli* from Polymicrobial Urinary Tract Infections in Comparison to Isolates from Monomicrobial Culture Samples

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Abstract

The current diagnostic standard procedure outlined by the HPA for urinary tract infections (UTI) in clinical laboratories does not report bacteria isolated from samples containing 3 or more different bacterial species. As a result many UTI go unreported and untreated, particularly in elderly patients, where polymicrobial UTI samples are especially prevalent. This study reports the presence of the major uropathogenic species in mixed culture urine samples from elderly patients, and of resistance to front line antibiotics, with potentially increased levels of resistance to ciprofloxacin and trimethoprim. Most importantly, the study highlights that *E. coli* present in polymicrobial UTI samples are statistically more invasive ($P < 0.001$) in *in vitro* epithelial cell infection assays than those isolated from monomicrobial culture samples. In summary this study suggests that the current diagnostic standard procedure for polymicrobial UTI samples needs to be re-assessed, and that *E. coli* present in polymicrobial UTI samples may pose an increased risk to human health.

Key words: Urinary tract infection, polymicrobial, *E. coli*, antimicrobial, invasion

Introduction

The annual incidence of UTI in the elderly population ranges from 10% in the community to as high as 30% of hospitalised patients (Cove-Smith & Almond, 2007). Mortality rates in elderly patients from bacteraemia as a result of UTI can be as high as 33% (Tal *et al*., 2005). A study by Plowman *et al*. (2001) found that over a 12-month period urinary tract infections had the highest incidence (35%) of all nosocomial infections in a district general hospital, and the majority of patients were over 60 years of age. UTI are also the most common infection in long term care facilities, where they account for 20-60% of all antibiotic prescriptions (Nicolle, 2001a). This large-scale prescription of antibiotics may well contribute to the levels of antibiotic resistance in urinary pathogens (Zhanel *et al*., 2005). The main etiologic agent of UTI is well documented as *E. coli* (Nicolle *et al*., 2001b, Farajnia *et al*., 2008, & Kumazawa & Matsumoto, 1997). The establishment of urinary tract pathogens is thought to begin with the invasion of the superficial bladder epithelium, where bacteria can form intracellular communities and receive a level of protection against the host immune system and antibiotic treatment (Anderson *et al*., 2003, Blango & Mulvey, 2010).

The diagnosis of UTI is routinely performed in the clinical laboratory by microbiological culture of a urine sample according to the national standard method developed and approved by the Health protection Agency (http://www.hpa-standardmethods.org.uk/documents/bsop/pdf/bsop41.pdf).

Infections caused by a single organism are usually treatable with an antibiotic regimen advised on the basis of antibiotic susceptibility tests. However, it has been estimated that 33% of urine cultures from elderly patients are polymicrobial

(Cove-Smith & Almond, 2007). Due to the difficulties of identifying the organisms present in these cultures clinical microbiology laboratories do not report organisms isolated from urine in mixed culture unless there is a significant count of a predominant organism

As a result there is insufficient information on the bacteria that cause polymicrobial UTI and the threat they pose to patient health. This study aimed to isolate the organisms present in polymicrobial urinary tract infection samples from a population of elderly patients, and compare their prevalence, phenotypic activity and pathogenic potential to monomicrobial culture isolates from the same population, with the aim of further understanding the potential threat posed by bacteria present in polymicrobial urine samples from elderly patients, and possibly reassessing the current diagnostic standard procedure.

Materials and Methods

Sample collection

Two hundred and fifty urine culture agar plates were collected from Nottingham University Hospitals (NUH) between October 2008 and June 2009. Cultures were collected anonymously (therefore no ethical approval or informed consent required) from patients aged 70 and over and assigned to one of the following categories; (1) non-catheterized male polymicrobial infection, (2) non-catheterized female polymicrobial infection, (3) catheterized male polymicrobial infection, (4) catheterized female polymicrobial infection, all of which contained 3 or more organisms with no predominant count of one species. A fifth group,

monomicrobial infection (uncatheterized or catheterized, males or females), was also included for use as a control group. Bacteria were isolated using standard microbiological identification procedures. Identification of isolates to species level was performed using the API identification systems, API 20E, API 20strep, API 20NE and API Staph (Biomerieux,).

Antibiotic susceptibility testing

A breakpoint method was employed to obtain antibiotic susceptibility profiles for all *E. coli, S. aureus, E. faecalis* and *P. mirabilis*. The BSAC method for antimicrobial susceptibility testing was followed to prepare standardised inocula (Andrews, 2009). The antibiotic panel used was as follows; Gentamicin (2µg/ml), Cefotaxime (1 µg/ml), Ceftazidime (1 µg/ml), Meropenem (2 µg/ml), Piperacillin-tazobactam (16 µg/ml), Co-amoxiclav (32 µg/ml), Trimethoprim (2 µg/ml), Ciprofloxacin (4 µg/ml), Cephradine (32 µg/ml), Nitrofurantoin (32 µg/ml), and Amoxicillin (32 µg/ml). All *P. aeruginosa* isolates were tested using the BSAC disc diffusion method (Andrews, 2009) and an antibiotic panel specific to *Pseudomonas*; Gentamicin (10µg/ml), Piperacillin-tazobactam (85 µg/ml), Ceftazidime (30 µg/ml), Meropenem (10 µg/ml) and Ciprofloxacin (1 µg/ml).

PCR detection of β-lactamase genes

All *E. coli, Klebsiella, Proteus, Pseudomonas, Enterobacter* and *Citrobacter* isolates were tested for the presence of the β-lactamase genes, bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$ and bla_{OXA} using a previously described multiplex PCR (Fang *et al*., 2008). GenElute extraction kits (SIGMA) were used to extract bacterial genomic DNA. Reference strains containing known β-lactamase types (NCTC 13351 *E.*

coli bla_{TEM-3}, NCTC 13353 *E. coli* bla_{OXA}, $bla_{CTX-M-15}$, and bla_{TEM} and NCTC 13368 *Klebsiella pneumoniae* bla_{TEM}, bla_{SHV-18}) were included as controls.

ESBL confirmation tests

ESBL combination ID discs were used to confirm the extended spectrum activity of selected β-lactamase positive strains. The BSAC method for antimicrobial susceptibility testing (Andrews, 2009) was followed to prepare a standardised inoculum, which was used to inoculate an Iso-Sensitest agar plate to produce a lawn of growth. Two sets of combination discs were used to optimise ESBL detection, Cefpodoxime/Cefpodoxime&clavulanate and Cefepime/Cefepime&clavulanate. An increase in zone of inhibition diameter of ≥5mm of the cephalosporin/clavulanic acid disc compared to the cephalosporin alone indicated the presence of an ESBL-producing organism. Reference organisms *E. coli* NCTC 13351, *E. coli* NCTC 13352 and *E. coli* NCTC 13353 and *E. coli* NCTC 10418 (HPA culture collections) were used for control purposes.

Detection of vancomycin resistant enterococci, MRSA and Panton-Valentine Leukocidin producing *S. aureus*

A previously published protocol, (Jayaratne *et al*., 1999) was used to screen all *E. faecalis* and *E. faecium* isolates for the presence of *van*A and *van*B genes. GenElute extraction kits (SIGMA) were used to extract bacterial genomic DNA. *E. faecalis* NCTC 12201 was used as a reference strain.

A real-time PCR protocol developed by Thomas *et al*, (2007) was used to detect the presence of the *mec*A gene, which confers resistance to methicillin, in all *Staphylococcus aureus* isolates. PCR was also performed for the detection of the

PVL-encoding gene, *lukF*, using a previously published protocol (Ribeiro *et al*., 2005). The BSAC disc diffusion protocol (Andrews, 2009) was followed to test all *S. aureus* isolates for phenotypic resistance to cefoxitin, which is indicative of methicillin resistance, and a selection of *Enterococcus* isolates for phenotypic resistance to vancomycin.

BOX-PCR

To confirm the organisms isolated from the UTI cultures were not all related to a single locally disseminated clone, BOX-PCR was performed using a protocol adapted from that of Koeuth *et al*, (1995). Extraintestinal pathogenic *Escherichia coli* (ExPEC) CFT073 was used as a reference strain. PCR products were examined using bionumerics software (BioNumerics v.3.5, Applied Maths), using CFT073 to monitor the repeatability of the experiment.

Cell cultures

T24 human epithelial cells (HPA cultures) were grown in McCoy's 5A modified media (Sigma, UK) supplemented with 10% fetal bovine serum (Sigma, UK) and 0.75% L-glutamine (Sigma, UK). Cells were grown in an atmosphere with 5% CO_2 at 37^{o}C and sub-cultured twice-weekly. Two days prior to cell infection assays, the T24 cells were seeded into 24-well plates

Association and invasion assays

All *E.* coli assays were performed in duplicate on different days, and in triplicate wells in each assay. Bacteria were cultured overnight in LB broth, harvested by centrifugation and re-suspended in supplemented tissue culture medium, which was then adjusted to $2x10^{7}$ cfu/ml, giving an MOI of 1:100. For *E. coli*, all 129 polymicrobial ExPEC and 21 monomicrobial ExPEC isolated in this study were

quantifiably assayed using classical gentamicin protection assays as described previously (McNally *et al*., 2007), using cultured T24 bladder cells. The invasive ExPEC type strain, CFT073 was used as a positive control strain in all assays and *E.coli* DH5α was used as a negative control strain. The mean number of invasive bacteria was determined by Miles & Misra plate counts from triplicate wells. Strains that showed more than a 10-fold increase in invasion compared to CFT073 were classed as highly-invasive strains. Those that showed more than a 10-fold decrease in invasion compared to CFT073 were deemed to be strains of limited invasive potential.

All *E. faecalis, P. mirabilis, P. aeruginosa* and *S. aureus* were subjected to a semi-quantitative screen performed in triplicate on different days to assess levels of invasion. Assays were completed using the above method, which was adapted for 96-well plates as described by Javed *et al*, (2009).

Statistical analysis

χ^2 tests were performed to compare invasion and antimicrobial resistance between the monomicrobial culture and mixed culture populations, and also bacterial prevalence in different patient groups.

Results

Similar bacterial species are isolated from monomicrobial and polymicrobial UTI samples

Urine culture plates were collected from 250 patients over a 9-month period from Nottingham University Hospitals (NUH) and were taken from both hospitalised and community patients, with the median age of patients being 83.5 years. Of the

200 polymicrobial cultures collected, 71 (36%) contained 2 organisms, 90 (45%) contained 3 organisms, 36 (18%) contained 4 organisms and 3 (1%) contained 5 organisms. Eighty three patients (33%) had previous history of UTI, 27 cases of which were designated as 'mixed' by the NUH clinical laboratory. Of these 27 patients presenting with a polymicrobial UTI, 13 went on to have further incidences of polymicrobial UTI after this study, 5 patients went on to have a UTI sample with a confirmed organism in monomicrobial culture and 9 had no further infections.

A total of 620 bacterial strains were isolated from the 250 urine cultures and identified to species level using API identification systems (figure 1). The most predominant organism in both catheterised and uncatheterised patients was *E. coli,* which was also far more prevalent in female patient cultures (83%) than male cultures (46%) $P<0.001$. In male samples *E. faecalis* was equally as ubiquitous as *E. coli*. These two organisms were also frequently associated in mixed cultures (36%). *E. coli* was isolated from 68% of polymicrobial cultures and 48% of monomicrobial cultures, and *E. faecalis* was isolated from 55% of polymicrobial cultures, but was significantly less frequently isolated from monomicrobial cultures (8%), $P<0.001$. *P. aeruginosa, P. mirabilis* and *S. aureus* were also frequently isolated from 23%, 25% and 10.5% of polymicrobial cultures respectively.

BOX-PCR profiles provide evidence of a genetically diverse population

In order to ensure that the organisms isolated were not epidemic clones, clonal relatedness of *E. coli, E. faecalis, P. mirabilis, P. aeruginosa* and *S. aureus* isolates was determined by BOX–PCR. BOX-group numbers were allocated

based upon a similarity threshold of 92% which was decided upon after reviewing previous publications (Proudy *et al*., 2008, & Yang *et al*., 2004). The *E.* coli strains exhibited varying BOX-profiles (figure 2) and did not appear to belong to a dominant epidemic clone. Nineteen distinct clonal groups of *E. coli* were assigned, the largest of which encompassed 71 of the total 150 *E. coli* strains in the collection. Eleven distinct BOX-groups of *E. faecalis* were detected, with the majority of isolates belonging to 2 of the groups, group 1 containing 53 isolates and group 3 containing 37 isolates. Eighteen of the 51 *P. aeruginosa* isolates were deemed to belong to the same BOX-group, and other isolates were also allocated to 11 other BOX-groups. *P. mirabilis* were allocated 5 BOX-groups in total, with 88% of strains found to belong to a single BOX-group. *S. aureus* were allocated to 7 BOX-groups with 50% of the total strains belonging to one group. These results suggest that the isolates are not derived from a single epidemic clone of each species, and are indeed individual strains which could reasonably be expected to possess varying phenotypic and genotypic properties.

Antibiotic resistance is comparable between bacteria from mixed and monomicrobial cultures

To determine the specific antibiotic resistances within the population of polymicrobial UTI organisms, 394 isolates of the 5 most commonly isolated species (*E. coli, E. faecalis, P. aeruginosa, P. mirabilis* and *S.* aureus) were subjected to antibiotic susceptibility tests using an antibiotic panel presently used in the NUH clinical laboratory (table 1). Results suggest that bacteria isolated from mixed culture samples exhibit comparable levels of resistance to front line antibiotics as that observed in isolates from monomicrobial culture samples,

regardless of species. With respect to *E. coli*, the percentage of mixed culture sample isolates exhibiting resistance to ciprofloxacin and trimethoprim was higher than that observed in monomicrobial culture sample isolates, and also higher than the levels observed routinely in clinical urinary tract infection isolates. The differences observed in this study are not statistically significant but require further specific investigation.

Increased detection of Extended Spectrum β-Lactamases in monomicrobial culture isolates

Due to the increasing prevalence of extended-spectrum β-lactamases (ESBLs) in clinical samples the UTI isolates were screened both for the possession of β-lactamase genes and also for any ESBL phenotypic activity. All *E. coli, Enterobacter*, *Citrobacter, Klebsiella, Proteus* and *Pseudomonas* strains (n=355) were screened for the presence of bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, and bla_{OXA} genes using a previously published protocol (Fang *et al*., 2008). Forty-nine percent of strains were found to possess a β-lactamase gene, and one fifth of the strains possessed multiple *bla* genes (table 2). Interestingly most of the strains containing multiple β-lactamases originated from monomicrobial culture infections (P=0.009). *Klebsiella* species were found to possess the most β-lactamases (93% positive), 61% of *E. coli* also possessed a form of β-lactamase and 12% possessed multiple β-lactamase genes. The extended spectrum β-lactamase, CTX-M was found significantly more frequently in monomicrobial culture isolates than in isolates from polymicrobial infections (P=0.014). To determine phenotypic expression of extended spectrum resistance against β-lactam antibiotics the double-disc method was used to screen all 173 β-

lactamase PCR positive strains. Sixteen strains (9%) exhibited phenotypic ESBL activity (12 *E. coli*, 3 *Pseudomonas* and 1 *Enterobacter*), most of which was in strains that possessed multiple □-lactamase genes, making it impossible to identify the β-lactamase gene responsible for the extended spectrum resistance.

Detection of MRSA and PVL-producing *S. aureus* in polymicrobial UTI samples

PCR detection of the *mecA* gene, confirmed 10 (45%) of the urinary *S. aureus* isolates to be MRSA. One of these was isolated from a monomicrobial culture UTI, and the remaining 9 confirmed MRSA were isolated from polymicrobial UTI samples. Phenotypic resistance tests confirmed all PCR MRSA strains to be resistant to cefoxitin, which is an indicator of methicillin resistance. The *lukF* gene, which encodes PVL production was detected in 2 methicillin sensitive *S. aureus* (MSSA) strains, which were both isolated from polymicrobial UTI samples. No vancomycin resistance genes were found in any of the *E. faecalis* isolates, but *vanA* was found in one *E. faecium* isolate. This isolate did not however show any phenotypic resistance to vancomycin.

Bacteria isolated from polymicrobial UTI samples exhibit increased pathogenic potential in *in vitro* cell invasion assays.

The ability to invade host epithelial cells is a critical factor in UTI. Therefore all *E. coli, E. faecalis, P. aeruginosa, P. mirabilis* and *S. aureus* strains were investigated to determine their ability to invade a human uroepithelial cell line. T24 human bladder cells were infected with a bacterial culture for 3 hours, after which external bacteria were killed by the addition of gentamicin and internalised bacteria were enumerated. In the case of the *E. coli* assays, strains were

designated as highly invasive if they showed a 10-fold increase in invasiveness compared to that of the invasive reference strain, *E. coli* CFT073, which exhibited variation of less than 1 log across all assays performed (less than 10 fold). Overall 52 strains (34.7%) were seen to exhibit the highly invasive phenotype and 21 strains (14%) exhibited a low invasive phenotype, more than a 10 fold reduction in invasion compared to that of CFT073. *E. coli* isolates from mixed culture samples exhibited increases in invasion as great as 1000 fold higher than that observed in the invasive type strain CFT073. No invasive capacity was observed in five strains, of which three were isolated from monomicrobial culture samples where they were reported as the infectious agent. Overall 45% of the polymicrobial *E. coli* strains assayed invaded to a similar level (less than a ten-fold increase or decrease) as that of CFT073 (figure 3). This is not significantly different to the monomicrobial culture isolates, 62% of which showed similar invasion capability to CFT073. In contrast 44% of polymicrobial *E. coli* strains were highly invasive, whereas no monomicrobial culture isolates invaded to a greater level than CFT073 and 11% of polymicrobial isolates were classified as 'low invasive' compared to 38% of monomicrobial culture isolates. This suggests that *E. coli* isolated from polymicrobial UTI samples may be significantly more invasive ($P<0.001$, χ^2 distribution of strains with high, normal, and low invasive capacity across polymicrobial and monomicrobial populations) in an *in vitro* uroepithelial cell infection model, than *E. coli* isolated as monomicrobial cultures from UTI samples. No association could be found between invasiveness and patient gender, previous history of UTI, catheterisation status, health status of patient, antibiotic resistance profile or BOX-PCR group.

Invasion assays were also performed on all *E. faecalis, P. mirabilis, P. aeruginosa* and *S. aureus* isolates. A wide range in invasion capability was noted in *E. faecalis* isolates, ranging from 10^6cfu/ml to 10^2cfu/ml bacteria recovered from invasion assays. *P. mirabilis, P. aeruginosa* and *S. aureus* showed a similar trend with a 3-log range in invasion. Similarly the increased/decreased invasion levels are not attributable to any patient characteristic, antibiotic resistance profile or BOX-PCR group.

Discussion

Urinary tract infection rates in elderly people in the community can be as high as 10% at any one time, and this figure can rise to 30% of hospitalised patients. Bacteraemic UTI in elderly patients can result in sepsis and death (Cove-Smith & Almond, 2007). A clinical microbiology laboratory will not routinely pursue or report organisms present in mixed culture from urine samples unless there is a significant count of a predominant organism. As up to 33% of samples can be polymicrobial a large proportion of infections from the elderly population may go untreated or indeed be treated with inappropriate antibiotics. This will only serve to encourage the development of antibiotic resistance in urinary pathogens.

The predominant organism in polymicrobial UTI samples was *E. coli*, which concurs with the general consensus among previously published data concerning monomicrobial culture UTI (Farajnia *et al.*, 2008, Tal *et al.*, 2005, Johnson, 1991, & Brzuszkiewicz *et al.*, 2006). *E. faecalis* was the second most commonly isolated organism, and was significantly more prevalent in polymicrobial cultures than monomicrobial cultures ($P<0.001$). *E. faecalis* and *E. coli* were found

306 together in 36% of cultures and co-infection by these two pathogens may pose
307 important questions for the antibiotic treatment of polymicrobial UTI as
308 *Enterococcus* is known to be intrinsically resistant to many antibiotics, including
309 several first choice antibiotics for the treatment of UTI. The presence of both this
310 organism and uropathogenic *E. coli* in an infection may not only create difficulties
311 in devising an antibiotic treatment regimen but also recent studies have
312 suggested *E. faecalis* may exacerbate the pathogenicity of *E. coli* (Lavigne *et al*.,
313 2008, & Montravers *et al*., 1997).
314 Prescription of ineffective antibacterial agents can increase selection pressure for
315 antibiotic resistant agents within an infection. Organisms present in polymicrobial
316 UTI cultures, that would not routinely be investigated, possessed antibiotic
317 resistance to front line antibiotics such as trimethoprim, ciprofloxacin and
318 amoxicillin. More importantly the percentage of mixed culture sample *E. coli*
319 isolates exhibiting resistance to trimethoprim and ciprofloxacin was higher than
320 that observed in monomicrobial culture sample isolates, and also higher than the
321 levels observed routinely in clinical isolates. Another issue raising concerns for
322 patient health is the presence of MRSA and PVL-producing MSSA in
323 polymicrobial UTI samples, which would not be detected under the current
324 guidelines for UTI diagnosis. The presence of MRSA in an infection limits the
325 choice of antibiotics available for treatment, and the cytotoxin PVL attacks white
326 blood cells and can cause severe tissue necrosis (Holmes *et al*., 2005). Other
327 specific antibiotic resistance traits such as extended-spectrum β-lactamases
328 were also detected, by PCR for known β-lactamase genes, although only a small
329 proportion showed phenotypic activity. This discrepancy re-emphasises the

importance of screening clinical isolates for ESBL gene carriage as opposed to phenotypic tests (Livermore & Hawkey, 2005, Tofteland *et al*., 2007, & Xu *et al*., 2005). Interestingly, the current increase in isolation of *E. coli* 025b-ST131 CTX-M^R from clinical samples (Lau *et al*., 2008, Nicolas-Chanoine *et al*., 2008, & Vincent *et al*., 2010) does not appear to be reflected in this study, with only 11% of *E. coli* containing the $bla_{CTX\text{-}M}$ gene. The full genetic diversity and lineage of the ExPEC strains isolated in this study is the current focus of intensive research.

It has been suggested that the critical step in UTI initiation is the attachment to and invasion of the superficial bladder epithelium, especially in the case of *E. coli* (Anderson *et al*., 2003, & Mulvey *et al*., 2001). By attaching to bladder epithelial cells *E. coli* are able to establish reservoirs known as intra-cellular bacterial communities, from which the invading bacteria receive some level of protection against the host immune system and also initiate recurrent infections. Therefore invasive bacteria are considered more proficient in instigating an infection. Significant differences were identified in the invasive capabilities of monomicrobial culture and polymicrobial culture isolates (figure 3). *E. coli* isolates of polymicrobial culture origin were significantly more invasive when compared to the invasion of the type strain, *E. coli* CFT073, than strains isolated from monomicrobial culture samples. The majority of isolates from monomicrobial infections were less invasive than *E. coli* CFT073 and none exhibited a highly invasive phenotype. The polymicrobial isolates that possessed increased invasive capacity did not belong exclusively to any specific patient group and were not shown to be associated with patient gender, catheterisation status, previous history of UTI and underlying medical issues, antibiotic resistance profile

354 or BOX-PCR group. This indicates the potential existence of a heterogeneous
355 group of highly invasive *E. coli* within polymicrobial UTI in the elderly, which
356 would not be diagnosed or treated due to limitations in the current diagnostic
357 standard procedure. A further study including larger numbers of ExPEC isolates
358 from monomicrobial infections is required to rule out any such associations, and
359 indeed to confirm the significance of the increased invasive phenotype
360 exclusively observed in the polymicrobial isolates from this study. This is
361 currently under investigation in several hospital labs examining equal numbers of
362 polymicrobial and monomicrobial isolates, including an examination of the clinical
363 nature of the infections, their association with complicated or uncomplicated UTI,
364 and the genotypic and phenotypic differences associated with the hyper-invasive
365 phenotype.
366 The frequent co-isolation of *E. coli* and *E. faecalis* from the clinical UTI samples
367 raises questions as to the possible contribution of Enterococci to the increased
368 invasive phenotype expressed by the ExPEC strains isolated in this study. There
369 have been reports of Enterococci exacerbating the pathogenicity of other
370 organisms including ExPEC in both *C. elegans* and rat models of infection
371 (Lavigne *et al*., 2008, & Montravers *et al*., 1997), and the promiscuous nature of
372 Enterococci with regards to gene transfer is well known. The possibility that
373 Enterococci can alter the genotype and/or phenotype of ExPEC during co-
374 infection of bladder epithelial cells is currently the subject of further investigation.
375 Polymicrobial urinary tract infections may pose a heightened threat to the health
376 and well being of the elderly population. This study found that the organisms
377 present in polymicrobial UTI possess traits such as antibiotic resistance akin to

that of their monomicrobial culture counterparts, and potentially with increased resistance to ciprofloxacin and trimethoprim which common front line antibiotics used for UTI treatment. Due to the complexities involved in the diagnosis and treatment in these infections many patients may receive inadequate antibiotic treatment or indeed a lack of treatment altogether. More worryingly for patient health, the majority of organisms isolated from polymicrobial cultures also exhibited increased human pathogenic potential as evidenced by *in vitro* cell infection assays. The diagnostic standard procedure for UTI should be reconsidered in light of the data presented here.

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Antibiotic concentration (µg/ml)	*E. coli* (%)		*E. faecalis* (%)		*P. mirabilis* (%)		*S. aureus* (%)		*P. aeruginosa* (%)	
	Poly (n=129)	Mono (n=21)	Poly (n=110)	Mono (n=4)	Poly (n=56)	Mono (n=1)	Poly (n=18)	Mono (n=4)	Poly (n=46)	Mono (n=5)
Gentamicin (2)	12.4	4.76	-	-	5.3	0.0	4.5	0	-	-
Cefotaxime (1)	17.8	14.29	-	-	33.9	100.0	-	-	-	-
Ceftazidime (1)	18.6	9.52	-	-	35.7	100.0	-	-	-	-
Meropenem (2)	0.0	0.00	15.5	0.0	0.0	0.0	-	-	-	-
Piperacillin-Tazobactam (16)	6.2	4.76	4.5	25.0	23.2	100.0	-	-	-	-
Co-amoxiclav (32)	5.4	0.00	2.7	0.0	16.1	100.0	0.0	0.0	-	-
Trimethoprim (2)	44.2	28.57	-	-	89.0	0.0	22.2	0.0	-	-
Ciprofloxacin (4)	23.3	9.52	28.2	0.0	0.0	0.0	55.5	50.0	-	-
Cephradine (32)	28.7	19.05	-	-	55.3	100.0	50.0	25.0	-	-
Nitrofurantoin (32)	17.1	9.52	10.9	0.0			11.0	0.0	-	-
Amoxicillin (32)	45.0	42.86	7.3	0.0	37.5	100.0	66.6	75.0	-	-
Gentamicin (10)	-	-	-	-	-	-	-	-	2.17	0.0
Piperacillin-tazobactam (85)	-	-	-	-	-	-	-	-	0.0	0.0
Ceftazidime (30)	-	-	-	-	-	-	-	-	2.17	00.0
Meropenem (10)	-	-	-	-	-	-	-	-	2.17	0.0
Ciprofloxacin (1)	-	-	-	-	-	-	-	-	4.35	00.0

Table 1. Prevalence of antibiotic resistance in UTI isolates

NOTE ‘-‘ indicates that particular species/antibiotic combination was not tested. The term ‘poly’ refers to strains of polymicrobial infection origin, whilst the term ‘mono’ refers to strains of monomicrobial infection origin.

Table 2. β-lactamase gene carriage in UTI isolates as determined by PCR

β-Lactamase carriage		Percentage of strains
Any β-lactamase	- monomicrobial culture isolates	57.1
	- polymicrobial isolates	48.4
SHV	- monomicrobial culture isolates	8.6
	- polymicrobial isolates	9.8
TEM	- monomicrobial culture isolates	45.7
	- polymicrobial isolates	36.4
CTX-M	- monomicrobial culture isolates	22.9*
	- polymicrobial isolates	9.5
OXA	- monomicrobial culture isolates	11.4
	- polymicrobial isolates	4.7
Multiple β-lactamases	- monomicrobial culture isolates	22.9#
	- polymicrobial isolates	8.8

Strains tested were all *E.. coli*, *Pseudomonas spp, Proteus spp, Citrobacter spp, Enterobacter spp* and *Klebsiella spp* isolated in this study. * P=0.016, #P=0.01, χ^2 test.

Figure 1.The relative prevalence of each of the main species associated with UTI isolated from the different types of sample collected in the study. Prevalence is presented as the percentage of samples collected which contain the given species.

* Indicates cultures taken from polymicrobial infections

Figure 2. BOX-PCR gel of *E. coli* strains isolated from UTI samples

Lanes marked * contain the BOX-PCR profile for *Escherichia coli* CFT073, lanes marked M contain molecular weight markers. Remaining lanes show a selection of BOX profiles obtained for *E. coli* isolates.

Figure 3. Invasive capabilities of UTI *E. coli* isolates compared to *E. coli* CFT073. CFT073 invasion is designated as 1.00. Polymicrobial sample isolates are statistically more invasive than monomicrobial culture sample isolates, $P<0.001$, χ^2 test.