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Antimicrobial resistance genotypes and phenotypes from multidrug-resistant bacterial wound infection isolates in Cambodia

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ABSTRACT

This study aimed to identify the molecular determinants responsible for antibiotic resistance among human wound isolates in Cambodia. *Staphylococcus* spp. ($n = 10$) and a variety of Gram-negative isolates ($n = 21$) were taken from a larger collection of wound isolates collected during 2011–2013 and were analysed for the presence of >230 resistance determinants using a broad-spectrum DNA microarray. These isolates were chosen to represent the species most commonly found in wound isolates referred during this time and to include some of the most resistant strains. Resistance determinants detected among the staphylococci included *blaZ* (90%), *mecA* (100%), *erm*(B) (70%), *erm*(C) (20%), *tet*(38) (90%), *tet*(K) (40%), *tet*(L$_p$) (10%), *tet*(M) (20%), *lnu*(A)/*lin*(A) and *lnu*(B)/*lin*(B) (10% each), *msr*(A)/*msr*(B)/*msr*(SA) (10%), *norA* (80%) and *dfrA* (10%). Eleven different β-lactamase genes were detected among the Gram-negative bacteria, including genes encoding the TEM (48%), CTX-M-1 (48%), CTX-M-9 (5%), SHV (5%) and VEB (10%) families of broad-spectrum and extended-spectrum β-lactamase enzymes, as well as the carbapenemase gene bla_{OXA-23}. Forty additional genes were also detected in the Gram-negative isolates conferring resistance to aminoglycosides (11 genes), phenicols (5 genes), macrolides [4 genes, including *mph*(A)/*mph*(K) (10%)], lincosamides [*lnu*(F)/*lin*(F), *lnu*(G)/*lin*(G)], tetracycline (4 genes), rifampicin [*arr* (29%)], quaternary amines [*qacEΔ1* (43%)], quinolones [*qnrS* (14%) and *qnrB* (5%)], sulfonamides [*sul1* (29%), *sul2* (38%) and *sul3* (10%)], streptothricin (*sat2*) and trimethoprim (6 genes). The results obtained here provide a snapshot of the broad variety of resistance determinants currently circulating within Cambodia.
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1. Introduction

Antimicrobial resistance (AMR) is of particular concern in Southeast Asia owing to ineffective antimicrobial stewardship, limited reporting of AMR, widespread availability of low-quality or counterfeit drugs and, in many places, poor infection control measures [1]. Whilst reports from Vietnam, Thailand, Indonesia, the Philippines and Malaysia are allowing researchers, clinicians and public health administrators to gain a greater understanding of resistance trends within these countries, AMR data for Cambodia are limited to only a few published studies [2–5].

In the present work, we sought to expand the limited molecular epidemiological data set for Cambodia by identifying the molecular determinants responsible for antibiotic resistance in a small set of Gram-positive and Gram-negative wound isolates. Using a broad-spectrum Antimicrobial Resistance Determinant Microarray (ARDM) [6–8], 31 human wound isolates (10 Gram-positive *Staphylococcus* spp. and 21 Gram-negative) were analysed for the presence of >230 resistance genes and families of genes. Whilst not intended as a diagnostic tool, the ARDM can be used to determine the presence/absence of a wide variety of genes useful in tracking the emergence and spread of new sources and mechanisms of resistance. The results obtained here provide a

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snapshot of the broad variety of resistance determinants that are currently circulating within Cambodia.

2. Materials and methods

A collection of 31 strains was selected from a population of 176 diagnostic wound isolates referred to the US Naval Medical Research Unit-2 (NAMRU-2) by clinicians at a non-profit surgical hospital in Phnom Penh (Cambodia) between 2011 and 2013, based on their broad range of AMR profiles. The select subset of strains was chosen to represent the genera most commonly found in wound isolates referred during this time (Ford, unpublished results) and to include the most resistant strains. Not surprisingly, it comprised most of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) [9]. No metadata were available describing the clinical presentation or history of the patients from whom the strains were isolated owing to the limitations of the research protocol under which they were collected; therefore, potential therapeutic indications and outcomes are unknown. Strains were identified using Gram-staining and standard biochemical analyses (API®; bioMérieux; http://www.biomerieux-usa.com/servlet/srt/bio/usa/dynPage?open=USA_PRD_LST&doc=USA_PRD_LST_G_PRD_USA_5&pubparams.sform=0&lang=en). Antimicrobial susceptibilities were determined by disk diffusion [10] using breakpoints based on Clinical and Laboratory Standards Institute (CLSI) standards [11] (Table 1).

The presence/absence of 238 different AMR determinants was determined for each of the 31 strains using the ARDM. The ARDM is an electrochemically interrogated microarray with 2240 DNA probes immobilised on individually addressable microelectrodes; ARDM content covers 238 different AMR determinants or families of determinants conferring resistance to >12 categories of antimicrobials directed both against Gram-negative and Gram-positive species; each determinant is represented by six to ten probes covering various loci of the gene sequence. Bacterial genomic DNA was extracted from individual colonies grown on non-selective agar medium using a QIAamp DNA Mini Kit (Dynamic Pharma Ltd., Phnom Penh, Cambodia). Purified DNA was then subjected to whole-genome amplification (GenomiPhi v.2 DNA Amplification Kit; GE Healthcare, Pittsburgh, PA), fragmentation using DNase I, biotinylation (Kreatech PlatinumBright Biotin Labeling Kit; LMS-Kreatech, Amsterdam, The Netherlands) and finally hybridisation on the ARDM v.2 [7,8]. Five isolates (*A. baumannii* EXT310/35, *Enterobacter cloacae* EXT389/49 and EXT413/50, *Escherichia coli* EXT351/67 and *K. pneumoniae* EST149/48) were also analysed on the ARDM v.3, which contains approximately double the content of the ARDM v.2 (542 determinants/determinant families) [6]. Isolates were deemed positive for the presence of a gene based on the number of positive gene-specific probes as previously described [7]. In a subset of samples, the presence of bla_{TEM}, bla_{CTX-M} and bla_{SHV} families of extended-spectrum β-lactamase (ESBL) genes was confirmed by PCR [4,12]. All isolations, characterisations and analyses,

Table 1
Phenotypic antimicrobial susceptibilities.

(a) Antimicrobial resistance phenotypes of Gram-positive strains[a]

Strain	MET	GEN	CLI	ERY	TET	VAN	CHL	CIP	LVX	SXT
Staphylococcus aureus EXT099/32 (MRSA)	R	R	R	R	R	S	S	R	R	S
S. aureus EXT108/33 (MRSA)	R	R	R	R	S	S	S	R	R	S
S. aureus EXT111/25 (MRSA)	R	R	R	R	S	S	S	R	R	S
S. aureus EXT168/23 (MRSA)	R	R	R	R	S	S	S	R	R	R
S. aureus EXT173/27 (MRSA)	R	R	R	R	R	S	R	R	I	R
S. aureus EXT192/24 (MRSA)	R	R	R	R	S	S	S	R	R	R
S. aureus EXT409/29 (MRSA)	R	R	R	R	R	S	S	R	R	R
S. aureus EXT410/26 (MRSA)	R	R	R	R	R	S	S	R	R	S
Staphylococcus sp. EXT085/30 (CoNS)	nd	R	R	R	R	S	R	R	R	R
Staphylococcus sp. EXT123/34 (CoNS)	nd	I	R	R	R	S	R	I	I	R

(b) Antimicrobial resistance phenotypes of Gram-negative strains

Strain	AMC	AMP	ATM	CFZ	FEP	CAZ	CRO	PIP	IPM	MEM	SAM	AMK	GEN	TET	CHL	CIP	LVX	SXT
Acinetobacter baumannii EXT310/35	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R
Escherichia coli EXT121/40	S	R	R	R	I	S	R	R	S	S	S	S	R	R	R	R	R	R
E. coli EXT191/43	I	R	R	R	R	R	R	R	S	S	I	S	R	R	S	S	S	R
E. coli EXT253/36	S	R	I	R	R	S	S	R	S	S	I	R	R	R	R	S	S	S
E. coli EXT301/41	R	R	R	R	R	R	R	R	S	S	R	S	S	S	S	R	R	S
E. coli EXT309/39	R	R	R	R	R	I	R	R	S	S	S	S	R	R	S	R	R	R
E. coli EXT311/37	I	R	R	R	R	R	R	R	S	S	S	S	R	R	R	R	R	R
E. coli EXT337/38	R	R	I	R	S	R	I	R	S	S	I	S	R	R	S	R	R	R
E. coli EXT351/67	R	R	R	R	R	I	R	R	S	S	R	I	R	R	nd	R	R	R
Enterobacter cloacae EXT389/29	R	R	I	R	S	S	R	R	S	S	R	S	R	R	R	R	R	R
E. cloacae EXT390/51	R	R	I	R	S	S	R	R	S	S	R	S	S	R	R	I	S	R
E. cloacae EXT413/50	R	R	R	R	R	I	R	R	S	S	R	S	R	R	R	R	R	R
Klebsiella pneumoniae EXT149/48	R	R	R	R	I	R	R	R	S	S	R	S	R	R	R	I	R	S
K. pneumoniae EXT183/46	R	R	R	R	R	R	R	R	S	S	R	I	S	R	R	R	S	R
K. pneumoniae EXT353/45	R	R	R	R	I	I	R	R	S	S	R	S	R	R	R	R	R	R
K. pneumoniae EXT386/47	S	R	R	R	R	I	R	R	S	S	S	S	R	R	R	S	S	R
Proteus mirabilis EXT353/59	R	R	S	I	S	S	S	R	S	S	I	S	R	R	R	R	R	R
P. mirabilis EXT369/58	R	R	S	I	S	S	S	S	S	S	I	S	R	R	R	R	R	R
P. mirabilis EXT386/57	R	R	S	R	I	S	R	R	S	S	R	S	R	R	R	R	S	R
Pseudomonas aeruginosa EXT170/54	nd	nd	R	nd	R	R	R	S	S	S	nd	S	R	nd	nd	R	R	nd
P. aeruginosa EXT407/53	nd	nd	R	nd	R	R	R	R	S	S	nd	S	R	nd	nd	S	R	nd

MET, meticillin; GEN, gentamicin; CLI, clindamycin; ERY, erythromycin; TET, tetracycline; VAN, vancomycin; CHL, chloramphenicol; CIP, ciprofloxacin; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole; AMC, amoxicillin/clavulanic acid; AMP, ampicillin; ATM, aztreonam; CFZ, cefazolin; FEP, cefepime; CAZ, ceftazidime; CRO, ceftriaxone; PIP, piperacillin; IPM, imipenem; MEM, meropenem; SAM, ampicillin/sulbactam; AMK, amikacin; MRSA, meticillin-resistant *S. aureus*; CoNS, coagulase-negative staphylococci; R, resistant; I, intermediate; S, sensitive; nd, not determined.

[a] Antimicrobial susceptibilities were determined by disk diffusion using breakpoints based on Clinical and Laboratory Standards Institute (CLSI) standards [11].