**Prevalence and antimicrobial susceptibilities of *Acinetobacter baumannii* and non-*baumannii* Acinetobacters from Terengganu, Malaysia and their carriage of carbapenemase genes**

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**ABSTRACT**

A total of 153 non-repeat *Acinetobacter* spp. clinical isolates obtained in 2015 from Hospital Sultanah Nur Zahirah (HSNZ) in Terengganu, Malaysia, were characterised. Identification of the isolates at species level was performed by ribosomal DNA restriction analysis (ARDRA) followed by sequencing of the *rpoB* gene. The majority of the isolates (*n* = 128; 83.7%) were *A. baumannii* while the rest were identified as *A. nosocomialis* (*n* = 16), *A. calcoaceticus* (*n* = 5), *A. soli* (*n* = 2), *A. berezeniae* (*n* = 1) and *A. variabilis* (*n* = 1). Multidrug resistance (MDR) was most prevalent in *A. baumannnii* (66.4%) whereas only one non-*baumannii* isolate (*A. nosocomialis*) was MDR. The *bla*OXA-23 gene was the predominant acquired carbapenemase gene (56.2%) and was significantly associated (*p* < 0.001) with carbapenem resistance. However, no significant association was found for carbapenem resistance and isolates that contained the IS*Aba1*-*bla*OXA-51 configuration.

**MAIN TEXT**

*Acinetobacter baumannii* is an important hospital-acquired pathogen that causes substantial mortality and economic burden, being responsible for a significant proportion of infections especially among patients in intensive care units [1, 2]. The major problem with *A. baumannii* is its extraordinary capacity to accumulate antimicrobial resistance genes and its potential to spread epidemically. Besides *A. baumannii*, four closely related *Acinetobacter* genomospecies, *A. nosocomialis*, *A. pittii*, *A. seifertii*, and *A. dijkshoorniae*, have been associated with nosocomial infections [3–7]. Along with *A. baumannii* and the soil bacterium *A. calcoaceticus*, these non-*baumannii* acinetobacters are often grouped together as part of the *A. baumannii-A. calcoaceticus* (Abc) complex due to difficulties in identifying these bacteria by routine biochemical methods [3, 5]. Besides the Abc complex, other non-*baumannii* acinetobacters such as *A. lwoffii*, *A. ursingii*, *A. johnsonii*, *A. haemolyticus*, *A. berezeniae* and *A. parvus* have been found to be clinically relevant [8, 9]. The List of Prokaryotic names with Standing in Nomenclature (LPSN) [10] currently lists a total of 62 described *Acinetobacter* species (including synonyms) [11]. Precise knowledge on the pathogenicity and occurrence of *Acinetobacter* species is currently limited due to the fact that its taxonomic description has undergone many changes over recent decades, while methods for the identification of newly described species have lagged behind or were not applicable in applied microbiology.

Epidemiological and clinical studies of *Acinetobacter* spp. often investigate the Abc complex as a single entity, which is practical, but limits the ability to differentiate the clinical features of infections due to *A. baumannii* and non­-*baumannii* isolates [3]. Amplified ribosomal DNA restriction analysis of the 16S rRNA gene (or ARDRA) was found to be sufficient to distinguish between *A. baumannii* and non-*baumannii* acinetobacters [12] although further identities of the non­-*baumannii* isolates were difficult to determine using this method. Methods for reliable species identification including *rpoB* sequencing [13–15] and matrix-assisted laser desorption-time of flight mass spectroscopy (MALDI-TOF MS) [5] have only recently been developed. These methods are now increasingly used, but are not yet widely available in diagnostic laboratories worldwide. Implementation of these methods will establish the clinical significance and epidemiology of non-*baumannii* *Acinetobacter* species [3, 16, 17].

Although Malaysia has an active national antimicrobial surveillance program, very few publications and little comprehensive data are available for *Acinetobacter* spp. infections [18], with none so far reporting on non-*baumannii* acinetobacters. Our previous study of *A. baumannii* isolated from Hospital Sultanah Nur Zahirah (HSNZ), the main tertiary hospital in the state of Terengganu, Malaysia in 2011, indicated a high incidence of multidrug resistance (>70%) with about 25% of the isolates categorized as extensive drug resistant (XDR) and displaying resistance to polymyxin B, considered one of the drugs of “last resort” for *Acinetobacter* infections [19, 20]. In this study, we examined *Acinetobacter* spp. isolates from the same tertiary hospital obtained throughout 2015 and identified the non-*baumannii* isolates by *rpoB* sequencing. Their antimicrobial susceptibility profiles were determined. Furthermore, carriage of carbapenemase-encoding genes was investigated since acquisition of these genes is the most important mechanism for dissemination of carbapenem resistance [21, 22]. This gives us a first comparative characterisation of *A.baumannii* and non-*baumannii* acinetobacters from a tertiary healthcare institution in Malaysia.

Ethical approval for this study was obtained from the Medical Research & Ethics Committee of the Malaysian Ministry of Health’s National Medical Research Register (approval no. NMRR-14-1650-23625-IIR). A total of 153 non-repeat *Acinetobacter* spp. isolates were collected from the Microbiology Laboratory, Department of Pathology, Hospital Sultanah Nur Zahirah (HSNZ), Kuala Terengganu, throughout 2015, and these were isolated from HSNZ as well as other district hospitals in the state of Terengganu. The strains were identified as *Acinetobacter* spp. by the Vitek 2 system (bioMérieux) at the hospital laboratory.

To determine if the isolates were *A. baumannii* or non-*baumannii* acinetobacters, their genomic DNA were subjected to amplified ribosomal DNA restriction analysis (ARDRA) with five different restriction enzymes [23, 24]. Further identification of the non-*baumannii* acinetobacters was performed by sequencing of the partial *rpoB* gene [15]. Suseptibility to fourteen antimicrobial agents (Oxoid Ltd., Basingstoke, UK) [i.e., gentamicin (10 µg), amikacin (30 µg), imipenem (10 µg), meropenem (10 µg), doripenem (10 µg), ciprofloxacin (5 µg), piperacillin-tazobactam (100/10 µg), ticarcillin-clavulanate (75/10 µg), cefotaxim (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefepime (30 µg), ampicillin-sulbactam (10/10 µg), tetracycline (30 µg),] was determined by the disc diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [25]. Minimum inhibitory concentration (MIC) values to imipenem, meropenem and doripenem (i.e., carbapenems), as well as tigecycline, were determined using M.I.C. Evaluator strips (Oxoid Ltd., Basingstoke, UK) with MIC breakpoints for tigecycline based on the US Food and Drug Administration guidelines [26]. Susceptibility to polymyxin B and colistin was determined by obtaining MIC values by the agar dilution method [19].

All isolates were screened for the presence of carbapenemase genes commonly found in *Acinetobacter* spp. by PCR using established primer sets (listed in Table S1). Statistical analyses were performed using SPSS (version 20.0) with parametric variables assessed using the chi-squared test whereas continuous variables were analysed using the Mann-Whitney test. A difference was considered statistically significant if the *p*-value was less than 0.05.

The majority of *Acinetobacter* spp. isolates were obtained from sputum (27.5%), blood (26.1%), and pus (19.6%) (Table S2). The median age of patients that were infected with *Acinetobacter* spp. in HSNZ was (52 ± 38) years and ranged from newborn to 91 years of age. The isolates were obtained from 90 male (58.8%) and 63 female (41.2%) patients.

The combination of banding patterns obtained from ARDRA (Figure S1) enabled the differentiation of *A. baumannii* (*n* = 128; 83.7%) and non-*baumannii* acinetobacters (*n* = 25; 16.3%). Fifteen isolates categorised as *A. baumannii* were randomly chosen along with 25 non-*baumannii* acinetobacters and subjected to PCR-amplification and sequencing of the *rpoB* gene. This validated the identity of all fifteen *A. baumannii* isolates (>98% sequence identity over the 330 nucleotides of the amplified partial *rpoB* gene). The majority of non-*baumannii* acinetobacter isolates were found to be *A. nosocomialis* (*n* = 16), with the rest identified as *A. calcoaceticus* (*n* = 5), *A. soli* (*n* = 2), *A. berezeniae* (*n* = 1), and *A. variabilis* (*n* = 1) (100% sequence identities with the *rpoB* sequences of the respective *Acinetobacter* spp. in the database). In contrast, an earlier study from Lieden University Medical Centre in the Netherlands showed that *A. baumannii* comprised only 27% of the 359 *Acinetobacter* spp. isolates obtained from 1999 – 2006 [27]. This illustrates that the clinical-epidemiological situation can differ significantly between hospitals and geographic areas. Part of the high prevalence of *A. baumannii* in HSNZ may be due to the endemic or even epidemic presence of one or more *A. baumannii* strains. Epidemic typing would be required to obtain insight into this matter.

The majority of the *A. baumannii* isolates were from intensive care units (ICUs) (32.0%), medical (31.3%) and surgical (19.5%) wards. Among the non-*baumannii* acinetobacters, eight of them were isolated from ICUs (six *A. nosocomialis,* one each of *A. calcoaceticus and A. soli*) (Table 1) with patients suffering from various additional co-morbidities. *A. nosocomialis* isolates were evenly distributed across the medical and surgical wards in HSNZ with four isolates originating from each ward, whereas the remaining two isolates were from other district hospitals or health clinics. However, due to the limited number of non-*baumannii* isolates that were obtained, we are unable to make any firm conclusion regarding their prevalence in HSNZ. For patients who were infected with *A. baumannii*, the mean duration of their hospital stay was 35.8 ± 3.6 with a median of 23 days. In comparison, the mean duration of hospital stay for patients infected with non-*baumannii* acinetobacters was 22.0 ± 7.3 with a median of 10 days. The relatively long stay in the hospital may increase the risk of acquisition of other nosocomial pathogens and contribute to the acquisition of endemic or epidemic *A. baumannii* strains.

Out of the 153 *Acinetobacter* spp. isolates, 86 were multidrug resistant (MDR; i.e., resistant to three or more classes of antimicrobials following proposed criteria [28]). However, among the 86 MDR isolates, only one was a non-*baumannii* acinetobacter, *A. nosocomialis*, which displayed resistance to carbapenems, cephalosporins (i.e., cefotaxime, ceftriaxone, ceftazidime and cefepime) and a β-lactam/β-lactamase inhibitor combination (ticarcillin/clavulanate). The remaining 85 MDR isolates were *A. baumannii* (out of 128, or a prevalence of 66.4%). Interestingly, four *Acinetobacter* spp. isolates were resistant only to carbapenems, three of which were *A. baumannii* and another one was *A. calcoaceticus*. Besides that, all the other non-*baumannii* acinetobacters were susceptible to the antimicrobials tested in this study (Table 2). The greater susceptibility of non-*baumannii* acinetobacters to antimicrobials as compared to *A. baumannii* has also been previously reported [29, 30].

For *A. baumannii*, the highest resistance rates were observed for the carbapenems at 68.8%, whereas the lowest was for the aminoglycosides at 60.9% (Table 2). E-test results indicated that the MIC values for these isolates for carbapenems were >32 µg/ml thus validating the carbapenem resistance state of these isolates. Lean et al. [19] reported on the resistance of *A. baumannii* isolated from HSNZ in 2011 and, in comparison, carbapenem resistance in 2015 (68.8%) was slightly lower (imipenem = 74.1%; meropenem = 77.8%). The prevalence of MDR was also lower among the *A. baumannii* isolates in 2015 (72.2% in 2011 compared to 66.4% in 2015).

There were no significant differences in the 2015 resistance levels of ciprofloxacin, cephalosporins and the combination of β-lactam/β-lactamase inhibitor when compared to the 2011 HSNZ isolates [19]. However, there was a marked reduction in the *A. baumannii* tetracycline resistance (64.8% in 2015) when compared to the 2011 isolates (87.0%). All *A. baumannii* and non-*baumannii* acinetobacter isolates in this study were susceptible to the polymyxins (i.e., polymyxin B and colistin) and tigecycline. This was in stark contrast to the 2011 isolates where a 25.9% polymyxin B resistance prevelance was reported; however, tigecycline susceptibility was not evaluated for the 2011 isolates [19].

All *A. baumannii* isolates in this study harboured the intrinsic *bla*OXA-51/*bla*OXA-51-like carbapenemase gene. Among the non-*baumannii* acinetobacter isolates, all were also positive for *bla*OXA-51/*bla*OXA-51-like with the exception of three *A. nosocomialis* isolates (Table S3; Figure S2). Despite their weak ability to hydrolyze carbapenems, the OXA-51/OXA-51-like enzymes that are intrinsic in *A. baumannii* may be involved in resistance to carbapenems through the insertion of IS*Aba1* upstream of the *bla*OXA-51/*bla*OXA-51-like gene which could lead to overexpression of the carbapenemase gene [31, 32]. In the Terengganu *Acinetobacter* spp.isolates, IS*Aba*1 was detected upstream of the   
*bla*OXA-51/*bla*OXA-51-like gene in 65.3% of the isolates. This configuration was found in three *A. nosocomialis*, two *A. calcoaceticus,* and one each of the *A. soli*, *A. bereziniae*,and *A. variabilis* isolates (Table S3; Figure S2) . However, the carriage of IS*Aba1*-*bla*OXA-51 was not associated at all with carbapenem resistance in the non-*baumannii* acinetobacters, as all the isolates that harboured IS*Aba1-bla*OXA-51 were carbapenem susceptible (Table S3). For the *A. baumannii* isolates, 17 were positive for IS*Aba1-bla*OXA-51 but were carbapenem susceptible (Table S3). The remaining 72 *A. baumannii* isolates that were carbapenem-resistant and harboured IS*Aba1*-*bla*OXA-51 also contained acquired carbapenemase genes, which made it difficult to ascertain if IS*Aba1*-*bla*OXA-51 was truly responsible for carbapenem resistance in these isolates. The non-association of *Acinetobacter* spp. isolates harbouring IS*Aba1*-*bla*OXA-51 with carbapenem resistance has also been reported elsewhere [33, 34].

As for the acquired carbapenemase genes, the majority of the *Acinetobacter* spp. isolates harboured the *bla*OXA-23 gene (*n* = 56; or 56.2%). A significant association (*p* < 0.001) was found between carbapenem resistance and the presence of the *bla*OXA-23 gene with 94.3% (*n* = 83) of the 88 carbapenem-resistant isolates harbouring the gene. Likewise, results from the 2011 *A. baumannii* isolates from HSNZ also showed significant association (*p* < 0.001) as 86.0% of the carbapenem-resistant isolates were positive for the *bla*OXA-23 gene [19]. Thus, there was a clear correlation between the presence of the   
*bla*OXA-23gene and carbapenem resistance in the Terengganu *Acinetobacter* spp. isolates.

The *bla*OXA-23 gene was also detected in the sole carbapenem-resistant *A. nosocomialis* isolate, Ans15/3 (Table S3; Figure S3). Likewise, *bla*OXA-23 was found in two *A. calcoaceticus* isolates, Aca15/2 and Aca15/4, but only one of them (Aca15/2) showed carbapenem resistance. This finding is remarkable since *A. calcoaceticus* is generally considered a harmless environmental species (although sometimes found in clinical specimens), which has not developed resistance to antibiotics over the past decades, in contrast to other species of the Abc complex. The only *A.variabilis* isolate, Ava15/1, also harboured IS*Aba1*-*bla*OXA-23 along with IS*Aba1*-*bla*OXA-51 (Table S3) but the isolate was intriguingly carbapenem susceptible. Only one *A. baumannii* isolate, Aba15/114, harboured the IS*Aba1*-*bla*OXA-23 configuration but was carbapenem-susceptible (Table S3); all other *A. baumannii* isolates that contained IS*Aba1*-*bla*OXA-23 were carbapenem-resistant. Isolates with *bla*OXA-23 but yet carbapenem susceptible were also found in the 2011 HSNZ *A. baumannii* isolates [19] and a previous study from Malaysia [34]. The reason for the carbapenem susceptibility of these isolates, despite containing *bla*OXA-23 or even IS*Aba1-bla*OXA-23, is unknown. Perhaps the IS*Aba1* is inserted in a position whereby its outward-directing promoter is non-functional, thereby the expression of *bla*OXA-23 remains low. Alternatively, the *bla*OXA-23 gene in these isolates could be mutated, resulting in a non-functional carbapenemase which renders the host isolate carbapenem susceptible. Wang et al. [35] suggested that the detection of IS*Aba*1-*bla*OXA-23 in carbapenem-susceptible isolates might be due to the downregulation of the IS element in the isolates or gene truncation at the 5’ terminus downstream of the *bla*OXA-23 gene. Researchers also suggested that gene down-regulation by the translational frameshift of IS*Aba*1 towards *bla*OXA-23 could occur depending on how IS*Aba1* was inserted upstream of the *bla*OXA-23 gene [36]. These should be investigated in future studies through full sequencing of these loci as well as measuring the expression levels of these carbapenemase genes.

Two other acquired carbapenemase genes detected in the Terengganu *Acinetobacter* isolates were *bla*OXA-24 and *bla*OXA-58. The *bla*OXA-24 gene was found in six *A. baumannii* and one *A. calcoaceticus* isolate, whereas *bla*OXA-58 was found in only two *A. baumannii* and one *A. nosocomialis* isolate (Table S3; Figure S3). *A. nosocomialis* Ans15/3 which co-harboured both *bla*OXA-58 and *bla*OXA-23 was carbapenem resistant. The *bla*OXA-58-positive *A. baumannii* isolates Aba15/20 and Aba15/21 also harboured IS*Aba1*-*bla*OXA-23 and were resistant to carbapenems. Likewise, *A. calcoaceticus* Ac15/2 contained both *bla*OXA-23 and *bla*OXA-24 genes and was also carbapenem resistant. However, three of the six *A. baumannii* isolates that harboured only the *bla*OXA-24 gene were carbapenem susceptible (isolates Aba15/37, Aba15/41 and Aba15/42); the other three *A. baumannii* isolates were carbapenem resistant but also co-harboured the *bla*OXA-23 gene.

None of the isolates were found to harbour the class B metallo-β-lactamase genes *bla*IMP and *bla*VIM. Nevertheless, the presence of the acquired *bla*OXA-23 and *bla*OXA-24 genes in carbapenem-susceptible *Acinetobacter* spp. isolates highlights the potential threat of undetected reservoirs of carbapenemase-encoding genes since the detection of resistance genes generally arises from phenotypically multidrug-resistant organisms [37].

**(2,455 words)**

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**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

**Abbreviations:** ARDRA, amplified ribosomal DNA restriction analysis; HSNZ, Hospital Sultanah Nur Zahirah; MDR, multidrug resistance

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**Table 1**: Distribution of the *Acinetobacter* spp. isolates (*n* = 153) according to the HSNZ hospital wards and other district hospitals and health clinics in Terengganu, Malaysia (2015)

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***Hospital Wards*** | ***Aba*** | | ***Ans*** | | ***Aca*** | | ***Aso*** | | ***Abr*** | | ***Ava*** | |
| ***n*** | ***%*** | ***n*** | ***%*** | ***n*** | ***%*** | ***n*** | ***%*** | ***n*** | ***%*** | ***n*** | ***%*** |
| Intensive Care Units (ICUs) | 41 | 32.0 | 6 | 37.5 | 1 | 20 | 1 | 50 | 0 | 0 | 0 | 0 |
| Medical Wards | 40 | 31.3 | 4 | 25.0 | 4 | 80 | 1 | 50 | 1 | 100 | 1 | 100 |
| Surgical Wards | 25 | 19.5 | 4 | 25.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Operation Theatres | 4 | 3.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Other district hospitals and health clinics in Terengganu | 18 | 14.0 | 2 | 12.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 128 | 100 | 16 | 100 | 5 | 100 | 2 | 100 | 1 | 100 | 1 | 100 |

**Abbreviations used: *Aba:*** *A. baumannii;*  ***Ans:*** *A. nosocomialis;* ***Aca:*** *A. calcoaceticus;*  ***Aso:*** *A. soli;*  ***Abr:*** *A. bereziniae;* ***Ava:*** *A. variabilis*

**Table 2**:Resistance rates of the *Acinetobacter* spp. isolates from HSNZ, Terengganu, Malaysia in 2015

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Antimicrobials** | **CONCENTRA-TION *a* (µg/disc)** | ***A. baumanni***  **(*n =* 128)** | | ***A. nosocomialis***  **(*n* =16)** | | ***A. calcoaceticus***  **(*n* = 5)** | | ***A. soli***  **(*n* = 2)** | | ***A. berezeniae***  **(*n* = 1)** | | ***A. variabilis***  **(*n* =1)** | |
| ***n*** | **%** | ***n*** | **%** | ***n*** | **%** | ***n*** | **%** | ***n*** | **%** | ***n*** | **%** |
| **Aminoglycosides** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Gentamicin | 30 | 78 | 60.9 | 1 | 6.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amikacin | 10 | 78 | 60.9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Carbapenems** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Imipenem | 10 | 88 | 68.8 | 1 | 6.3 | 1 | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| Meropenem | 10 | 88 | 68.8 | 1 | 6.3 | 1 | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| Doripenem | 10 | 88 | 68.8 | 1 | 6.3 | 1 | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Fluroquinolones** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Ciprofloxacin | 5 | 84 | 65.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **β-lactam/β-lactamase inhibitor combination** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Piperacillin/Tazobactam | 100/10 | 85 | 66.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ticarcillin/Clavulanate | 75/10 | 86 | 67.2 | 2 | 12.5 | 1 | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ampicillin/Sulbactam | 10/10 | 84 | 65.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Cephalosporins** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Cefotaxime | 30 | 87 | 68.0 | 1 | 6.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceftriaxone | 30 | 86 | 67.2 | 1 | 6.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceftazidime | 30 | 85 | 66.4 | 1 | 6.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cefepime | 30 | 85 | 66.4 | 1 | 6.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Tetracyclines** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Tetracycline | 30 | 83 | 64.8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Glycylcylines** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Tigecycline*b* | NA*d* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Lipopeptides (Polymyxins)** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Polymyxin B*c* | NA*d* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Colistin*c* | NA*d* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*a* Susceptibilities were determined using the disc diffusion method following the guidelines from the Clinical and Laboratory Standards Institute (CLSI) [25] with the exception of tigecycline and the polymyxins

*b* Tigecycline susceptibility was determined by the E-test which measures the MIC values and the breakpoints were obtained from the United States Food and Drug Administration (FDA) as outlined by Nicolau et al. (2015) [26]

*c* Polymyxin B and colistin susceptibility were determined by obtaining the MIC values by the agar dilution method [19] and the breakpoints from CLSI [25].

*d* NA = not applicable