

In vitro activity of colistin in antimicrobial combination against carbapenem-resistant *Acinetobacter baumannii* isolated from patients with ventilator-associated pneumonia in Vietnam

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Acinetobacter baumannii has become one of the major infection threats in intensive care units (ICUs) globally. Since 2008, *A. baumannii* has been the leading cause of ventilator-associated pneumonia (VAP) in our ICU at an infectious disease hospital in southern Vietnam. The emergence of this pathogen in our setting is consistent with the persistence of a specific clone exhibiting resistance to carbapenems. Antimicrobial combinations may be a strategy to treat infections caused by these carbapenem-resistant *A. baumannii*. Therefore, we assessed potential antimicrobial combinations against local carbapenem-resistant *A. baumannii* by measuring *in vitro* interactions of colistin with four antimicrobials that are locally certified for treating VAP. We first performed antimicrobial susceptibility testing and multilocus variable number tandem repeat analysis (MLVA) genotyping on 74 *A. baumannii* isolated from quantitative tracheal aspirates from patients with VAP over an 18-month period. These 74 isolates could be subdivided into 21 main clusters by MLVA and >80 % were resistant to carbapenems. We selected 56 representative isolates for *in vitro* combination synergy testing. Synergy was observed in four (7 %), seven (13 %), 20 (36 %) and 38 (68 %) isolates with combinations of colistin with ceftazidime, ceftriaxone, imipenem and meropenem, respectively. Notably, more carbapenem-resistant *A. baumannii* isolates (36/43; 84 %) exhibited synergistic activity with a

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Abbreviations: FIC, fractional inhibitory concentration; FICI, fractional inhibitory concentration index; HCMC, Ho Chi Minh City; HTD, Hospital for Tropical Diseases; ICU, intensive care unit; MDR, multidrug-resistant; MLVA, multilocus variable number tandem repeat analysis; TA, tracheal aspirate; VAP, ventilator-associated pneumonia; VNTR, variable number tandem repeat.

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combination of colistin and meropenem than carbapenem-susceptible *A. baumannii* isolates (2/13; 15 %) ($P=0.023$; Fisher's exact test). Our findings suggest that combinations of colistin and meropenem should be considered when treating carbapenem-resistant *A. baumannii* infections in Vietnam, and we advocate clinical trials investigating combination therapy for VAP.

INTRODUCTION

Acinetobacter baumannii has emerged as one of the most important Gram-negative nosocomial pathogens affecting critically ill patients, and is one of the most common causes of ventilator-associated pneumonia (VAP) worldwide (Taylor *et al.*, 2012; Schultz *et al.*, 2013). The treatment of *A. baumannii* infections is complicated because of increasing resistance to antimicrobial agents including carbapenems. Outbreaks of healthcare-associated infections caused by carbapenem-resistant *A. baumannii* have been reported, and carbapenem-resistant organisms are now widespread (Kim *et al.*, 2012; McGrath *et al.*, 2011; Song *et al.*, 2011). As a consequence of carbapenem resistance, the polymyxin drug colistin is becoming an alternative treatment for antimicrobial-resistant *A. baumannii* infections (De Pascale *et al.*, 2010; Lim *et al.*, 2011a, b; Kofteridis *et al.*, 2010; Lin *et al.*, 2010; Nakwan *et al.*, 2011). However, colistin is also an imperfect alternative, and colistin-resistant *A. baumannii* are becoming more frequently reported (Adams *et al.*, 2009; López-Rojas *et al.*, 2011; Mofatt *et al.*, 2010, 2011; Park *et al.*, 2009; Rodriguez *et al.*, 2009; Soon *et al.*, 2011).

In the intensive care unit (ICU) at the Hospital for Tropical Diseases (HTD), Ho Chi Minh City (HCMC), Vietnam, an apparent clonal replacement of a carbapenem-resistant *A. baumannii* strain in 2008 (Nhu *et al.*, 2014) has led to colistin being used as the first-line treatment for VAP caused by these infections. If these multidrug-resistant (MDR) *A. baumannii* isolates also become colistin resistant, they will be untreatable with locally available antimicrobials. Therefore, we hypothesize that antimicrobial combinations may be a therapeutic strategy that improves outcome against these MDR *A. baumannii* (Gordon *et al.*, 2010; Kiratisin *et al.*, 2010; Lim *et al.*, 2011b; Sheng *et al.*, 2011; Sopirala *et al.*, 2010; Tan *et al.*, 2011; Wareham *et al.*, 2011). We investigated the *in vitro* activity of colistin in combination with a variety of commonly used antimicrobial agents against carbapenem-resistant *A. baumannii* strains isolated from the ICU of the HTD. Our data showed that colistin used in combination with either third-generation cephalosporins or carbapenems may have a synergistic effect on treating infections caused by MDR *A. baumannii*.

METHODS

Study site and patients. This study was performed on bacterial isolates collected as part of diagnostic tests performed as a standard of care at our hospital. The data were anonymized before analysis and individual patient consent was not required. The site of the study was

the HTD in HCMC in the south of Vietnam. HTD is a 550-bed hospital that serves as a primary and secondary facility for the surrounding local population in HCMC and as a tertiary referral centre for infectious diseases for the 17 southern provinces of the country; it has a catchment population of approximately 40 million people. Nearly 70 % of HTD admissions are resident in HCMC, with the remainder resident in the surrounding provinces. Patients without infectious diseases, including those with surgical requirements, tuberculosis, cancer, primary haematological disorders or immunosuppression (other than human immunodeficiency virus infection) are generally referred to other healthcare settings in the city.

Sample collection and microbiological culture. All strains were isolated from tracheal aspirate (TA) specimens taken from patients with suspected VAP in the ICU of the HTD in HCMC from January 2011 to June 2012. The criteria for analysis were: admission to the ICU, intubated for mechanical ventilation (due to respiratory failure), with a TA collected because of suspected VAP. VAP is defined as pneumonia where the patient was on mechanical ventilation for >2 days when pneumonia was recorded, with day 1 being the first day of mechanical ventilation. If the patient was admitted to or transferred to the ICU on a ventilator, the day of admission was considered as day 1.

TAs were collected according to the local standard operating procedures of HTD. Patients were pre-oxygenated and a standard 500 mm, 14-gauge tracheal aspiration catheter (Argyle Sherwood Medical) was attached to a 20 ml syringe filled with 20 ml sterile saline. The distal end was lubricated with sterile gel, introduced via the tracheostomy or endotracheal tube, and advanced until significant resistance was encountered. The saline was instilled over 10–15 s, the tube was then withdrawn 10–20 mm, the saline was immediately re-aspirated and the catheter was then removed. In total, 5–10 ml fluid was recovered. No further aspiration was attempted during removal of the catheter to avoid contamination with tracheal secretions. Samples were transported to the microbiology laboratory, placed in the fridge at 4 °C and processed within 2 h of collection. The TA samples were examined by a Gram stain and the aspirate fluid was diluted 1 : 1 with Sputasol (Oxoid) and incubated at 37 °C, with periodic agitation, until liquefaction. The sample was then diluted (1 : 1, 10^{-1} and 10^{-2}) using maximum recovery diluent (Oxoid), and 20 µl of 1 : 1 diluent was inoculated onto blood agar and chocolate agar base plates. Additionally, 20 µl of the 10^{-1} and 10^{-2} dilutions was plated onto MacConkey medium and blood agar base (all media were supplied by Oxoid Unipath). Inoculated media were incubated at 37 °C and examined after 24 and 48 h of incubation. The threshold used to discriminate between infection and colonization was $\geq 1 \times 10^5$ c.f.u. ml $^{-1}$ (i.e. >20 colonies on either medium from the 10^{-2} dilution). Colonies above this threshold were identified using an in-house bacteriological identification (biochemical short-set) kit and/or by API 20E and API 20NE kits following the manufacturer's guidelines (bioMérieux).

Acinetobacter spp. identified using API 20NE were confirmed by a previously described PCR method to detect *bla*_{OXA-51} (Woodford *et al.*, 2006). PCR amplifications were performed in 20 µl reaction volumes, containing 1 µl template genomic DNA, 0.2 µM each primer, 2 U Taq DNA polymerase, 200 µM each dNTP and 1.5 mM MgCl₂ in 1 × PCR buffer (all PCR reagents were supplied by Bioline). PCR amplifications were visualized on 1 % agarose gels under UV

light after staining with ethidium bromide and were compared with the predicted sizes. All *A. baumannii* isolates were stored in glycerol at -70°C until the synergistic testing was performed.

Antimicrobial susceptibility testing. Antimicrobial susceptibilities were determined at the time of isolation by the modified Bauer–Kirby disc diffusion method, as recommended by the CLSI guidelines (CLSI, 2012). The antimicrobials tested were piperacillin/tazobactam (100/10 μg), imipenem (10 μg), meropenem (10 μg), amikacin (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), ticarcillin/clavulanic acid (75/10 μg), cefepime (30 μg), levofloxacin (5 μg) and co-trimoxazole (1.25/23.75 μg). Mueller–Hinton agar and antimicrobial discs were purchased from Oxoid. For colistin, meropenem, imipenem, ceftazidime and ceftriaxone, MICs were determined by E-test based on the manufacturer's recommendations (bioMérieux). The results were interpreted as resistant or sensitive according to current CLSI guidelines (CLSI, 2012). *Escherichia coli* ATCC 25922 was used as the control for these assays. For isolates that had an MIC above the range of the E-test, MICs were determined by the broth dilution method (Wiegand *et al.*, 2008). Briefly, 5×10^5 c.f.u. (ml bacteria) $^{-1}$ were inoculated into a series of Mueller–Hinton broths containing two-fold dilutions of the antimicrobial agent. Following inoculation, the broth was incubated at 37°C for 18–24 h. The MIC was defined as the lowest concentration of antimicrobial that inhibited the growth of bacteria.

Multilocus variable number tandem repeat (MLVA) genotyping. DNA was extracted from the 74 *A. baumannii* selected for further analysis using a Wizard Genomic DNA Extraction kit (Promega). The quality and concentration of the DNA were assessed using a Nanodrop Bioanalyser spectrophotometer (Thermo Scientific). Genomic DNA from all strains was standardized to a concentration of 25 ng μl^{-1} for further use. The 74 selected strains of *A. baumannii* were genotyped using the MLVA method developed by Pourcel *et al.* (2011) with some modifications. Briefly, genomic DNA from each of the 74 *A. baumannii* was subjected to three multiplex PCR amplifications (in a total volume of 50 μl), in which the annealing temperature was set at 50°C . The sizes of the amplicons [corresponding to eight variable number tandem repeats (VNTRs)] at each locus were determined by capillary electrophoresis fragment analysis using an ABI 3130 XL capillary electrophoresis system (Applied Biosystems). For fragment analysis, 0.5 μl PCR amplicon was mixed with 9.25 μl Hi-Di Formamide and 0.25 μl GeneScan LIZ500 size standard (Applied Biosystems). The mixture was incubated for 3 min at 95°C , chilled for 10 min and analysed. Resulting fragment analysis data were analysed using GeneMapper v.4.0 (Applied Biosystems). Furthermore, to determine the number of repeating units, the different-sized amplicons at each locus were DNA sequenced. PCR amplicons were purified using a PCR purification kit (Qiagen) and sequenced using a BigDye Terminator Sequencing kit (Applied Biosystems). All data were analysed using a numeric coefficient in BioNumerics software (Applied Maths) and trees were drawn using Dendroscope v.2.3.

Synergy testing by chequerboard assay. The *in vitro* activity of colistin in combination with meropenem, imipenem, ceftazidime or ceftriaxone was assessed in a microtitre plate chequerboard assay (Petersen *et al.*, 2006). All antimicrobials were obtained from Sigma-Aldrich and stock solutions of 10 000 mg l^{-1} were prepared in sterile water and stored at -20°C until use. The range of final working concentrations of each antimicrobial varied and depended on the MIC of each strain. The concentration range for colistin was 0.03–2 mg l^{-1} , for carbapenem was 0.13–8 μg ml^{-1} and the for the cephalosporins was 1–64 mg l^{-1} . The final concentration of the test strain was approximately 5×10^5 c.f.u. ml^{-1} in a final volume of 100 μl in each well. The *A. baumannii* were incubated at 37°C for 18–24 h. The chequerboard titration of each combination was carefully

performed with positive (with bacteria) and negative (without bacteria) controls.

The fractional inhibitory concentration (FIC) to determine synergy between antimicrobial agents was calculated as follows: FIC of drug A=(MIC of drug A in combination)/(MIC of drug A alone); FIC of drug B=(MIC of drug B in combination)/(MIC of drug B alone). The FIC index (FICI) was defined as the FIC of drug A added to the FIC of drug B. The FICI was interpreted as follows: synergistic, FICI ≤ 0.5 ; indifferent, $0.5 < \text{FICI} < 4$; and antagonistic, FICI ≥ 4.0 (Hall *et al.*, 1983). The assay was repeated on three occasions for each isolate.

RESULTS

Microbial culture of TAs

From January 2011 to June 2012, there were 156 patients with suspected VAP in the ICU at HTD in HCMC. From these, a total of 228 TAs were collected for bacteriological assessment, of which 166 (72.8 %) produced a significant bacterial culture result (defined as $\geq 10^5$ c.f.u. ml^{-1} of a potential VAP pathogen). The predominant bacterial species were the *Acinetobacter* spp., accounting for nearly half of the isolated bacteria (94/195), of which 74 (78.7 %) were identified as *A. baumannii* by PCR amplification of the *bla*_{OXA-51} gene.

The 74 *A. baumannii* strains were subjected to antimicrobial susceptibility testing against 11 antimicrobials (Table 1). The *A. baumannii* isolates exhibited substantial antimicrobial resistance, with 54 % of the isolates resistant to all antimicrobials tested except colistin. According to current CLSI breakpoints, colistin was the only active

Table 1. Antimicrobial susceptibilities of 74 *A. baumannii* isolates from VAP patients

CO, colistin; IMP, imipenem; MEP, meropenem; CAZ, ceftazidime; CRO, ceftriaxone; TZP, piperacillin/tazobactam; AK, amikacin; TCC, ticarcillin/clavulanic acid; SXT, co-trimoxazole; FEP, cefepime; LEV, levofloxacin; NT, not tested.

| Antimicrobial | Resistant (%) [*] | MIC (mg l^{-1}) | | |
|---------------|----------------------------|---------------------------|-------------------|-------------------|
| | | Range | MIC ₅₀ | MIC ₉₀ |
| CO | 0 (0 %) | 0.047–0.75 | 0.19 | 0.38 |
| IMP | 61 (82 %) | 0.25–512 | 64 | 64 |
| MEP | 62 (84 %) | 0.19–128 | 32 | 64 |
| CAZ | 64 (86 %) | 2–4096 | 256 | 512 |
| CRO | 65 (88 %) | 2–2048 | 1024 | 2048 |
| FEP | 69 (93 %) | NT | NT | NT |
| TZP | 66 (89 %) | NT | NT | NT |
| AK | 61 (82 %) | NT | NT | NT |
| TCC | 66 (89 %) | NT | NT | NT |
| SXT | 60 (80 %) | NT | NT | NT |
| FEP | 69 (93 %) | NT | NT | NT |
| LEV | 60 (80 %) | NT | NT | NT |

^{*}Resistance breakpoints (mg l^{-1}): colistin, 4; imipenem, 16; meropenem, 16; ceftazidime, 32; ceftriaxone, 64.

agent against all *A. baumannii* isolates, while >80 % of the *A. baumannii* isolates were resistant to at least three different antimicrobial classes, including carbapenems, cephalosporins and fluoroquinolones. Notably, the MIC₉₀ for meropenem and imipenem was >32 mg l⁻¹, and was >256 mg l⁻¹ for ceftriaxone and ceftazidime.

Genotyping of *A. baumannii*

MLVA was performed on eight VNTR loci to determine relatedness among the 74 *A. baumannii* isolates associated with VAP. With an arbitrary cut-off set at 95 % similarity, the MLVA patterns classified these isolates into 21 discrete clusters (Fig. 1). Three main groups were observed (labelled 1, 4 and 6 in Fig. 1). However, one clone (group 4) dominated and was responsible for a third of all the VAP cases caused by *A. baumannii* during this period. All of these isolates were resistant to meropenem, imipenem, ceftazidime, ceftriaxone and levofloxacin. A total of 56 *A. baumannii* isolates were selected for synergy testing with an array of antimicrobials through comparison of antimicrobial and MVLA profiles in order to cover a range of genotypes. The selected strains and their associated resistance profile are summarized in Fig. 1.

In vitro synergy testing of colistin in combination with other antimicrobials

The 56 *A. baumannii* isolates were subjected to *in vitro* synergy testing using a checkerboard method to identify potential activity between colistin and the other antimicrobial agents currently available and licensed for treating VAP in Vietnam: meropenem, ceftazidime, ceftriaxone and imipenem. Data from the 224 combination assays are shown in Table 2 and Fig. 1. Sixty-nine (31 %) of the assays demonstrated a synergistic interaction, with the remaining 155 (69 %) assays showing indifferent interactions. No antagonism was identified with any combinations of antimicrobials (Table 2). Synergistic activity was observed between colistin and ceftazidime (4/56 isolates; 7 %), colistin and ceftriaxone (7/56 isolates; 13 %), and colistin and imipenem (20/56 isolates; 36 %). The greatest degree of synergistic activity was observed between colistin and meropenem, with 38/56 (68 %) demonstrating a decreased FICI.

To assess the potential impact of the combination of colistin and meropenem in treating carbapenem-resistant *A. baumannii* VAP infections, the data resulting from the synergy assays were stratified into two groups, carbapenem-resistant and carbapenem-susceptible isolates (Fig. 1, Table 3). Synergism was observed more frequently (in any colistin/antimicrobial combination) in the carbapenem-resistant *A. baumannii* isolates than in the carbapenem-susceptible isolates. Furthermore, there was a significant synergistic effect between colistin and meropenem in carbapenem-resistant *A. baumannii* (36/43; 84 %) compared with the carbapenem-susceptible isolates

(2/13; 15 %) ($P=0.023$, Fisher's exact test). Notably, synergy between colistin and carbapenems was observed in all the dominant MLVA groups (including group 4) and across a broad range of the MLVA subgroups.

DISCUSSION

A. baumannii is a ubiquitous environmental organism that can cause a wide range of opportunistic infections in healthcare settings, including septicaemia, pneumonia, endocarditis, meningitis, skin infections, wound infections and urinary tract infections (Bergogne-Bérézin & Towner, 1996). Indeed *A. baumannii* is now widely acknowledged as an important cause of VAP (Choi *et al.*, 2010; Peleg *et al.*, 2008). *A. baumannii* is a substantial threat in critically ill patients in ICUs and other high-dependency healthcare units (Barnaud *et al.*, 2010; Jung *et al.*, 2010; McGrath *et al.*, 2011). In Vietnam, *A. baumannii* is recognized as a substantial problem in healthcare facilities, causing infections in intubated adults and critically ill children and neonates (Kruse *et al.*, 2013; Schultz *et al.*, 2013; Tada *et al.*, 2013). We have found that the proportion of patients at our hospital infected with MDR *A. baumannii* has been steadily increasing over the last decade (Nhu *et al.*, 2014). The increase in *A. baumannii* in Vietnam has led to widespread use of polymyxins, particularly colistin, for the treatment of severe infections with this organism. Additional data from Vietnam is currently limited, but we speculate that this trend is widespread in other less-well-equipped healthcare settings across the country.

In this study, we determined the potential *in vitro* efficacy of colistin in combination with other antimicrobial agents against a range of *A. baumannii* isolated from VAP patients in our ICU. Several studies have evaluated the ability of other antimicrobial agents to induce synergy when combined with colistin *in vitro* (Kruse *et al.*, 2013; Paul *et al.*, 2004; Petrosillo *et al.*, 2008; Tada *et al.*, 2013; Zusman *et al.*, 2013). We assessed the synergistic potential of colistin with ceftazidime, ceftriaxone, imipenem and meropenem against 56 clinical isolates of *A. baumannii*, collected over an 18-month period. Our data showed that synergistic activity between colistin in combination with either cephalosporins (ceftazidime and ceftriaxone) or carbapenems (imipenem and meropenem) was highly variable. Overall, there was limited synergistic interaction between colistin and ceftazidime/ceftriaxone, with marked *in vitro* synergy between colistin and ceftazidime in only 7 % and ceftriaxone in 13 % of isolates. Synergy between colistin and third-generation cephalosporins has been reported previously for both MDR *A. baumannii* and *Pseudomonas aeruginosa* (Gunderson *et al.*, 2003; Kroeger *et al.*, 2007). In contrast, we found that synergy between colistin and carbapenems was more common, with 68 % of *A. baumannii* isolates exhibiting a synergistic effect between colistin and meropenem, and 36 % of isolates showing synergy between colistin and imipenem. The synergistic activity of colistin and meropenem was significantly more

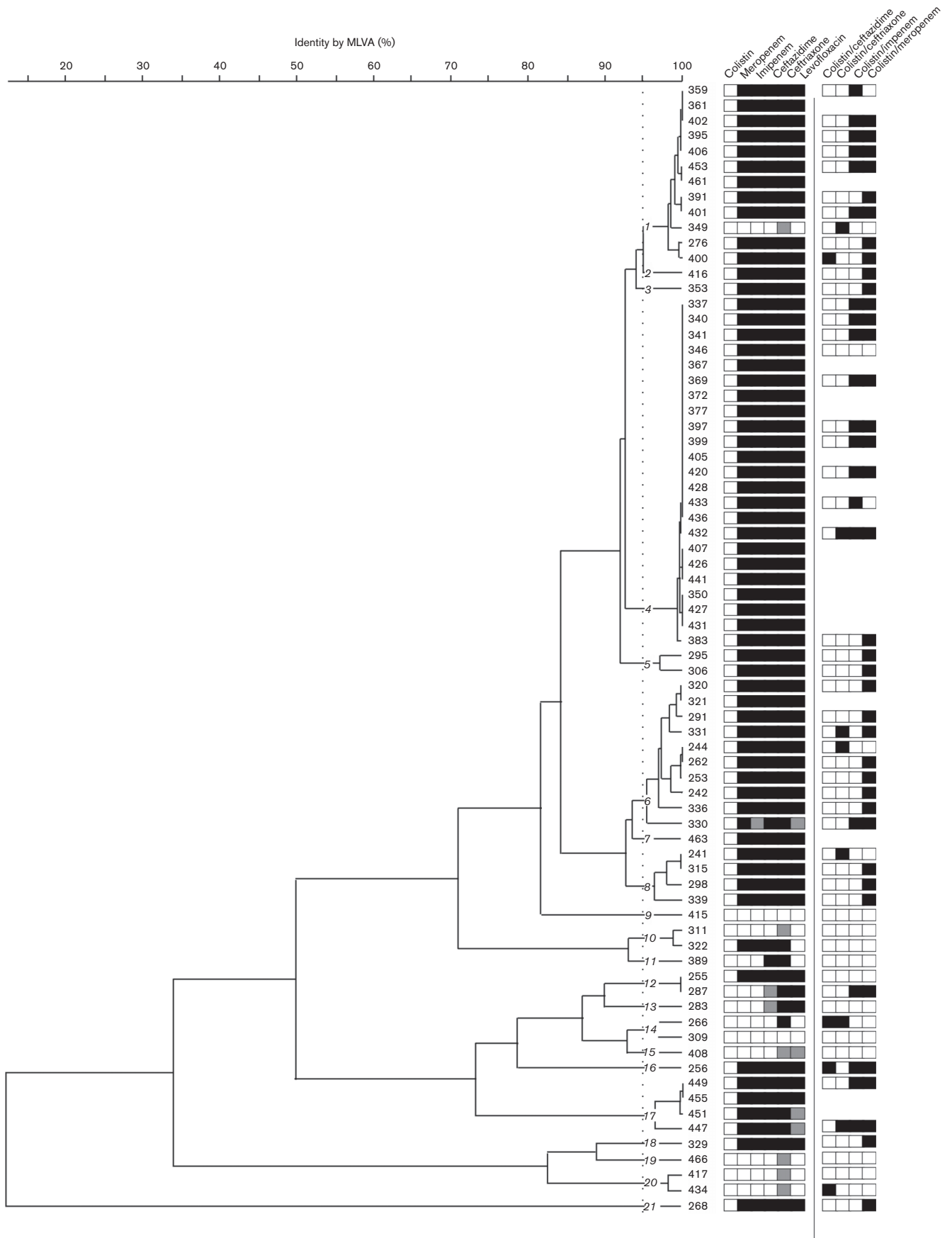


Fig. 1. MLVA genotyping of 74 Vietnamese *A. baumannii* isolates. Dendrogram created from MLVA using eight VNTR loci from 74 *A. baumannii* strains isolated from the ICU at the HTD in HCMC between January 2011 and June 2012. The strain numbers are shown to the right of the dendrogram and the 21 strain clusters are highlighted (arbitrary cut-off of >95 % identity). Associated metadata include susceptibility to colistin, meropenem, imipenem, ceftazidime, ceftriaxone and levofloxacin (white, susceptible; grey, intermediate; black, resistant) and synergy between colistin and ceftazidime, ceftriaxone, imipenem or meropenem (black, synergism; white, indifference).

Table 2. Antimicrobial combinations against *A. baumannii* using the checkerboard titration assay

| Antimicrobial combination | No. tested | Nature of interaction [n (%)]* | |
|---------------------------|------------|--------------------------------|--------------|
| | | Synergism | Indifference |
| Ceftazidime/colistin | 56 | 4 (7) | 52 (93) |
| Ceftriaxone/colistin | 56 | 7 (13) | 49 (87) |
| Imipenem/colistin | 56 | 20 (36) | 36 (64) |
| Meropenem/colistin | 56 | 38 (68) | 18 (32) |
| Total | 224 | 69 (31) | 155 (69) |

*Interaction: FICI ≤0.5, synergism; 0.5<FICI<4, indifference.

common in carbapenem-resistant *A. baumannii* isolates compared with carbapenem-susceptible *A. baumannii* isolates, with 84 versus 15 % of isolates displaying synergy, respectively. This difference in synergistic interaction was not observed with the combinations of colistin and ceftazidime, or colistin and ceftriaxone.

Our results support the findings of a recent systemic review and meta-analysis of *in vitro* synergy of polymyxins and carbapenems, which found significant advantages of combining meropenem or doripenem with colistin, rather than imipenem, for *A. baumannii* infections (Zusman *et al.*, 2013). Both meropenem and imipenem induce bacterial lysis in susceptible organisms by high-affinity binding to high-molecular-mass penicillin-binding proteins (Zhanel *et al.*, 1998). Meropenem has a higher affinity for

penicillin-binding proteins than imipenem in Gram-negative organisms, which may account for the enhanced performance of meropenem in our checkerboard assays (Zhanel *et al.*, 1998). Further evidence for the potential value of colistin combination therapy arose from a clinical study that measured resistance development in colistin monotherapy compared with colistin combination therapy (Paul *et al.*, 2004). This study found that colistin resistance could develop in the first 24 h of treatment with colistin monotherapy, and that colistin resistance was suppressed and delayed by combination therapy.

The current study indicates that combinations of colistin with ceftazidime, ceftriaxone, imipenem or meropenem have *in vitro* synergistic activity against local carbapenem-resistant *A. baumannii* strains in Vietnam. We found that colistin/meropenem had the greatest potential synergistic effect. Antimicrobial combinations may improve outcome by broadening the spectrum of antimicrobial activity, minimizing the potential emergence of antimicrobial-resistant organisms and by achieving a stronger antimicrobial effect through synergy. We cannot assume *in vivo* efficacy due to potential variability in pharmacokinetic effects of these drugs in the host, and different bacterial and drug concentrations in the specific sites of infection. In addition, these *in vitro* studies did not examine bactericidal activity, which is particularly relevant with respect to colistin combination therapy. Clinical investigations are required to elucidate the mechanism responsible for this effect and to explore its therapeutic potential, and we suggest that a suitable combination of these drugs should be tested rigorously in clinical trials of infections caused by MDR *A. baumannii*.

Table 3. Antimicrobial combinations against carbapenem-resistant and -susceptible *A. baumannii*

| Antimicrobial combination | Nature of interaction [n (%)] in carbapenem-resistant <i>A. baumannii</i> (n=43)* | | Nature of interaction [n (%)] in carbapenem-sensitive <i>A. baumannii</i> (n=13)* | |
|---------------------------|---|--------------|---|--------------|
| | Synergism | Indifference | Synergism | Indifference |
| Ceftazidime/colistin | 2 (5) | 41 (95) | 2 (15) | 11 (85) |
| Ceftriaxone/colistin | 5 (12) | 38 (88) | 2 (15) | 11 (85) |
| Meropenem/colistin | 36 (84) | 7 (16) | 2 (15) | 11 (85) |
| Total | 43 (33) | 86 (67) | 6 (15) | 33 (85) |

*Interaction: synergism, FICI ≤0.5; indifference, 0.5<FICI<4.

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