Globally dispersed mobile drug-resistance genes in Gram-negative bacterial isolates from patients with bloodstream infections in a US urban general hospital

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Mobile drug-resistance genes with identical nucleic acid sequences carried by multidrug-resistant *Escherichia coli* strains that cause community-acquired infections are becoming increasingly dispersed worldwide. Over a 2-year period, we analysed Gram-negative bacterial (GNB) pathogens from the blood of inpatients at an urban public hospital to determine what proportion of these isolates carried such globally dispersed drug-resistance genes. Of 376 GNB isolates, 167 (44 %) were *Escherichia coli*, 50 (13 %) were *Klebsiella pneumoniae*, 25 (7 %) were *Pseudomonas aeruginosa*, 25 (7 %) were *Proteus mirabilis* and 20 (5 %) were *Enterobacter cloacae*; the remainder (24 %) comprised 26 different GNB species. Among *E. coli* isolates, class 1 integrons were detected in 64 (38 %). The most common integron gene cassette configuration was *dfrA17-aadA5*, found in 30 (25 %) of 119 drug-resistant *E. coli* isolates and in one isolate of *Moraxella morganii*. Extended-spectrum β -lactamase (ESBL) genes were found in 16 *E. coli* isolates (10 %). These genes with identical sequences were found in nearly 40 % of bloodstream *E. coli* isolates in the study hospital, as well as in a variety of bacterial species from clinical and non-clinical sources worldwide. Thus, a substantial proportion of bloodstream infections among hospitalized patients were caused by *E. coli* strains carrying drug-resistance genes that are dispersed globally in a wide variety of bacterial species.

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INTRODUCTION

Multidrug-resistant Gram-negative bacteria (GNB) that cause community-onset infections are increasingly being reported from many regions of the world (Ajiboye *et al.*, 2009; Coque *et al.*, 2008; Johnson *et al.*, 2008, 2010; Kumarasamy *et al.*, 2010; Peirano & Pitout, 2010; Pitout *et al.*, 2005; Suzuki *et al.*, 2009; Woerther *et al.*, 2010). The sources of these drug-resistant GNB causing extra-intestinal infections, such as urinary tract infection (UTI), are not obvious. There is increasing evidence that drug-resistant extra-intestinal *Escherichia coli* infections can cause outbreaks in community settings (Enne *et al.*, 2001; Manges *et al.*, 2001, 2006; Olesen *et al.*, 1994; Smith *et al.*, 2008). These organisms carry mobile drug-resistance genes that are also recognized globally. Such global spread may be

facilitated by widely distributed food products contaminated with drug-resistant GNB that originate in food animal reservoirs, or by person-to-person transmission following foreign travel, including ‘medical tourism’ (Ajiboye *et al.*, 2009; Johnson *et al.*, 2009; Kumarasamy *et al.*, 2010; Peirano & Pitout, 2010; Vincent *et al.*, 2010).

Hospitals and outpatient clinical settings are important reservoirs and sources of drug-resistant GNB pathogens and drug-resistance genes. In hospitals, antimicrobial drugs can select for drug-resistant GNB strains that acquire resistance by gene mutation. Antimicrobial agents used in these clinical settings may also facilitate mobile drug-resistance genes to transfer across different lineages of the same bacterial species or across different bacterial species by horizontal gene transfer. The prevalence of drug-resistant GNB infections among inpatients in hospitals is thus affected by antimicrobial drug-use practices.

It is also recognized that drug-resistant GNBs and their drug-resistance genes circulating in the community can be introduced into hospitals. However, in most hospitals, the

Abbreviations: BSI, bloodstream infection; ESBL, extended-spectrum β -lactamase; GNB, Gram-negative bacteria; NCBI, National Center for Biotechnology Information; SFGH, San Francisco General Hospital; SNP, single-nucleotide polymorphism; TMP, trimethoprim-sulfamethoxazole; UTI, urinary tract infection.

relative proportion of infections caused by GNB strains whose resistance is selected in the hospital versus those caused by strains newly introduced into the hospital is not known. This is an important piece of information for infection control in hospitals. In the former, antimicrobial drug-use stewardship may help control such infections. However, if a substantial proportion of GNB infections in a hospital are caused by resistant GNB pathogens newly introduced into the hospital, such an infection-control practice may have a limited effect. Here, we sought to address this question in a large urban hospital, where antimicrobial selective pressures are high. We prospectively examined GNB isolates from patients diagnosed with bloodstream infections (BSIs) and specifically compared the nucleic acid sequences of mobile drug-resistance genes with those genes already recognized to be circulating globally.

METHODS

Strain collection. The clinical microbiology laboratory of San Francisco General Hospital (SFGH) is one of the largest in the Bay Area, and the cultures originated from all hospital wards, as well as from jail clinics and San Francisco's city outpatient clinics. All GNB isolates from BSIs at the clinical microbiology laboratory of SFGH are stored on agar slants for up to 3 months. Every 3 months, these isolates were provided to us for analysis. We examined all consecutively collected GNB BSI isolates from patients admitted as an inpatient to SFGH between July 2007 and September 2009. This study was approved by the University of California, San Francisco Committee on Human Research.

Information on the dates of admission and first blood culture that yielded the GNB pathogen was available for most of the isolates. In this study, we considered BSI to be a community-onset infection if the time period between the date of admission and the first blood culture that grew a GNB pathogen was ≤ 48 h (Friedman *et al.*, 2002).

Strain characterization and susceptibility testing. At SFGH, the BSI isolates were speciated biochemically using API 20E (bioMérieux) for fermenters or API 20NE (bioMérieux) for non-enteric bacteria. Antimicrobial susceptibility tests were performed using a Microscan WalkAway Gram-Negative panel (Dade Behring/Siemens). For *E. coli* organisms, multiple drug resistance was defined as resistance to one or more agents in three or more classes of tested drugs (Johnson *et al.*, 2010; Márquez *et al.*, 2008). Intermediate susceptibility was classified as resistant. Extended-spectrum β -lactamase (ESBL) production was confirmed by a double-disc diffusion assay according to Clinical and Laboratory Standards Institute (CLSI, 2011) guidelines with cefotaxime and ceftazidime on Luria–Bertani (LB) agar plates with and without clavulanic acid.

DNA extraction and PCR amplification for integron gene cassettes and ESBL gene detection. PCR analysis to detect class 1 integrons was conducted on all isolates. Gene cassette PCR was conducted on all class 1 integrase-positive isolates. PCR analysis to detect β -lactamase genes was conducted on isolates suspected to be ESBL producers by double-disc diffusion assay results. The bacterial DNA was extracted from a single colony on either an LB agar plate or a tryptic soy agar plate using a freeze–thaw method. The colony was suspended in 500 μ l water, boiled for 15 min, frozen overnight at -80°C , reboiled for 15 min and centrifuged for 30 s at 2000 r.p.m.

in a bench microfuge. Two microlitres of the supernatant was used as template DNA in a 25 μ l reaction buffer containing 1.5 mM MgCl_2 , 0.4 mM dNTPs, 1 U *Taq* polymerase (Invitrogen), $1\times$ PCR buffer (Invitrogen) and 2 μ M each primer. AmpliTaq Gold (Applied Biosystems) was used for gene cassette PCR. PCR products were visualized on a 1% agarose gel stained with ethidium bromide.

Primer sequences. The primer sequences used have been published previously (Table 1). To detect empty integrons, we used the 3'-CS primer in conjunction with *Int₂F*. We used primers described by others to detect β -lactamase genes (Dallenne *et al.*, 2010; Shibata *et al.*, 2006). As a positive PCR control for the class 1 integrase PCR, we amplified 16S rRNA gene sequences. For a positive control for the gene cassette and β -lactamase gene PCRs, we used strains confirmed previously by sequencing to contain cassette and β -lactamase genes.

Sequencing amplified gene cassette products. For each PCR product that appeared as a unique band when visualized by gel electrophoresis, 2 μ l PCR product was cleaned with 2 μ l of a 1:10 dilution of ExoSAP-IT (Affymetrix), which was then diluted 100-fold and submitted for direct sequencing. Sequencing was carried out at the University of California Berkeley DNA sequencing facility. The DNA sequences were inspected visually, edited and assembled using BioEdit version 7.0.1. CLUSTAL W was used to perform multiple alignment analyses of the sequences. Sequences were compared for single-nucleotide polymorphisms (SNPs) with gene sequences deposited in the National Center for Biotechnology Information (NCBI) database using an updated version of the BLAST program.

Statistics. Categorical variables were compared by χ^2 (with Yates' correction) or Fisher's two-tailed exact tests.

RESULTS

Of 376 BSI isolates collected between July 2007 and September 2009, 167 (44%) were *E. coli*, 50 were *Klebsiella pneumoniae* (13%), 25 were *Pseudomonas aeruginosa* (7%), 25 were *Proteus mirabilis* (7%) and 20 were *Enterobacter cloacae* (5%). Together, these accounted for 76% of all isolates. In total, 31 distinct GNB species were identified (Table 2).

Of 359 isolates with susceptibility data, 279 (78%) were resistant to at least one antimicrobial class. Of 165 *E. coli* isolates, 86 (52%) were multidrug-resistant. The ESBL gene *bla*_{CTX-M} was identified by PCR in 16 *E. coli* isolates (Table 3); three were *bla*_{CTX-M-1}, four were *bla*_{CTX-M-14} and nine were *bla*_{CTX-M-15}. The β -lactamase gene *bla*_{TEM-1} was found in seven *E. coli*, one *Citrobacter freundii* and one *Enterobacter aerogenes*. *bla*_{SHV} was not detected in any of the isolates. For those isolates showing phenotypic resistance to broad-spectrum antimicrobial agents but lacking the above β -lactamase genes, we performed PCR analysis for plasmid-borne *ampC*-type genes. CIT group and DHA group genes were detected in six such isolates (Table 3). Additionally, two *E. coli* isolates with *bla*_{CTX-M} and one *C. freundii* isolate with *bla*_{TEM} also harboured plasmid-borne *ampC*-type genes.

By PCR analysis, we identified class 1 integrons in 90 isolates (24%). The five most frequently isolated species accounted for 85 (94%) of the class 1 integron-containing isolates (Table 3). Class 1 integrons were found in 64

Table 1. PCR primers used in this study

F, Forward primer; R, reverse primer.

Primer target	Primer name(s)	Sequence (5'→3')	Size (bp)	Reference
Class 1 integrase	201F 202R	CCTCCCGCACGATGATC TCCACGCATCGTCAGGC	280	Ajiboye <i>et al.</i> (2009)
Class 1 gene cassette, variable region	5' CS 3' CS	GGCATCCAAGCAGCAAG AAGCAGACTTGACCTGA	Variable	Lévesque <i>et al.</i> (1995)
<i>fimH</i> SNP	<i>fimH</i> F <i>fimH</i> R	TCGAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	506	Tartof <i>et al.</i> (2007)
16S rDNA, positive control	16S8F 16S8R	AGAGTTTGATCCTGGCTCAG GGACTACCAGGGTATCTAATCC	800	Ajiboye <i>et al.</i> (2009)
Empty integron detection	Int ₂ F	TCTCGGGTAACATCAAGG	Variable	Martinez-Freijo <i>et al.</i> (1998)
β-Lactamase multiplexes:				Dallenne <i>et al.</i> (2010)
TEM variants		CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	800	
SHV variants		AGCCGCTTGAGCAAATTAAC ATCCCGCAGATAAATCACCAC	713	
OXA-1, -4, -30		GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCCTGTAAGTG	564	
ACC-1, -2		CACCTCCAGCGACTTGTTAC GTTAGCCAGCATCACGATCC	346	
FOX-1-FOX-5		CTACAGTGCGGGTGGTTT CTATTTGCGGCCAGGTGA	162	
MOX-1, -2, CMY-1, -8 to -11, -19		GCAACAACGACAATCCATCCT GGGATAGGCGTAACTCTCCCAA	895	
DHA-1, -2		TGATGGCACAGCAGGATATTC GCTTTGACTCTTTCGGTATTCTG	997	
LAT-1, -2, -3; BIL-1, CMY-2 to -7, -12 to -18, -21 to -23		CGAAGAGGCAATGACCAGAC ACGGACAGGGTTAGGATAGY	538	
ACT-1, MIR-1		CGGTAAAGCCGATGTTGCG AGCCTAACCCTGATACA	683	
CTX-M universal	UnivF UnivR	TTTGCGATGTGCGAGTACCAGTA CTCCGCTGCCGGTTTATC	500	Shibata <i>et al.</i> (2006)
CTX-M-1 all	1AllF 1AllR	ATGGTTAAAAAATCACTGCG TTACAAACCGTCGGTGACGAT	876	Shibata <i>et al.</i> (2006)
CTX-M-9	9F 9R	GCAGATAATACGCAGGTG CGGCGTGGTGGTGTCTCT	392	Shibata <i>et al.</i> (2006)

(38 %) of 167 *E. coli* isolates and in only two (8 %) of 25 *Pseudomonas aeruginosa* isolates. Of 117 drug-resistant *E. coli* isolates, 62 (53 %) harboured class 1 integrons. Among 48 *E. coli* isolates that were susceptible to all drugs tested, only one (2 %) contained a class 1 integron bearing no detectable gene cassette. Using primers to amplify the integron variable region, we detected gene cassettes in 69 (77 %) of the integron-harboring isolates. *E. coli* comprised 48 (70 %) of these. Twenty-one (23 %) of the integrons were 'empty', harbouring no detectable gene cassettes. Four gene cassettes contained partial or non-resistance genes. We were unable to identify conclusively the gene in five of the cassettes.

The most frequent integron cassette genes identified were dihydrofolate reductase A (*dfrA*) genes *dfrA12*, *dfrA15*, *dfrA16* and *dfrA17*, which confer resistance to trimethoprim-sulfamethoxazole (TMP), and aminoglycoside

adenyltransferase (*aad*) genes *aadA1*, *aadA2*, *aadA5* and *aadB*, which confer resistance to aminoglycosides (Table 3). *aadA2* was found in combination with *dfrA12* in three isolates and alone in three others. There were seven alleles of *aadA1*, and they were detected in 17 (25 %) of the 69 gene cassettes. One *aadA1* allele was found among seven *E. coli* strains. Another *aadA1* allele occurred in one *E. coli* and one *K. pneumoniae* strain. The other alleles were mostly unique, distributed among two different GNB species, whilst the other *aad* genes were found in only one GNB species each. *aad* cassettes were detected in 23 (58 %) of 40 aminoglycoside-resistant GNB isolates and in 19 (66 %) of 29 aminoglycoside-resistant *E. coli* isolates, indicating that all strains carrying *aad* were resistant to an aminoglycoside.

Of 120 BSI isolates that were resistant to TMP, 39 (33 %) were confirmed by PCR to harbour *dfr* cassette configurations; all

Table 2. Antimicrobial resistance profiles of the BSI isolates

Antimicrobial susceptibility data were not available for 19 of 376 isolates. Results are shown as number (%) of isolates resistant to each antibiotic for which data were available. GENT, aminoglycoside class: gentamicin, tobramycin, amikacin; AMP, ampicillin; CEFZ, cefazolin; CEFX, cefuroxime and/or cefotetan; CEFT, ceftriaxone, cefotaxime, ceftazidime; AZT, aztreonam; CIP, fluoroquinolones: ciprofloxacin, levofloxacin, moxifloxacin; TMP, trimethoprim-sulfamethoxazole; CARB, carbapenems: ertapenem, imipenem, meropenem; NA, susceptibility data not available; NI, susceptibility data not interpretable for this species.

Species	n (%)	GENT	AMP	CEFZ	CEFX	CEFT	AZT	CIP	TMP	CARB
<i>Escherichia coli</i>	167 (44)	29 (17)	109 (65)	37 (22)	24 (14)	18 (11)	17 (10)	44 (26)	74 (44)	0 (0)
<i>Klebsiella pneumoniae</i>	50 (13)	1 (2)	49 (98)	3 (6)	5 (10)	0 (0)	0 (0)	1 (2)	5 (10)	0 (0)
<i>Pseudomonas aeruginosa</i>	25 (6.6)	4 (16)	NI	NI	NI	20 (80)	10 (40)	2 (8)	24 (96)	5 (20)
<i>Proteus mirabilis</i>	25 (6.6)	2 (8)	5 (20)	3 (12)	1 (4)	1 (4)	1 (4)	2 (8)	6 (24)	1 (4)
<i>Enterobacter cloacae</i>	20 (5.3)	2 (10)	15 (75)	15 (75)	11 (55)	6 (30)	4 (20)	1 (5)	4 (20)	2 (10)
<i>Serratia marcescens</i>	14 (3.7)	0 (0)	11 (79)	13 (93)	13 (93)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Citrobacter freundii</i>	10 (2.7)	0 (0)	5 (50)	7 (70)	2 (20)	2 (20)	1 (10)	1 (10)	1 (10)	0 (0)
<i>Stenotrophomonas maltophilia</i>	9 (2.4)	NI	NI	NI	NI	NI	NI	NI	1 (11)	NI
<i>Klebsiella oxytoca</i>	7 (1.9)	0 (0)	7 (100)	4 (57)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Acinetobacter lwoffii</i>	6 (1.6)	0 (0)	NI	1 (17)	1 (17)	2 (33)	2 (33)	0 (0)	0 (0)	0 (0)
<i>Acinetobacter baumannii</i>	5 (1.3)	0 (0)	NI	1 (20)	1 (20)	1 (20)	1 (20)	0 (0)	0 (0)	1 (20)
<i>Enterobacter aerogenes</i>	5 (1.3)	0 (0)	4 (80)	3 (60)	3 (60)	2 (40)	1 (20)	0 (0)	0 (0)	0 (0)
<i>Morganella morganii</i>	5 (1.3)	1 (20)	5 (100)	5 (100)	5 (100)	0 (0)	0 (0)	2 (40)	3 (60)	0 (0)
<i>Enterobacter agglomerans</i>	4 (1.1)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Serratia liquefaciens</i>	3 (0.8)	0 (0)	NI	NI	NI	1 (33)	1 (33)	0 (0)	1 (33)	0 (0)
<i>Salmonella</i> species	3 (0.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Aeromonas</i> species	2 (0.5)	0 (0)	NI	NI	NI	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Providencia rettgeri</i>	2 (0.5)	0 (0)	1 (50)	2 (100)	1 (50)	1 (50)	1 (50)	1 (50)	0 (0)	0 (0)
<i>Proteus vulgaris</i>	2 (0.5)	0 (0)	2 (100)	2 (100)	2 (100)	1 (50)	0 (0)	0 (0)	1 (50)	0 (0)
<i>Alcaligenes</i> species	1 (0.3)	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Azorhizobium</i> species	1 (0.3)	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Burkholderia cepacia</i>	1 (0.3)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Brevundimonas</i> species	1 (0.3)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Citrobacter koseri</i>	1 (0.3)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Flavimonas oryzihabitans</i>	1 (0.3)	0 (0)	NI	NI	NI	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
<i>Leclercia adecarboxylata</i>	1 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Myroides</i> species	1 (0.3)	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Providencia stuartii</i>	1 (0.3)	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Salmonella enteritidis</i>	1 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Salmonella newport</i>	1 (0.3)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
<i>Serratia</i> species	1 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	376	40 (11)	218 (58)	101 (27)	75 (20)	64 (17)	41 (11)	57 (15)	120 (32)	9 (2)

39 showed the phenotype of TMP resistance. Of 74 *E. coli* isolates resistant to TMP, 35 (47%) were confirmed by PCR to carry the *dfr* gene cassette.

The most frequent gene cassette configuration was *dfrA17-aadA5*, a 1.8 kb sequence. Thirty (48%) of 62 drug-resistant *E. coli* strains carrying an integron harboured this cassette configuration. An identical sequence was also found in one strain of *Morganella morganii* (Table 3).

SNPs of *fimH*, a gene that encodes the adhesin subunit of *E. coli* type I pilus, were used to screen *E. coli* genotypes (Dias *et al.*, 2009; Tartof *et al.*, 2007). Of 17 distinct *fimH* SNP types comprising 82 of 87 multidrug-resistant *E. coli* isolates (five could not be typed), strains belonging to five distinct SNP types carried identical *dfrA17-aadA5* sequence.

Of 353 patients with admission date information, 243 (69%) had a GNB cultured from blood obtained ≤ 48 h

Table 3. Integron gene cassette and β -lactamase gene analysis of the BSI isolates

Gene	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>E. cloacae</i>	Other species
<i>aadA1</i> (n=16)*	9	3	0	3	0	1
<i>aadA1</i> , <i>bla</i> _{OXA} (n=1)	1	0	0	0	0	0
<i>aadA2</i> (n=3)	0	0	0	0	3	0
<i>aadB</i> (n=1)	0	0	1	0	0	0
<i>dfrA1-aadA1</i> (n=1)	0	1	0	0	0	0
<i>dfrA12-orfF-aadA2</i> (n=3)	3	0	0	0	0	0
<i>dfrA15</i> (n=1)	0	1	0	0	0	0
<i>dfrA16</i> (n=1)	0	0	0	1	0	0
<i>dfrA17-aadA5</i> (n=31)*	30	0	0	0	0	1
<i>dfrA7</i> (n=2)	2	0	0	0	0	0
Could not identify (n=5)	3	2	0	0	0	0
None, 'empty' (n=21)	16	2	1	1	1	0
Partial/non-resistance genes (n=4)†	0	0	0	0	1	3
<i>bla</i> _{CTX-M} (n=16)	16	0	0	0	0	0
<i>bla</i> _{TEM-1} (n=9)	7	0	0	0	0	2
<i>bla</i> _{CTX-M} + <i>bla</i> _{TEM} (n=2)	2	0	0	0	0	0
Plasmid-borne <i>ampC</i> (n=9)	2	0	0	0	0	7

**Salmonella newport* (n=1) carried the *aadA1* gene cassette and *M. morganii* (n=1) carried the *dfrA17-aadA5* gene cassette.

†*Alcaligenes* species, *Burkholderia cepacia* and *Stenotrophomonas maltophilia* each had one strain in this category.

after admission (Table 4); seven (29 %) of the *Pseudomonas aeruginosa* cases and 236 (72 %) of all other GNB BSI cases had the first positive blood culture obtained ≤ 48 h after admission ($P < 0.05$) (Table 4). Among 30 patients infected with *E. coli* harbouring the most common drug-resistant cassette configuration (*dfrA17-aadA5*), 68 % had the first positive blood culture obtained ≤ 48 h after admission; 53 % were cultured < 24 h after admission. The blood culture from one patient that grew *M. morganii* harbouring *dfrA17-aadA5* was obtained 21 h after admission.

DISCUSSION

Among 167 *E. coli* BSIs identified between July 2007 and September 2009, 63 (38 %) were caused by strains that

harboured three integron cassette genes (*aadA1*, *aadA2* and *dfrA17-aadA5*) and ESBL genes (*bla*_{CTX-M}). These genes were 100 % identical in sequence to those already dispersed globally in a variety of bacterial strains and species isolated from non-hospital sources, including food, water, animals and healthy humans, deposited in the NCBI database. By the definition we used, 78 % of the *E. coli* BSIs identified at SFGH were community-onset infections (Table 4). Among those patients infected with an *E. coli* strain carrying the most common cassette configuration (*dfrA17-aadA5*), 68 % were already infected at the time of admission to the study hospital. This large cassette sequence (1.8 kb) was 100 % identical in all of the strains. As this cassette was found in a wide variety of *fimH* SNP types, it was not likely to have been spread by some biologically 'fit' or epidemic strain in the hospital.

Table 4. Timing of collection of first positive blood culture after hospitalization by GNB isolates

Results are shown for strains with available date of admission information.

GNB species and drug-resistance gene cassettes	Total	≤ 48 h	> 48 h	P value
All GNB isolates	353	243 (69 %)	110 (31 %)	–
<i>Escherichia coli</i>	164	128 (78 %)	36 (22 %)	0.001
<i>Klebsiella pneumoniae</i>	49	32 (65 %)	17 (35 %)	0.565
<i>Proteus mirabilis</i>	24	17 (71 %)	7 (29 %)	0.827
<i>Pseudomonas aeruginosa</i>	24	7 (29 %)	17 (71 %)	< 0.0005
All other GNB species	92	59 (64 %)	33 (36 %)	0.257
<i>E. coli</i> with <i>dfrA17-aadA5</i>	30	20 (68 %)	10 (32 %)	0.788
<i>E. coli</i> with other integron gene cassettes	18	16 (89 %)	2 (11 %)	0.069
<i>E. coli</i> with <i>bla</i> _{CTX-M} or <i>bla</i> _{TEM}	21	12 (57 %)	9 (43 %)	0.342

In contrast, 32 % of the *E. coli* strains carrying *dfrA17-aadA5* were detected in *E. coli* cultured from blood after >48 h of hospitalization. It is possible that these strains acquired the cassette by horizontal gene transfer after hospitalization. However, even this observation suggests that this particular cassette configuration was introduced into the hospital sometime earlier and spread subsequently. Interestingly, most (71 %) of the *Pseudomonas aeruginosa* BSIs appeared to have been hospital acquired; none had the *dfrA17-aadA5* configuration.

In fact, *dfrA17-aadA5* was the most frequently observed integron cassette configuration found in this study. In the NCBI database, there are 13 different *dfrA17* alleles; nine of these differ by 1 nt and four differ by 2–5 nt. There are 16 different *aadA5* alleles in the NCBI database, which differ by a 1 nt substitution in most. Therefore, if the mutation frequency of this cassette configuration was identical, over 200 alleles of the *dfrA17-aadA5* configuration should be represented in the database. The observation that only one allele of this configuration was represented among SFGH GNB isolates and among many bacterial species and sources around the world suggests that it has undergone horizontal gene transfer as a single 1.8 kb block across many bacterial species.

A study of UTI *E. coli* isolates collected from a university health centre in the San Francisco Bay Area between 1999 and 2001 also reported *dfrA17-aadA5* as the most frequently detected gene cassette configuration (Solberg *et al.*, 2006). This configuration was found among food animal *E. coli* isolates from different regions of the USA (Ajiboye *et al.*, 2009). The most common subtype of this array (GenBank accession no. FJ381673) found in that study was 100 % identical in sequence to the *dfrA17-aadA5* sequence found in this BSI study. A study from Korea found this *dfrA17-aadA5* sequence to be the most frequently detected type during the period 1996–2002, replacing *dfrA12*, which was previously found to be the most commonly detected drug-resistance cassette from 1980 to 1985 in that region (Yu *et al.*, 2004). *dfrA17-aadA5* was the only gene cassette isolated from healthy farm workers in China (Li *et al.*, 2010). Studies of commensal species isolated from healthy humans in Switzerland and Spain detected this same allele, although they reported the *dfrA1-aadA1* arrangement to have a higher frequency (Cocchi *et al.*, 2007; Vinué *et al.*, 2008).

The second most common drug-resistance gene that we found in this study was the ESBL gene *bla*_{CTX-M}, which was found in 16 (9.6 %) of the *E. coli* BSI isolates. Of these, more than half (56 %) belonged to *bla*_{CTX-M-15}, which is the most common CTX-M type found in ESBL-producing *E. coli* strains circulating globally, many of which are associated with community-acquired UTIs (Peirano & Pitout, 2010; Pitout & Laupland, 2008; Pitout *et al.*, 2005). It appears that patients with BSIs diagnosed at our study hospital have become part of this global pandemic caused by CTX-M-expressing *E. coli*.

There were several limitations to this study. A large proportion (44 %) of BSIs in this study were caused by *E. coli*. Thus, our observation that a large proportion of drug-resistant BSI cases were community-onset infections is limited to *E. coli* infections and cannot be generalized to other GNB infections. Another limitation is that we did not have detailed information on the setting of infections of the study subjects. The so-called community-onset cases could have had prior health-care-setting exposures. However, even if many of these patients acquired their *E. coli* strains harbouring drug-resistance genes at a health-care setting elsewhere prior to being admitted to the study hospital, the fact remains that they were already infected at the time of admission and that for them, the antimicrobial stewardship practised at this hospital would not have prevented these infections.

Thus, it appears that the prevalence of drug-resistant BSIs in a local setting, such as a hospital in San Francisco, can be affected by globally circulating mobile drug-resistance genes, in addition to the antimicrobial drug selective pressure in the hospital. The BSI prevalence we estimated to be due to drug-resistant *E. coli* strains newly introduced into the hospital may be an underestimate, as we only examined integrons and some of the ESBL genes. We did not examine other mobile genes known to be dispersed globally, such as those encoding metallo- β -lactamases (Kumarasamy *et al.*, 2010; Peleg & Hooper, 2010; Pitout & Laupland, 2008). More attention needs to be paid to the global factors that contribute to the spread of these drug-resistance determinants that enter local health-care settings.

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