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# Conditional probability analysis of multidrug resistance in Gram-negative bacilli isolated from tertiary medical institutions in South Korea during 1999–2009[§]

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**Multidrug resistance of Gram-negative bacilli is a major problem globally. However, little is known about the combined probability of resistance to various antibiotics. In this study, minimum inhibitory concentrations of widely used antibiotics were determined using clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, randomly chosen from strain collections created during 1999–2009 in tertiary medical institutions in Seoul, South Korea. To analyze combined efficacy of antibiotics against a subgroup of isolates, conditional probabilities were determined based on arbitrary, non-independent patterns of antimicrobial susceptibility and resistance. Multidrug resistance, defined as resistance to three or more classes of antibiotics, was observed in the following order: *A. baumannii* (96%), *P. aeruginosa* (65%), *E. coli* (52%), and *K. pneumoniae* (7%). *A. baumannii* strains resistant to gentamicin were found to be resistant to a number of antibiotics, except for colistin and polymyxin B. Resistance to gentamicin following exposure to this antibiotic was highly likely to lead to multidrug resistance in all four microbes. This study shows a causal relationship between gentamicin resistance and the prevalence of multidrug resistance in clinical isolates of Gram-negative bacilli in South Korea during 1999–2009 and suggests the importance of prudent use of gentamicin in hospitals.**

***Keywords***: condtional probability, multidrug resistance, Gram-negative bacilli, aminoglycoside resistance

## Introduction

Multidrug resistance (MDR) is a growing problem worldwide. It is particularly concerning in connection with resistance to broad- and extended-spectrum antibiotics in Gram-negative bacilli (GNBs), such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (Talbot *et al.*, 2006; Souli *et al.*, 2008). These pathogens present high risks of nosocomial infections that range from mild urinary infections to severe bacteremia and pneumonia, resulting in a high rate of mortality and morbidity in hospitals (Gieske *et al.*, 2008; Peleg and Hooper, 2010).

Despite efforts to limit their spread, MDR-GNBs continue to increase worldwide. MDR-GNBs are widespread in tertiary care hospitals, which are suspected to be reservoirs of infectious bacteria, leading to extensive drug resistance or pandrug resistance to available antibiotics, including aminoglycosides, β-lactams, cephalosporins, monobactam, carbapenems, clavulanic acid, tetracyclines, quinolones, and polymyxins (Garnacho-Montero *et al.*, 2003; Falagas *et al.*, 2005). Except for the re-administration of polymyxins (Garnacho-Montero *et al.*, 2003; Falagas and Kasiakou, 2005; Falagas *et al.*, 2005; Li *et al.*, 2006) and tigecycline (Rubinstein and Vaughan, 2005), there are as yet no specific drugs for the treatment of patients infected with MDR-GNBs.

The prospects of discovering new drugs for GNBs are low because of prolonged colonization effects, low permeability membrane barriers, and multidrug efflux pumps of these bacteria (Nikaido, 1994, 1998; O'Fallon *et al.*, 2009; Fischbach and Walsh, 2009). It is difficult to identify clinical MDR-GNBs by molecular techniques since adaptive resistance to a broad spectrum of antibiotics can be caused by non-clonal changes in bacterial phenotypes that revert to the original states after the removal of antibiotics (Daikos and Jackson, 1990; Skiada *et al.*, 2011). In this study, therefore, antibiotic resistance is broadly defined as the ability of a strain to grow in the presence of antibiotics at concentrations greater than the breakpoint above the minimum inhibitory concentrations (MICs), determined by testing the antibiotics against a subgroup of isolates.

The purpose of this study was to develop a probabilistic model of antibiotic resistance patterns of MDR-GNBs in subgroups of clinical isolates of *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *A. baumannii* obtained from various clinical samples in several tertiary medical institutions in Seoul, South Korea, collected during 1999–2009. After log2 transformation and normalization of non-negative MIC+1 values for each species of GNB, the likelihood (similarity) of antimicrobial susceptibility patterns was estimated by Pearson's correlation coefficients. Conditional probabilities of developing MDR were examined after setting a breakpoint to detect resistance above the cut-off MIC value for each antibiotic. This study shows that gentamicin resistance is commonly observed in all subgroups of MDR-GNBs, except those resistant to colistin or polymyxin B, and suggests that this fact

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should be considered while prescribing antibiotics to patients infected with MDR-GNBs or those who have been exposed to gentamicin earlier.

## Materials and Methods

### Strains and culture conditions

Clinical isolates of *E. coli* ($n$ = 141), *P. aeruginosa* ($n$ = 100), *K. pneumoniae* ($n$ = 100), and *A. baumannii* ($n$ = 50) were randomly selected from the Culture Collection of Antimicrobial Resistant Microbes (CCARM), Seoul Women's University, South Korea. These strains were isolated from various clinical samples at several tertiary medical institutions in Seoul during 1999–2009 (Supplementary data Table S1). Identification and antimicrobial susceptibility tests of clinical isolates were performed by following the standards and guidelines of the Clinical and Laboratory Standards Institute (www.clsi.org). All strains were cultivated in Luria-Bertani (LB) medium (BD) under aerobic conditions with shaking (180 rpm) at 37°C. To identify the response chain of aminoglycoside resistance, isolates were streaked on LB agar plates containing gentamicin (20 mg/L), kanamycin (80 mg/L), or streptomycin (40 mg/L), singly or in combination ('triple mix'), at concentrations above their susceptibility-resistance breakpoints and incubated at 37°C.

### Antimicrobial susceptibility tests

Antimicrobial susceptibility tests for the determination of MICs were performed with the following initial concentrations of antibiotics: 128 mg/L ampicillin (AMP); 128 mg/L cephalothin (CEF); 128 mg/L cefoxitin (FOX); 128 mg/L cefotaxime (CTX); 128 mg/L ceftazidime (CAZ); 128 mg/L aztreonam (ATM); 128, 256, or 400 mg/L piperacillin (PIP); 32 mg/L colistin (CST); 32 mg/L polymyxin B (PMB); 128 mg/L amikacin (AMK); 128 or 256 mg/L gentamicin (GEN); 512 mg/L kanamycin (KAN); 512 mg/L streptomycin (STR); 128 mg/L norfloxacin (NOR); 128 mg/L ciprofloxacin (CIP); 128 mg/L levofloxacin (LVX); 128 mg/L gatifloxacin (GAT); and 128 mg/L moxifloxacin (MXF). Exponentially growing cells were harvested from cultures growing in LB medium at an optical density ($OD_{600}$) of ~0.2 and were suspended in 100 µl Hinton-Mueller broth (BD) at a final $OD_{600}$ of 0.01 (~$10^6$ cells/ml) in 96-well microplates. Each row of the microplates contained 2-fold serial dilutions of antibiotics in 11 consecutive wells and also had an antibiotic control well without addition of antibiotics. Plates were sealed with sterile adhesive tape and incubated on a rotary shaker (200 rpm) at 37°C, and MICs were determined as the lowest antimicrobial concentrations that inhibited bacterial growth (OD600) measured at 10 h and 24 h. The MIC results are shown in Supplementary data Table S1.

### Conditional probability analysis of antibiotic resistance

MDR was determined by the ability of a strain to grow in selective media containing three or more different antibiotics. It was assumed that MDR is mediated by acquisition of genetic elements or non-clonal changes of bacterial phenotypes that confer resistance to different antibiotics. If MDR is caused by a mobile genetic element like R plasmid (Hawkey and Jones, 2009), it is likely to be clonally transmitted within a generation. Thus, simultaneous resistance to different antibiotics probably arises from horizontal gene transfer or co-expression of MDR determinants in a subgroup of isolates, whereas heterogeneous resistance arises from multiple gene transfers or differential expression patterns of several MDR determinants over time. The former patterns of drug resistance are considered to be dependent on each other, while the latter patterns are independent events that do not affect each other. The probability of MDR ($X_i$), which accounts for the entire spectrum of MDR, is calculated by the multiplication rule of probability to find the intersection of dependent and independent events for different sets of antibiotic resistance patterns, $x_i$, which are defined as distinct random variables, the so-called breakpoints above the MIC values between susceptibility and resistance. Over a long period, however, the probability of MDR can be affected by any previous antibiotic exposure, which causes a condition favorable or unfavorable for acquiring resistance to different antibiotics, as seen in a 10-year retrospective study of bloodstream infections with GNBs (Wong *et al.*, 2014). Thus, it is considered that there is a conditional dependence of MDR on past exposure to antibiotics, which has the potential to change the probability of MDR in a subgroup of isolates over a long period. Conditional probabilities between two antibiotic resistance events, A and B, were estimated based on the arbitrary, non-independent features of the MIC values as follows: $P(B|A) = P(B \cap A)/P(A)$, where $P(A) > 0$, and $P(A|B) = P(A \cap B)/P(B)$, where $P(B) > 0$. The different probabilities, $P(B|A) \neq P(A|B)$, indicate that a route goes from A to B or from B to A in a subgroup of isolates by increasing the probability of detection. In general, $P(A|B)$ (conditional probability of A given B) is not equal to $P(B|A)$, unless the two events are synchronized. However, there is not always a causal or temporal relationship between A and B. For example, when A and B are independent, $P(A|B) = P(A)$ and $P(B|A) = P(B)$. If $P(A) = P(B)$, Bayes' theorem gives a constant probability, $P(B|A)P(A) = P(B \cap A) = P(A \cap B) = P(A|B)P(B)$. This indicates that there is no cause-and-effect relationship between the two events, even though it is mathematically difficult to prove the simultaneous, interdependent relationship because of the small size of the data.

### Statistics

Antibiotic MIC values resulting from three independent culture experiments were log2-transformed with the addition of +1 in order to eliminate negative values and were normalized to be in the [0, 1] interval between the control and tests wells for each antibiotic. Similarities of the normalized MIC profiles for each species of GNB were expressed as percentages of Pearson's correlation coefficients.

## Results

### Antibiotic resistance patterns of GNBs

Antimicrobial susceptibilities varied in subgroups of clinical isolates of *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *A.*

**Table 1. Antibiotic resistance patterns of Gram-negative bacilli isolated from clinical samples in South Korea during 1999-2009**

| Antibiotics | Breakpoint[a] > MIC (mg/L) | *Escherichia coli* (*n* = 141) | *Pseudomonas aeruginosa* (*n* = 100) | *Klebsiella pneumoniae* (*n* = 100) | *Acinetobacter baumannii* (*n* = 50) |
|---|---|---|---|---|---|
| Ampicillin | >64 | 82.3% | | 24% | 96% |
| Cephalothin | >16 | 43.3% | | 19% | 100% |
| Cefoxitin | >32 | | | | 96% |
| Cefotaxime | >32 | | | | 92% |
| Ceftazidime | >8 | | 56% | | 92% |
| Aztreonam | >32 | | | | 92% |
| Piperacillin | >128 | | 33% | | |
| Colistin | >4 | 0.7% | 20% | 3% | 0% |
| Polymyxin B | >8 | 1.4% | 6% | 3% | 0% |
| Amikacin | >16 | | | | 86% |
| Gentamicin | >16 | 45.4% | 78% | 10% | 50% |
| Kanamycin | >32 | 46.8% | | | 94% |
| Streptomycin | >16 | 75.9% | | | |
| Norfloxacin | >16 | 51.8% | 79% | 2% | 92% |
| Ciprofloxacin | >0.5 | | | | 92% |
| Levofloxacin | >2 | | | | 92% |
| Gatifloxacin | >1 | | | | 92% |
| Moxifloxacin | >2 | | | | 92% |

[a] Breakpoint above the minimum inhibitory concentration of susceptibility. The antimicrobial MIC data for each species are shown in Supplementary data Table S1. Blanks, not determined.

*baumannii* obtained over a decadal period of 1999–2009. Prevalence of MDR-GNBs, defined as the *in vitro* ability to grow in the presence of three or more classes of antibiotics, was highest in *A. baumannii* (96%), followed by *P. aeruginosa* (65%), *E. coli* (52.4%), and *K. pneumoniae* (7%). Cases of infection with MDR-GNBs were highly associated with infectious diseases of the lungs (*A. baumannii* and *P. aeruginosa*), urinary tract (*E. coli* and *P. aeruginosa*), skin (*P. aeruginosa*), and gastrointestinal tract (*E. coli*), and were also detected in the bloodstream (*P. aeruginosa*) and cerebrospinal fluid (*P. aeruginosa*), as shown in Supplementary data Table S1. MDR-GNBs were resistant to various classes of antibiotics, including β-lactam, cephalosporins, monobactam, expanded-spectrum β-lactam, polymyxins, aminoglycosides, and quinolones.

Antibiotic resistance patterns for each species of GNB are summarized in Table 1. Based on the *in vitro* MIC values of clinical isolates shown in Supplementary data Table S1, colistin was found to be more efficacious against *E. coli* (MIC ≤ 0.5 mg/L) and *A. baumannii* (MIC ≤ 2 mg/L) than polymyxin B was against *P. aeruginosa* (MIC ≤ 4 mg/L). The number of isolates resistant to polymixins (colistin and polymy-

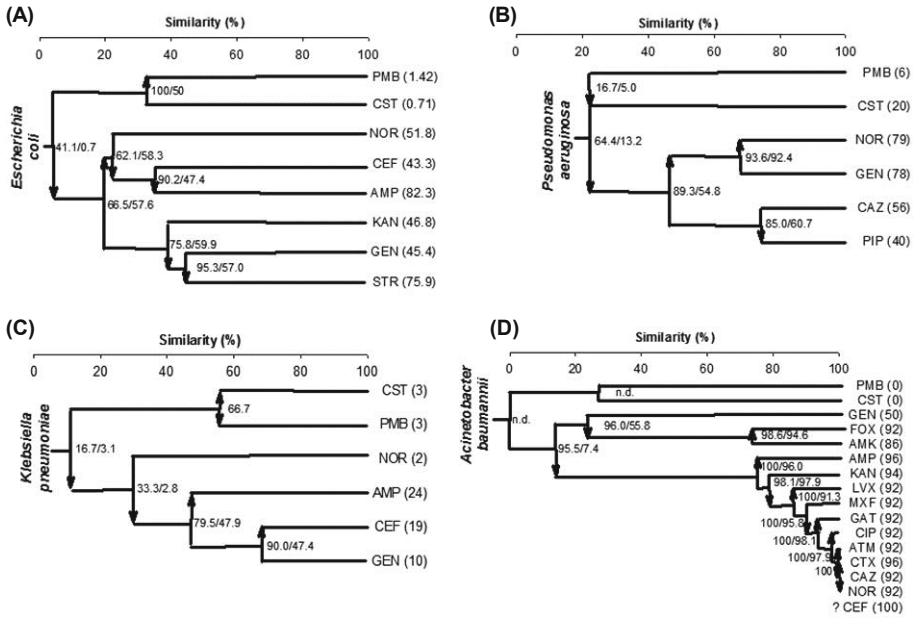


**Fig. 1. Dendrograms of similarity of antimicrobial susceptibilities expressed as percentages of Pearson's correlation coefficients.** (A) *Escherichia coli* (*n* = 141), (B) *Pseudomonas aeruginosa* (*n* = 100), (C) *Klebsiella pneumoniae* (*n* = 100), (D) *Acinetobacter baumannii* (*n* = 50). The MIC values shown in Supplementary data Table S1 were log2-transformed with the addition of +1 to eliminate negative values for normalization, as described in 'Materials and Methods'. Conditional probabilities of resistance to two or more different antibiotics, given resistance to an antibiotic, are shown by arrows at the branch nodes, indicating good/reverse signals of a relationship between antibiotic resistance patterns and multidrug resistance in subgroups of Gram-negative bacilli. The prevalence of resistance to individual antibiotics in clinical isolates is expressed as percentages in parentheses at the terminal nodes in each species of GNB.

xin B) was much lower in *A. baumannii* (0%) than in *E. coli* (0.71–1.42%), *K. pneumonia* (3%), and *P. aeruginosa* (6–20%).

### Conditional probability analysis of antibiotic resistance patterns

The *in vitro* MIC values of isolates were log2-transformed by the addition of +1 in order to eliminate negative values for normalization. Normalized data in the [0,1] interval were used to calculate the likelihood (similarity) of antibiotic susceptibility/resistance patterns for each species of GNB by Pearson's correlation coefficients, as shown in Fig. 1. Relatedness determined by the neighbor-joining method was categorized according to a percentage of nodes connected with branch lines of the MIC profiles. Although there were large differences in antimicrobial susceptibility/resistance patterns of bacterial species, conditional probability analyses showed conditional dependence relations that were connected to the causal chain involving gentamicin resistance in all subgroups of isolates of GNBs.

The joint probability tended to decrease as the similarity was lowered. The conditional probabilities of resistance to two or more different antibiotics given resistance to gentamicin appeared to be high in all subgroups, but there were large variations in the sequences of MDR in subgroups of isolates that showed low similarities between adjacent MIC profiles or clusters of combined sets. This indicates that gentamicin resistance was common, but not prevalent, and strongly varied among subgroups of MDR-GNBs during 1999–2009 in South Korea. Of particular concern to the high mortality of hospitalized patients with nosocomial infections, gentamicin-resistant *A. baumannii* strains were highly resistant to β-lactam, cephalosporins, monobactam, an expanded β-lactam antibiotic, aminoglycosides, and quinolones, but they were sensitive to colistin and polymyxin B. The other isolates of MDR-GNBs belonging to *E. coli*, *K. pneumoniae*, and *P. aeruginosa* showed a relationship between polymyxin resistance and MDR to different antibiotics, but with low conditional dependence. The sample sizes of polymyxin-resistant GNBs were not large enough to result in a statistically significant relationship with MDR-GNBs during 1999–2009.

### Combined analysis of aminoglycoside resistance patterns

It has been considered that aminoglycoside resistance is highly linked to MDR in clinically important GNBs. Until date, no single gene or mutation responsible for co-resistance to different aminoglycoside antibiotics, such as gentamicin, kanamycin, and streptomycin, that contain different aminocyclitol moieties, streptamine and 2-deoxystreptamine, has been identified (Shaw *et al.*, 1993; Davies, 1994; Honoré and Cole, 1994; Meier *et al.*, 1994; Davies and Wright, 1997; Mingeot-Leclercq *et al.*, 1999; Fluit *et al.*, 2001; Jana and Deb, 2006). Therefore, it was considered possible that adaptive resistance to "all" aminoglycoside antibiotics developed in subgroups of GNBs after exposure to an aminoglycoside antibiotic (Karlowsky *et al.*, 1997; Xiong *et al.*, 1997; MacDougall and Chambers, 2011). To calculate the probability of aminoglycoside resistance, isolates of GNBs were streaked on LB agar plates containing gentamicin (20 mg/L), kanamycin (80 mg/L), and streptomycin (40 mg/L), individually or in combination, at concentrations exceeding the breakpoints above the MIC values of susceptibility. Results for individual and combined aminoglycoside antibiotics tested in this study are shown in Supplementary data Table S1.

Figure 2 shows Venn diagrams of aminoglycoside resistance detected in subgroups of isolates of GNB. Although the prevalence of aminoglycoside resistance varied with species of GNBs, the conditional probabilities of resistance to kanamycin and streptomycin, given resistance to gentamicin, were greater than those calculated by the use of the antibiotics in combination and by the multiplication rule for the independent events in all species of GNBs. The combined probability of aminoglycoside resistance patterns in subgroups of isolates of GNBs showed a causal relationship of gentamicin resistance in the sequences for acquired resistance to kanamycin and streptomycin. This suggests that there was a conditional or temporal dependence: GNBs are likely to acquire resistance to kanamycin and streptomycin if they are resistant to gentamicin. However, simultaneous resistance to the three aminoglycoside antibiotics was unlikely to occur together. This supports the conclusion that gentamicin resistance was at least in part associated with the prevalence of MDR-

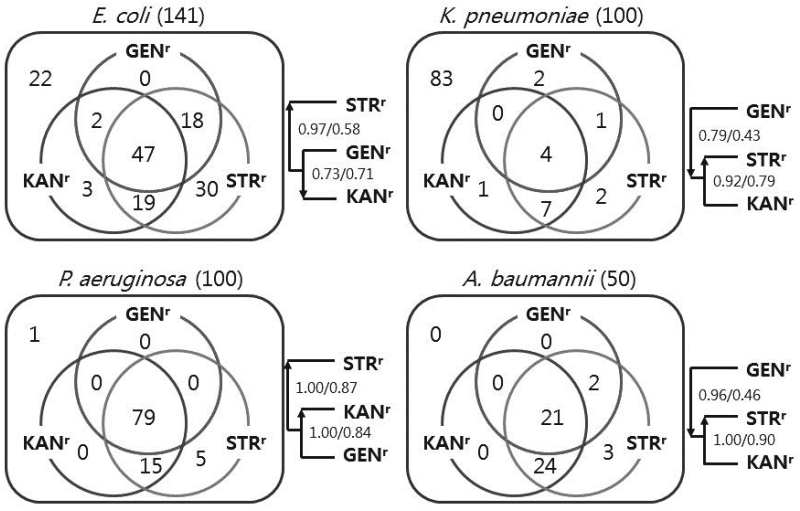


**Fig. 2. Venn diagrams for the combined analysis of aminoglycoside resistance patterns.** Subgroups of isolates resistant to gentamicin (GEN$^r$), kanamycin (KAN$^r$), or streptomycin (STR$^r$) were determined using clinical isolates of *Escherichia coli* ($n$ = 141), *Pseudomonas aeruginosa* ($n$ = 100), *Klebsiella pneumoniae* ($n$ = 100), and *Acinetobacter baumannii* ($n$ = 50), as shown in Supplementary data Table S1. A route to resistance to the three aminoglycoside antibiotics is shown by arrows at the branch nodes in the right panels, showing the causal relationship of gentamicin resistance with aminoglycoside resistance in all subgroups of the four species.

GNBs isolated during 1999–2009 in South Korea.

## Discussion

Antimicrobial treatments increase patient susceptibility to colonization with resistant bacteria during or after treatment (Austin *et al.*, 1999; Lipsitch *et al.*, 2000). Intrinsically resistant bacteria can emerge in commensal microflora of children and wild rodents without antibiotic intake (Calva *et al.*, 1996; Gilliver *et al.*, 1999). They can also arise under certain stress conditions as seen for multidrug-resistant *P. aeruginosa*, which is associated with accumulation of multiple mutations under oxidative stress conditions caused by chronic lung inflammation in cystic fibrosis patients (Ciofu *et al.*, 2005). The capability of certain bacteria to adapt to xenobiotics and hydrocarbons is often associated with antibiotic resistance due to horizontal transfers of mobile genetic elements or with the fitness costs resulting from the phenotypic variability in the community (Top and Springael, 2003; Kang and Park, 2010). Identification of a rapid mutation or change, conferring a novel MDR phenotype in a subgroup of GNBs, boosts efforts to find a significant relationship between antibiotic use and resistance.

To analyze antibiotic resistance patterns of MDR-GNBs that are greater than expected under independence assumption, a mathematical approach is required to estimate the conditional probability of resistance to two or more different antibiotics, given resistance to each antibiotic, which will promote natural selection of bacteria under antibiotic treatments. A neighbor-joining dendrogram based on antimicrobial susceptibilities of clinical isolates supported the retrospective MDR analysis by a root-cause test using conditional probability for establishing a relationship between antibiotic resistance patterns. From this analysis, it was found that gentamicin resistance was strongly associated with MDR in all subgroups of GNBs isolated during 1999–2009 in South Korea. Combined analysis of aminoglycoside resistance showed that gentamicin resistance occurred in most subgroups of GNBs with a conditional dependence structure, although the prevalence was different from the four microbes. The resistance to the three aminoglycoside antibiotics is similar with previous studies (Barclay *et al.*, 1992; Barclay and Begg, 2001; Mohamed *et al.*, 2011), which suggested that adaptive resistance to "all" aminoglycoside antibiotics develops in subpopulations of GNBs within a few hours after the first exposure to gentamicin. Over a prolonged period, aminoglycoside resistance can also arise due to horizontal transfer of aminoglycoside resistance genes in MDR-GNBs, as seen with multidrug-resistant isolates of *A. baumannii* carrying pan-European clones I and II that played an important role in the dissemination of aminoglycoside resistance in European countries between 1982 and 2001 (Nemec *et al.*, 2004). These results indicate that aminoglycoside resistance contributes to both clonal and non-clonal subpopulations of MDR-GNBs.

The conditional probability analysis of antibiotic resistance patterns is useful for establishing a causal relationship for MDR in clinical isolates with no training history of patients with infectious diseases. The major drawback in the use of old patient samples is that it is difficult to calculate the conditional probability of an all-or-nothing form of resistance. For example, it was difficult to establish a relationship between cephalothin resistance and MDR for the *A. baumannii* isolates tested in this study because all such isolates were resistant to cephalothin. It was also difficult to identify a response chain involving polymyxin resistance in all subgroups of MDR-GNBs because of the small number of isolates resistant to colistin and polymyxin B in the study period. It has been argued that extensive or inadequate use of colistin in the past few years might have contributed to non-clonal emergence of colistin-resistant MDR-GNBs in intensive care units of South Korea during the 2000s (Park *et al.*, 2009; Suh *et al.*, 2010; Lee *et al.*, 2011). However, it is unclear how polymyxin resistance affects selection and transmission of MDR-GNBs in patients.

Clinical MDR-GNBs represent a substantial and serious issue in hospitals and societies (Gieske *et al.*, 2008; Peleg and Hooper, 2010). It is one of the major infectious complications, which is independently associated with a higher rate of overall mortality. A surveillance study in neonatal intensive care units reported that 92 infants, who received ampicillin/sulbactam and gentamicin most frequently, did not show significant susceptibility to MDR-GNB colonization (Mammina *et al.*, 2007). A US surveillance study from 1994 to 2002 showed a rapid increase in nosocomial MDR-GNBs, with the most common pattern of resistance to quinolones, third-generation cephalosporins, and aminoglycosides (D'Agata, 2004). Another US surveillance study from 1994 to 2000 documented that the broad resistance to quinolones occurred concurrently with the increased use of quinolones (ciprofloxacin, levofloxacin, and ofloxacin) in the 1990s (Neuhauser *et al.*, 2003). A cohort study showed that admission to an intensive care unit previously occupied by a patient infected with multidrug-resistant *P. aeruginosa* or *A. baumannii* was an independent risk factor for acquisition of these bacteria by subsequent room occupants (Nseir *et al.*, 2010). In neonatal intensive care units, an 8-year cohort study has shown that neonates with MDR-GNB bacteremia more likely received inadequate initial antibiotic therapy, compared to admissions with non-MDR-GNB bacteremia (Tsai *et al.*, 2014). Conditional logistic regression analysis showed that antibiotic exposure of residents in long-term care facilities was significantly associated with acquisition of MDR-GNBs, showing the most common resistance pattern to extended-spectrum penicillins, ciprofloxacin, and gentamicin (O'Fallon *et al.*, 2010). Accumulated evidence suggests that MDR-GNB cases are increasing due to extensive or inadequate use of some types of antibiotics in patients in developed countries. These factors appear to be independently associated with higher rates of MDR-GNB acquisition and overall fatality.

Previous exposure to broad-spectrum antibiotic therapy and underlying disease may be high risk factors for acquisition of MDR-GNBs, contributing to cross-transmission between patients or accumulation of mutations in highly resistant cells that can give rise to new phenotypes, similar to those of *Staphylococcus aureus* (Klevens *et al.*, 2007; Mwangi *et al.*, 2008; Dordel *et al.*, 2014). In addition, this study shows that gentamicin resistance is an extremely significant factor leading to the development of MDR in clinically important

GNBs, probably by selecting resistance to aminoglycosides, penicillins, and fluoroquinolones. Although further studies will be required to determine the genetic relationship between aminoglycoside resistance and MDR in these bacteria, this finding suggests that it is important to consider the prudent use of gentamicin, especially in tertiary care hospitals, to treat patients infected with MDR-GNBs or exposed to gentamicin prior to treatment.

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