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 *bla_Z* (90%), *mecA* (100%), *erm*(B) (70%), *erm*(C) (20%), *tet*(38) (90%), *tet*(K) (40%), *tet*(L_p) (10%), *tet*(M) (20%), *Inu*(A)/*lin*(A) and *Inu*(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 [*Inu*(F)/*lin*(F), *Inu*(G)/*lin*(G)], tetracycline (4 genes), rifampicin [*arr* (29%)], quaternary amines [*qacE 1* (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.

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

2. Materials and methods

A collection of 31 strains were 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, including microarray analysis, were performed at facilities in Phnom Penh.

3. Results and discussion

The bacterial isolates analysed in this study were selected to represent the genera most commonly isolated from wound specimens collected during 2011–2013 and to include some of the most resistant strains. They include five of the ESKAPE pathogens

responsible for a significant proportion of nosocomial infections worldwide and whose AMR profiles often present serious therapeutic dilemmas for clinicians [9]. Most of the tested isolates demonstrated phenotypic resistance to at least three different classes of antimicrobial compounds and were classified as multidrug-resistant (Table 1).

Amongst the 10 Gram-positive and 21 Gram-negative isolates tested, the ARDM identified 76 unique resistance determinants potentially responsible for resistance to 14 classes of antimicrobial compounds (Table 2). In general, the *Staphylococcus* spp. and *E. coli* isolates harboured the fewest number of determinants each, with a mean of 5.5–5.6 resistance genes per isolate, respectively. As the subset of Gram-negative species covered six genera, it was not surprising that the Gram-negative isolates harboured a much wider variety of resistance determinants, with a total of 59 different genes detected.

3.1. Gram-positive isolates: *Staphylococcus* spp.

Ten Gram-positive isolates were included in the subset of analysed wound isolates; eight of the ten *Staphylococcus* spp. isolates were identified as *S. aureus* and were subsequently defined as methicillin-resistant *S. aureus* (MRSA) based on their oxacillin resistance phenotypes. In addition, all of the isolates were resistant or had diminished susceptibility to gentamicin, clindamycin, erythromycin and two quinolones tested, but were susceptible to vancomycin.

Overall, a total of 13 resistance genes were detected amongst the staphylococci, potentially conferring resistance to six different classes of antimicrobial compounds (Table 2). In general, there was relatively little variability, with each strain harbouring between five and eight resistance genes and showing similar resistance profiles. The *mecA* gene was detected in all MRSA isolates as well as in the coagulase-negative staphylococci (CoNS); carriage of *mecA* by the two CoNS strains was not surprising as methicillin-resistant CoNS are becoming more prevalent among healthcare-associated infections and may serve as reservoirs of *mecA* for community-acquired MRSA [13].

In agreement with susceptibility phenotypes, no *van* genes were detected, and all ciprofloxacin-resistant staphylococci harboured *norA*.

The class of antimicrobials with the broadest variety of resistance determinants amongst the *Staphylococcus* spp. isolates was the tetracyclines (four genes). Resistance to tetracycline was highly correlated with the presence of *tet*(K), *tet*(L_p) and/or *tet*(M), but not *tet*(38); only a single isolate harbouring *tet*(38) alone was resistant to tetracycline. Somewhat unexpectedly, *tet*(K) was detected in a large proportion of the tested isolates (40%). The *tet*(M) gene has been reported as the most prevalent staphylococcal tetracycline resistance determinant in Southeast Asia, with *tet*(K) only rarely being identified alone, and most often in the presence of *tet*(M) [14]. Furthermore, to our knowledge, this is the first report of *tet*(L_p) in a *Staphylococcus* sp. within Southeast Asia.

Nine of the 10 *Staphylococcus* isolates were found to harbour *erm*(B) or *erm*(C), which can confer the macrolide/lincosamide/streptogramin B (MLS_B) resistance phenotype, and all nine were phenotypically resistant to erythromycin and clindamycin. The tenth strain (*Staphylococcus* sp. EXT 085/30) displayed the same apparent resistance phenotype, likely due to co-carriage of *msr*(A)/*msr*(B)/*msr*(SA) genes (MS_B phenotype) and *Inu*(A)/*lin*(A) (L phenotype). Whilst potentially not reflective of the population as a whole, the relative proportions of *erm*(B)-positive ($n = 7$) and *erm*(C)-positive ($n = 2$) strains observed here contrasted with other studies of *Staphylococcus* spp. in Southeast Asia documenting high carriage of *erm*(A) (not detected here), a lower prevalence of *erm*(C) and rarity of *erm*(B) [14,15]. Similarly, we were also surprised to observe *Inu*(A)/*lin*(A) and *Inu*(B)/*lin*(B) in two isolates [one isolate (10%) each]; these genes are infrequently observed in *Staphylococcus* spp., especially in Southeast Asia (<http://faculty.washington.edu/marilynr/ermweb4.pdf>; [15,16]). Genes encoding other mechanisms of resistance to macrolides, lincosamides and/or streptogramins were not detected.

With the exception of one strain harbouring the *dfrA* gene, molecular mechanisms for resistance to gentamicin, chloramphenicol and trimethoprim/sulfamethoxazole could not be determined for the staphylococcal isolates using the ARDM v.2 microarray. Analysis of these strains on the ARDM v.3 chip (used here with select Gram-negative isolates; see below) may reveal additional resistance determinants amongst the staphylococci that are responsible for phenotypic resistance to these categories of antimicrobials. The

ARDM v.3 content includes 23 additional resistance determinants for gentamicin, chloramphenicol and trimethoprim derived from *Firmicutes* spp.

3.2. Gram-negative isolates

The subset of Gram-negative isolates studied included 18 Enterobacteriaceae, one *A. baumannii* and two *P. aeruginosa* strains. All were resistant to at least three different categories of antimicrobial compounds (Table 1) and, with a single exception isolate (*E. coli* EXT311/37), all harboured multiple AMR determinants. A total of 59 different genes or gene families were detected amongst the Gram-negative isolates (Table 2). Overall, the *Klebsiella* and *Proteus mirabilis* isolates harboured the greatest variety of determinants identified (23 and 24 unique determinants detected, respectively).

Determinants conferring resistance to β -lactam antibiotics demonstrated the greatest variety and included six families of determinants encoding ESBLs (*bla*_{TEM}, *bla*_{CTX-M-1}, *bla*_{CTX-M-9}, *bla*_{OXA-1}, *bla*_{OXA-10/PSE}, *bla*_{SHV} and *bla*_{VEB}). In agreement with other studies of this region [3,4,17,18], genes encoding TEM- and CTX-M-1-type β -lactamases predominated; two-thirds of these strains were positive for *bla*_{TEM}, *bla*_{CTX-M-1} or both. On the other hand, only one strain harboured carbapenemase genes (*bla*_{OXA-51} and *bla*_{OXA-23}), which was the only strain exhibiting phenotypic carbapenem resistance (*A. baumannii* EXT310/35).

Eleven different aminoglycoside resistance determinants were detected. Almost one-half of the isolates harboured from two to six different genes. Detected genes included

those encoding all three classes of aminoglycoside-modifying enzymes (acetyltransferases, nucleotidyltransferases and phosphotransferases), but not rRNA methylase genes (e.g. *rmt* genes, *armA*). There was a significant disparity between aminoglycoside resistance phenotypes and the observed genotypes, which underscores the limitations of this (and any) molecular test for diagnostic use; three isolates resistant to amikacin and/or gentamicin were negative for the presence of all 42 aminoglycoside resistance determinants on the ARDM v.2 (121 determinants on ARDM v.3), whilst eight amikacin-susceptible strains harboured *aac(6')-Ib*, which typically confers amikacin resistance.

In agreement with other studies in this region [2,19,20], diminished susceptibility to at least one fluoroquinolone, tetracycline or chloramphenicol was observed in all of the Gram-negative strains tested. The presence of *tet(A)*, *tet(B)*, *tet(D)* and *tet(J)* genes always corresponded with phenotypic tetracycline resistance, and the presence of *catA1/cat4*, *floR* and *cmiA* genes with chloramphenicol resistance. However, phenotype could not always be predicted by the presence or absence of specific genes. For example, the presence of *qnrB* and *qnrS* in four isolates could also not be accurately correlated with phenotypic quinolone resistance (potentially due to low-level resistance) and there were a number of isolates resistant to tetracycline, chloramphenicol or quinolones for which no antimicrobial-specific resistance determinant was detected. Whilst phenotypic quinolone resistance can potentially be due to point mutations in *gyrA*, *gyrB*, *parC* or *parE* (not tested here), cross-category resistance can arise from the presence and overexpression of a number of multidrug efflux pumps. Whilst only one of

the seven isolates harbouring *cmr/mdfA* (encoding a multidrug efflux pump) was resistant to all three categories, transcriptional analysis of this gene and others encoding multidrug efflux pumps (e.g. *acrAB*, *ade*, *mex*) may potentially elucidate the mechanism(s) responsible for phenotypic cross-category resistance to these antimicrobials.

Whilst not clinically relevant for treatment of wound infections, several macrolide and lincosamide resistance determinants were also detected amongst the Enterobacteriaceae strains tested here. The presence of *mph(A)/mph(K)* genes (azithromycin resistance) in two Enterobacteriaceae isolates may be somewhat concerning; *E. coli* has been postulated as a potential reservoir for transfer of macrolide resistance genes to other species responsible for travellers' diarrhoea, typhoid fever and shigellosis [21], where azithromycin is used as an alternative therapeutic. Lincosamide resistance determinants [*Inu(F)/lin(F)* and *Inu(G)/lin(G)*] were also detected in strain *E. cloacae* EXT389/49. Given the >90% sequence identity between the *Inu(F)/lin(F)* and *Inu(G)/lin(G)* genes, it is likely that these results represent detection of only one gene, rather than both. Rarely reported, *Inu(F)/lin(F)* and *Inu(G)/lin(G)* have been recently described in Enterobacteriaceae strains isolated from this region or from travellers returning from Southeast Asia [22,23]. We anticipate that as screening of Enterobacteriaceae strains for *Inu(F)/lin(F)* and *Inu(G)/lin(G)* becomes more commonplace, heretofore unknown reservoirs for transfer of these and other resistance genes may be revealed, facilitating epidemiological mapping.

Trimethoprim/sulfamethoxazole resistance was observed in 16 isolates, 11 of which harboured at least one sulfonamide or trimethoprim resistance determinant. Six *dfrA* genes, including the recently described *dfrA32* and the *Acinetobacter*-specific *folA* gene conferring trimethoprim resistance, were detected. Unexpectedly, *dfrA12*, commonly detected in Southeast Asia [24,25], was not observed here. We were also surprised to detect all three *sul* genes, including *sul3* in two *P. mirabilis* strains; *sul3* has been reported infrequently globally but has recently been identified in high prevalence in *Salmonella* isolates from Thai livestock [26].

The *eptA* gene, strongly associated with colistin resistance, was detected in *A. baumannii* EXT310/35. Although colistin susceptibility was not determined here, the presence of this gene, combined with this strain's resistance to all other antibiotic compounds tested, may indicate a very limited number of chemotherapeutic options available for this strain.

The presence of oft-linked genes suggested the possible presence of a number of antimicrobial determinant-harboring genetic structures such as integrons and plasmids which, whilst not specifically identified here, can be useful in molecular epidemiology. Six Gram-negative isolates harboured both *sul1* and *qacE 1*, genes commonly associated with class 1 integrons and suggesting the presence of class 1 integrons within these strains. However, the absence of *sul1* in several *qacE 1*-positive strains and independent carriage of *int1* and *qacE 1/sul1* in isolates *E. coli* EXT351/67 and *A. baumannii* EXT310/35 support recent reports of alternative class 1 integron structures in

Southeast Asia [27,28]. The co-carriage of *sat2*, *aadA1/A2* and *dfrA1* in *P. mirabilis* EXT353/59, commonly associated with class 2 integrons, similarly suggested the presence of a class 2 integron.

Also of interest were *E. cloacae* EXT413/50 and EXT390/51 that both carried at least eight resistance determinants found on plasmid pKpn2146b [29]: *dfrA14*; *qnrB6* family (ARDM v.3); *aac(6)-Ib* family; *bla_{OXA-1}*; *bla_{CTX-M-1}* family; *bla_{TEM}* family; *aph3/strA*; *aph6/strB*; and *sul2*. The remaining two resistance determinants on pKpn2146b [*aac(3)-III* and *bla_{SHV}*] were missing from one or both. Future sequencing efforts and plasmid transfer experiments will ascertain whether a plasmid related to pKpn2146b is present in these two strains.

The overall purpose of this study was to demonstrate the utility of the ARDM, previously used only in a research setting, in a public health laboratory to determine the prevalence and distribution of AMR determinants in a variety of pathogenic species. Whilst the ARDM's inability to detect point mutations (e.g. in fluoroquinolone resistance) and to assess expression levels of various genes (e.g. RND pumps, inducible RNA methylation) limits its function as a diagnostic tool, its broad content can provide valuable information useful for epidemiological purposes. Although the presence of some genes may not be clinically relevant to the particular strains harbouring them (e.g. macrolide or lincosamide resistance genes in Enterobacteriaceae), they may indeed be relevant to related species or other species occupying the same niche, especially if gene transfer has been documented. Furthermore, this technology can be used to

identify unexplored reservoirs of genes not commonly assessed [6,30] whose roles in the emergence and spread of regional resistance have not yet been fully defined.

4. Conclusion

This study, conducted in its entirety in Phnom Penh (Cambodia), provides a snapshot of the broad variety of resistance determinants that are currently circulating among bacterial wound isolates in this environment. Whilst the determinants detected in this study have all previously been associated with the same bacterial species in other parts of the world, the prevalence of these genes within Cambodia had not previously been defined. The data described here can serve as a baseline for larger-scale molecular epidemiological studies in this region. An understanding of how, when, where and why AMR is spreading—information that genotypic, but not phenotypic, tests can furnish—can be used to track local trends and provide guidance for more effective public health infection control policies and outbreak preparation.

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Government' as defined as 'prepared by a military service member or employee of the US Government as part of that person's official duties.'

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Table 1(a) Antimicrobial resistance phenotypes of Gram-positive strains ^a

Strain	MET	GEN	CLI	ERY	TET	VAN	CHL	CIP	LVX	SXT
<i>Staphylococcus aureus</i> EXT099/32 (MRSA)	R	R	R	R	R	S	S	R	R	S
<i>S. aureus</i> EXT108/33 (MRSA)	R	R	R	R	S	S	S	R	R	S
<i>S. aureus</i> EXT111/25 (MRSA)	R	R	R	R	S	S	S	R	R	S
<i>S. aureus</i> EXT168/23 (MRSA)	R	R	R	R	S	S	S	R	R	R
<i>S. aureus</i> EXT173/27 (MRSA)	R	R	R	R	R	S	R	R	I	R
<i>S. aureus</i> EXT192/24 (MRSA)	R	R	R	R	S	S	S	R	R	R
<i>S. aureus</i> EXT409/29 (MRSA)	R	R	R	R	R	S	S	R	R	R
<i>S. aureus</i> EXT410/26 (MRSA)	R	R	R	R	R	S	S	R	R	S
<i>Staphylococcus</i> sp. EXT085/30 (CoNS)	nd	R	R	R	R	S	R	R	R	R
<i>Staphylococcus</i> 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
<i>Acinetobacter baumannii</i> EXT310/35	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R
<i>Escherichia coli</i> EXT121/40	S	R	R	R	I	S	R	R	S	S	S	S	R	R	R	R	R	R
<i>E. coli</i> EXT191/43	I	R	R	R	R	R	R	R	S	S	I	S	R	R	S	S	S	R

<i>E. coli</i> EXT253/36	S	R	I	R	R	S	S	R	S	S	I	R	R	R	R	S	S	S
<i>E. coli</i> EXT301/41	R	R	R	R	R	R	R	R	S	S	R	S	S	S	S	R	R	S
<i>E. coli</i> EXT309/39	R	R	R	R	R	I	R	R	S	S	S	S	R	R	S	R	R	R
<i>E. coli</i> EXT311/37	I	R	R	R	R	R	R	R	S	S	S	S	R	R	R	R	R	R
<i>E. coli</i> EXT337/38	R	R	I	R	S	R	I	R	S	S	I	S	R	R	S	R	R	R
<i>E. coli</i> EXT351/67	R	R	R	R	R	I	R	R	S	S	R	I	R	R	nd	R	R	R
<i>Enterobacter cloacae</i> EXT389/29	R	R	I	R	S	S	R	R	S	S	R	S	R	R	R	R	R	R
<i>E. cloacae</i> EXT390/51	R	R	I	R	S	S	R	R	S	S	R	S	S	R	R	I	S	R
<i>E. cloacae</i> EXT413/50	R	R	R	R	R	I	R	R	S	S	R	S	R	R	R	R	R	R
<i>Klebsiella pneumoniae</i> EXT149/48	R	R	R	R	I	R	R	R	S	S	R	S	R	R	R	I	R	S
<i>K. pneumoniae</i> EXT183/46	R	R	R	R	R	R	R	R	S	S	R	I	S	R	R	R	S	R

<i>K. pneumoniae</i> EXT353/45	R	R	R	R	I	I	R	R	S	S	R	S	R	R	R	R	R	R
<i>K. pneumoniae</i> EXT386/47	S	R	R	R	R	I	R	R	S	S	S	S	R	R	R	S	S	R
<i>Proteus mirabilis</i> EXT353/59	R	R	S	I	S	S	S	R	S	S	I	S	R	R	R	R	R	R
<i>P. mirabilis</i> EXT369/58	R	R	S	I	S	S	S	S	S	S	I	S	R	R	R	R	R	R
<i>P. mirabilis</i> EXT386/57	R	R	S	R	I	S	R	R	S	S	R	S	R	R	R	R	S	R
<i>Pseudomonas aeruginosa</i> EXT170/54	nd	nd	R	nd	R	R	R	S	S	S	nd	S	R	nd	nd	R	R	nd
<i>P. aeruginosa</i> 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].

Table 2Resistance determinants detected in bacterial wound infection isolates ^a

Strain no.	ARDM determinants directed against:										
	β-Lactams	Aminoglycosides	Macrolides, lincosamides, streptogramins	Tetracyclines	Ansamycins	Phenicol	Quinolones	Quaternary amines	Streptothricin	Sulfonamide	Diaminopyrimidine
<i>Staphylococcus aureus</i> EXT099/32	<i>blaZ/bla</i> PC-1 family, <i>pbp/m</i> <i>ecA</i>		<i>erm</i> (B)	<i>tet</i> (38), <i>tet</i> (K)			<i>norA</i>				
<i>S. aureus</i> EXT108/33	<i>blaZ/bla</i> PC-1 family, <i>pbp/m</i> <i>ecA</i>		<i>erm</i> (B)	<i>tet</i> (38)			<i>norA</i>				
<i>S. aureus</i> EXT111/25	<i>blaZ/bla</i> PC-1 family, <i>pbp/m</i> <i>ecA</i>		<i>erm</i> (B)	<i>tet</i> (38)			<i>norA</i>				
<i>S. aureus</i> EXT168/23	<i>blaZ/bla</i> PC-1 family, <i>pbp/m</i> <i>ecA</i>		<i>erm</i> (B)	<i>tet</i> (38)			<i>norA</i>				

<i>S. aureus</i> EXT173/27	<i>blaZ/bla</i> PC-1 family, <i>pbp/m</i> <i>ecA</i>	<i>erm(B)</i>	<i>tet(38)</i> , <i>tet(L_p)</i> , <i>tet(M)</i>	<i>norA</i>
<i>S. aureus</i> EXT192/24	<i>blaZ/bla</i> PC-1 family, <i>pbp/m</i> <i>ecA</i>	<i>erm(B)</i>	<i>tet(38)</i>	<i>norA</i>
<i>S. aureus</i> EXT409/29	<i>pbp/me</i> <i>cA</i>	<i>erm(C)</i> , <i>lnu(B)/lin(B)</i>	<i>tet(38)</i> , <i>tet(K)</i> , <i>tet(M)</i>	<i>norA</i>
<i>S. aureus</i> EXT410/26	<i>blaZ/bla</i> PC-1 family, <i>pbp/m</i> <i>ecA</i>	<i>erm(B)</i>	<i>tet(38)</i> , <i>tet(K)</i>	<i>norA</i>
<i>Staphylococ</i> <i>cus</i> sp. EXT085/30	<i>blaZ/bla</i> PC-1 family, <i>pbp/m</i> <i>ecA</i>	<i>msr(A)/msr(B)/ms</i> <i>r(SA)</i> family, <i>lnu(A)/lin(A)</i>	<i>tet(38)</i>	<i>norA</i>
<i>Staphylococ</i> <i>cus</i> sp. EXT123/34	<i>blaZ/bla</i> PC-1 family, <i>pbp/m</i> <i>ecA</i>	<i>erm(C)</i>	<i>tet(K)</i>	<i>dfrA</i>

<i>Acinetobact</i> <i>er</i> <i>baumannii</i> EXT310/35 b	<i>ampC</i> , <i>bla</i> _{OXA-} 23, <i>bla</i> _{OXA-} 51-like, <i>bla</i> _{ADC-} 1	<i>aacC1</i> , <i>aadA1/A2</i> family, <i>aphA6</i>	<i>tet(A)</i>	<i>catA1/c</i> <i>at4</i> family	<i>qacEΔ1</i> , <i>qacE</i>	<i>sul1</i>	<i>folA</i>
<i>Escherichia</i> <i>coli</i> EXT121/40	<i>bla</i> _{CTX-M-} 1 family	<i>aac(3)-III</i>		<i>cmr</i>			
<i>E. coli</i> EXT191/43	<i>bla</i> _{CTX-M-} 1 family	<i>aac(3)-III</i>		<i>cmr</i>	<i>qnrS</i>		
<i>E. coli</i> EXT253/36	<i>bla</i> _{TEM} family , <i>bla</i> _{CTX-} M-9 family			<i>cmr</i>			
<i>E. coli</i> EXT301/41	<i>bla</i> _{TEM} family	<i>aac(6)-Ib</i> family		<i>cmr</i>			
<i>E. coli</i> EXT309/39	<i>bla</i> _{TEM} family , <i>bla</i> _{CTX-} M-1 family	<i>aac(3)-III</i>	<i>mac(A)</i>	<i>cmr</i>			<i>dfrA17</i>

<i>E. coli</i> EXT311/37	<i>bla</i>_{CTX-M-1} family						
<i>E. coli</i> EXT337/38	<i>bla</i> _{CMY/L_{AT}} family, <i>bla</i>_{TEM} family	<i>aac(3)-III</i> , <i>aph(6)-Id</i>	<i>mac(A)</i> , <i>mac(B)</i> , <i>mph(A)/mph(K)</i> family		<i>cmr</i>	<i>qacEΔ1</i>	<i>sul2</i> <i>dfrA17</i>
<i>E. coli</i> EXT351/67 ^c	<i>bla</i> _{OXA-1} , <i>bla</i>_{TEM} family , <i>bla</i> _{CTX-M-1} family	<i>aac(3)-III</i> , <i>aac(6)-Ib</i> family, <i>aphA1</i>	<i>mac(A)</i>	<i>tet(B)</i>	<i>catA1/c</i> <i>at4</i> family, <i>cmr</i>		
<i>Enterobacter cloacae</i> EXT389/49 ^d	–	<i>aadA1/A2</i> family	<i>lnu(F)/lin(F)</i> , <i>lnu(G)/lin(G)</i>		<i>qnrS</i>		
<i>E. cloacae</i> EXT390/51	<i>bla</i> _{OXA-1} , <i>bla</i> _{TEM} family, <i>bla</i> _{CTX-M-1} family	<i>aac(6)-Ib</i> family, <i>aadA1/A2</i> family, <i>aph(3'')</i> , <i>aph(6)-Id</i>			<i>catA1/c</i> <i>at4</i> family	<i>sul2</i>	<i>dfrA14</i>

<i>E. cloacae</i> EXT413/50 e	<i>bla</i> _{OXA-1} , <i>bla</i> _{TEM} family, <i>bla</i> _{CTX-} M-1 family	<i>aac(3)-III</i> , <i>aac(6)-Ib</i> family, <i>aadA1/A2</i> family, <i>aph(3'')</i> , <i>aph(6)-Id</i>				<i>catA1/c</i> <i>at4</i> family		<i>sul2</i>	<i>dfrA14</i>
<i>Klebsiella pneumoniae</i> EXT149/48 f	<i>ampC</i> , <i>bla</i> _{DHA}			<i>tet(A)</i>			<i>qnrS</i>		
<i>K. pneumoniae</i> EXT183/46	<i>bla</i> _{OXA-1} , <i>bla</i>_{SHV} family , <i>bla</i>_{TEM} family , <i>bla</i>_{CTX-} M-1 family	<i>aac(6)-Ib</i> family, <i>aph(3'')</i> , <i>aph(6)-Id</i>		<i>tet(A)</i> , <i>tet(D)</i>		<i>catA1/c</i> <i>at4</i> family		<i>sul2</i>	<i>dfrA14</i>
<i>K. pneumoniae</i> EXT353/45	<i>bla</i>_{TEM} family , <i>bla</i>_{CTX-} M-1 family	<i>aac(3)-III</i> , <i>aac(6)-Ib</i> family, <i>aphA1</i> , <i>aph(3'')</i> , <i>aph(6)-Id</i>	<i>mph(A)/mph(K)</i> family	<i>tet(D)</i>	<i>arr</i>	<i>catA1/c</i> <i>at4</i> family	<i>qacEΔ1</i>	<i>sul1, sul2</i>	<i>dfrA27, dfrA5</i>

<i>K. pneumoniae</i> EXT386/47	<i>bla</i> _{CTX-M-1} family						<i>qacEΔ1</i>			
<i>Proteus mirabilis</i> EXT353/59	<i>bla</i> _{OXA-1} , <i>bla</i> _{TEM} family	<i>aac(3)-I</i> , <i>aac(3)-IVa</i> , <i>aadA1/A2</i> family		<i>tet(J)</i>	<i>arr</i>	<i>cmlA</i>	<i>qacEΔ1</i>	<i>sat2</i>	<i>sul1</i> , <i>sul2</i> , <i>sul3</i>	<i>dfrA1</i>
<i>P. mirabilis</i> EXT369/58	<i>bla</i> _{OXA-1}	<i>aac(3)-IVa</i> , <i>aac(6)-Ib</i> family, <i>aadA1/A2</i> family, <i>aphA1</i> , <i>aph(6)-Id</i> , <i>aphA6</i>		<i>tet(J)</i>	<i>arr</i>	<i>floR</i> family, <i>cmlA</i>	<i>qacEΔ1</i>		<i>sul1</i> , <i>sul2</i> , <i>sul3</i>	
<i>P. mirabilis</i> EXT386/57	–	<i>aac(3)-IVa</i>	<i>erm(H)/car(B)</i>	<i>tet(J)</i>	<i>arr</i>		<i>qacEΔ1</i>		<i>sul2</i>	
<i>Pseudomonas aeruginosa</i> EXT170/54	<i>bla</i> _{OXA-10/PSE-1} , <i>bla</i> _{VEB}	<i>aac(3)-III</i> , <i>aac(6)-Ib</i> family, <i>aadA1/A2</i> family, <i>aadB</i> , <i>aph(6)-Id</i> , <i>aphA6</i>			<i>arr</i>	<i>cmlA</i>	<i>qacEΔ1</i>		<i>sul1</i>	

<i>P.</i>	<i>bla</i> _{OXA-}	<i>aac</i> (3)-III,	<i>arr</i>	<i>cmlA</i>	<i>qacE</i> Δ1	<i>sul1</i>
<i>aeruginosa</i>	10/PSE,	<i>aac</i> (6)-Ib				
EXT407/53	<i>bla</i> _{VEB}	family,				
		<i>aadA</i> 1/A2				
		family,				
		<i>aadB</i> ,				
		<i>aph</i> (6)-Id,				
		<i>aphA</i> 6				

ARDM, Antimicrobial Resistance Determinant Microarray.

^a PCR-confirmed genes shown in bold.

^b Analysed on ARDM v.3 chip. Additional genes detected: *aac*(2)-Ib, *eptA*.

^c Analysed on ARDM v.3 chip. Additional genes detected: *intl1*, *catB3*, *satG*, *dfrA32*.

^d Analysed on ARDM v.3 chip. Additional genes detected: *qnrS2*.

^e Analysed on ARDM v.3 chip. Additional genes detected: *qnrB6* family.

^f Analysed on ARDM v.3 chip. Additional genes detected: *catA2*, *qnrS2*.