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Original Article

Bacterial profile, and independent predictors for healthcare-associated pneumonia persistently caused by multidrug-resistant Gram-negative bacteria for patients with the preceding multidrug-resistant Gram-negative pneumonia in Taiwan

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KEYWORDS
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Multidrug-resistant;

Abstract *Objectives:* To understand the microbial profile and investigate the independent predictors for healthcare-associated pneumonia (HCAP) pertinaciously caused by isolates of multidrug-resistant (MDR) Gram-negative bacteria (GNB).
Methods: Multicenter ICU patients who received appropriate antibiotic treatments for preceding pneumonia due to MDR GNB isolates and subsequently developed HCAP caused by either

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Klebsiella pneumoniae;
Acinetobacter baumannii complex;
Pseudomonas aeruginosa

MDR GNB (n = 126) or non-MDR GNB (n = 40) isolates in Taiwan between 2018 and 2023 were enrolled. Between the groups of patients with HCAP due to MDR GNB and non-MDR GNB, the proportions of the following variables, including demographic characteristics, important comorbidities, nursing home residence, physiological severity, intervals between two hospitalizations, steroid use, the tracheostomy tube use alone, ventilator support, and the predominant GNB species involving HCAP, were analyzed using the chi-square test. Logistic regression was employed to explore the independent predictors for HCAP persistently caused by MDR GNB in the aforementioned variables with a *P*-value of <0.15 in the univariate analysis.

Results: MDR-*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* complex were the three predominant species causing HCAP. Chronic structural lung disorders, diabetes mellitus, intervals of $\leq$30 days between two hospitalizations, use of the tracheostomy tube alone, and prior pneumonia caused by MDR *A. baumannii* complex were shown to independently predict the HCAP tenaciously caused by MDR GNB. Conversely, the preceding pneumonia caused by MDR *P. aeruginosa* was a negative predictor.

Conclusion: Identifying predictors for HCAP persistently caused by MDR GNB is crucial for prescribing appropriate antibiotics.

Introduction

Pneumonia is a primary cause of hospitalization in intensive care units (ICU). In 2019, it reportedly accounted for over 1.5 million fatalities and represented a significant contributor to global antimicrobial resistance.[1] Pneumonia types are categorized based on the acquisition environments, history of the latest hospitalization, and ventilator use.[2] Two decades ago, healthcare-associated pneumonia (HCAP) was specifically considered to encompass infections acquired in a long-term or subacute/intermediate healthcare facility, or following recent hospitalizations (discharged within 90 days of current admission) by the pulmonary experts.[3]

The 2016 guidelines for hospital-acquired pneumonia (HAP) suggest that patients with HCAP are not at risk for multidrug-resistant (MDR) pathogens.[2] Nevertheless, a Korean study investigating residents of nursing homes showed that both the rates of mortality and drug-resistant pathogens regarding nursing home-acquired pneumonia, one of the important types of HCAP, exceeded 20%.[4] Thus, many Asian countries still emphasize the importance of enhanced treatment of HCAP. Moreover, as witnessed globally[5–8] and in Taiwan,[9–12] the susceptibility profiles of clinical Gram-negative bacterial (GNB) isolates have been considerably altered with the arrival of an increasing frequency of MDR or extensively drug-resistant (XDR) phenotypes in these pathogens. This condition poses a substantial risk of initial ineffective antibiotic therapy for patients with HCAP.

To elucidate the bacterial profile and identify predisposing factors contributing to HCAP caused by MDR GNB, we conducted this survey to comprehend the distribution of GNB species, assess the proportions of XDR phenotypes among MDR GNB isolates responsible for HCAP, and investigate independent predictors for HCAP persistently caused by MDR or XDR GNB pathogens in the ICU-hospitalized patients who previously had received appropriate antibiotic therapies for pneumonia due to MDR/XDR GNB.

Material and methods

Patient source, selection and exclusion criteria

We conducted a review of patient data from three hospitals in Taiwan: Far Eastern Memorial Hospital, a 1408-bed academic medical center located in New Taipei City; Mackay Memorial Hospital, a 1046-bed university-affiliated academic medical center in Taipei; and Min-Sheng General Hospital, a 776-bed accredited regional teaching hospital in Taoyuan. The data covered admissions to the ICU for pneumonia treatment between January 2018 and December 2023. Each of the three hospitals contributed an equal number of patients to this survey. Pneumonia was defined as the presence of lung infiltrates and clinical evidence, including fever, purulent sputum as indicated in smear and culture results, leukocytosis or leukopenia, and a decline in oxygenation as per the established criteria.[3]

Patients eligible for enrollment in this survey were those who had subsequent HCAP caused by GNB and a history of preceding pneumonia also caused by GNB within three months of the admission for HCAP management. Additionally, patients with pneumonia were excluded if any Gram-positive bacteria were cultured from their sputum or the secretion of the lower respiratory tract.

This survey was an observational investigation that did not involve any adjustment of therapy. Therefore, patient consents were waived in each participating hospital following the recommendations of the Institutional Review Board. This study was approved by the Institutional Review Board of each participating hospital.

Susceptibility data, potential resistance mechanisms of MDR/XDR GNB isolates involving pneumonia, the definition of species concordance, and PCR screening for various β-lactamase genes among Enterobacterales and non-fermenting GNB isolates

We assessed the antibiotic susceptibility of the MDR and XDR GNB isolates that caused pneumonia using the breakpoints of minimum inhibitory concentration and the formal recommendations of the Clinical and Laboratory Standards Institute (CLSI).[13] The BD Phoenix™ automated microbiology system (Becton, Dickinson and Company, Maryland, USA) was primarily used for species identification and in vitro susceptibility testing of these pneumonia-causing GNB isolates across the three hospitals participating in this survey. In cases where polymicrobial GNB species were cultured from sputum or lower respiratory tract secretions, the GNB species with the most abundant colonies was considered the primary etiological bacterium for pneumonia analysis.[3] In this survey, the acquired MDR or XDR phenotypes (after exclusion of the intrinsic resistance) in the studied GNB isolates were interpreted following the criteria proposed by Magiorakos et al.[14]

Furthermore, in-hospital patients who had evidence of infection consistent with pneumonia— radiographically, symptomatically, and with compatible laboratory results[3]— were immediately prescribed antibiotic therapy. To assess the appropriateness of antibiotics used for the preceding pneumonia treatment in the enrolled patients during their former hospitalizations, the potential resistance mechanisms (such as the production of carbapenemase, extended-spectrum β-lactamase, and/or hyper-production of AmpC β-lactamase, porin disappearance, etc., as suggested by De Angelis et al.[15]) in the studied GNB isolates were inferred from the respective results of antibiotic susceptibility testing. These were primarily interpreted using the CLSI criteria[13] by the consultant infectious disease (ID) specialists within two days of consultations at each participating hospital. The regimens and dosages of prescribed antibiotics for pneumonia therapy mostly followed the 2018 Taiwan Guidelines for the Management of Pneumonia[16] as well as the existing guidelines of the ID Society of America. In addition, species concordance was defined as the identification of the same GNB species that was validated to cause both the preceding pneumonia and subsequent HCAP in two consecutive hospitalizations for a given patient.

In the HCAP study protocol, the genes encoding resistant β-lactamases for the concordant MDR/XDR GNB isolates involved in HCAP were surveyed at each participating hospital using multiplex PCR. For isolates of Enterobacterales species and *Pseudomonas aeruginosa*, five groups of β-lactamase genes were examined: (1) bla_{TEM}, bla_{SHV}, bla_{VEB}, bla_{PER}, bla_{GES}; (2) $bla_{CTX\text{-}M\text{-}group\text{-}1}$, $bla_{CTX\text{-}M\text{-}group\text{-}2}$, $bla_{CTX\text{-}M\text{-}group\text{-}9}$; (3) $bla_{CTX\text{-}M\text{-}group\text{-}8}$, $bla_{CTX\text{-}M\text{-}group\text{-}25}$; (4) bla_{ACC}, bla_{CMY}, bla_{MOX}, bla_{ACT}, bla_{MIR}, bla_{DHA}, bla_{FOX}, bla_{PDC}; (5) bla_{KPC}, bla_{GIM}, bla_{SPM}, bla_{NDM}, bla_{IMP}, bla_{VIM}, and $bla_{OXA\text{-}48}$. Additionally, bla_{PER}, bla_{VEB}, $bla_{OXA\text{-}24}$-like, $bla_{OXA\text{-}51}$-like, $bla_{OXA\text{-}58}$, and $bla_{OXA\text{-}72}$ were examined for MDR/XDR *Acinetobacter baumannii* complex isolates. All detected genes encoding β-lactamases were amplified and sequenced as described previously.[9]

Risk factors for patients with HCAP persistently caused by MDR GNB

Many factors documented in the PubMed database have been identified as characteristic predispositions for hosts to acquire pneumonia caused by MDR pathogens (see as follows). Therefore, among the patients included in our survey who had a history of previous pneumonia caused by MDR GNB isolates, our initial investigation focused on examining the relationships between the presence of important variables and the subsequent development of HCAP also caused by MDR GNB.

The clinical variables under analysis included diabetes mellitus (DM),[17] chronic obstructive pulmonary disease, chronic structural lung disorders (including bronchiectasis and pulmonary parenchymal fibrosis; both interfere with the clearance of airway secretion),[18] chronic kidney disorder requiring long-term hemodialysis,[19] neurological disorders,[20] dementia,[21] malignancy, use of immunosuppressant drugs, or long-term high-dose corticosteroid therapy ($\geq$0.3 mg/kg prednisolone dose equivalent for $\geq$3 weeks),[22] residence at a nursing home,[23] and use of a tracheostomy tube alone or combined with mechanical ventilation support.[24] Additionally, we also analyzed the following factors: age ($\geq$65 vs. $<$ 65 years old), gender, Charlson co-morbidity index (CCI) score ($\geq$4 vs. $<$ 4 points), clinical severity on the HCAP episode in terms of the Acute Physiologic and Chronic Health Evaluation II score (APACHE II of $\geq$30 vs. $<$ 30 points), intervals between the date of last discharge and the next admission for subsequent HCAP treatment ($>$30 days vs. $\leq$ 30 days), and the predominant three GNB species involving pneumonia.

Statistical analyses, including independent predictors for patients with HCAP persistently caused by MDR GNB

Categorical variables were presented as percentages of the total number of isolates. Differences in proportions of the examined factors between the two subsets of patients with HCAP caused by either MDR GNB or non-MDR GNB were analyzed using the chi-square test, as appropriate. The independent predictors for the persistent development of HCAP due to MDR/XDR GNB were investigated using logistic regression analysis on the factors with P values $\leq$ 0.15 in the univariate analysis.

All statistical calculations were two-tailed, and a P value of $<$0.05 was considered statistically significant. The statistical analyses were performed using SPSS version 17.0 (Chicago, Armonk, NY, USA).

Results

Steps for the selection of patients with HCAP caused by GNB

Fig. 1 outlines the steps concerning the selection of ICU-hospitalized patients with healthcare-associated factors and pneumonia caused by GNB between January 2018 and December 2023. It specifically highlights the process of

identifying patients with GNB-related HCAP who previously had received appropriate antibiotics for the treatment of the preceding pneumonia caused by MDR GNB during their former hospitalizations. In total, 166 ICU-hospitalized patients with GNB-causing HCAP were eventually enrolled in this survey.

Distributions of the MDR GNB isolates that caused pneumonia in the enrolled ICU-hospitalized patients between two hospitalizations

Table 1 presents the distribution of MDR/XDR GNB species among patients who had received appropriate antibiotic(s) for the management of preceding pneumonia caused by MDR GNB. The table compares patients who subsequently developed HCAP caused by concordant species of GNB regardless of resistance phenotypes (n = 166, consisting of 98 [59%] isolates of Enterobacterales species and 68 [41%] isolates of glucose non-fermenting GNB), with those who experienced HCAP persistently caused by concordant MDR GNB species (n = 126, comprising 74 [60.7%] isolates of Enterobacterales species and 48 [39.3%] isolates of glucose non-fermenting GNB).

In summary, *Klebsiella pneumoniae* [39.3% (n = 48)], followed by *P. aeruginosa* [15.9% (n = 20)], and *A. baumannii* complex [23.0% (n = 28)] were the three predominant HCAP species of MDR GNB isolates concordant with etiologies of the preceding pneumonia. Among these MDR isolates, the rates

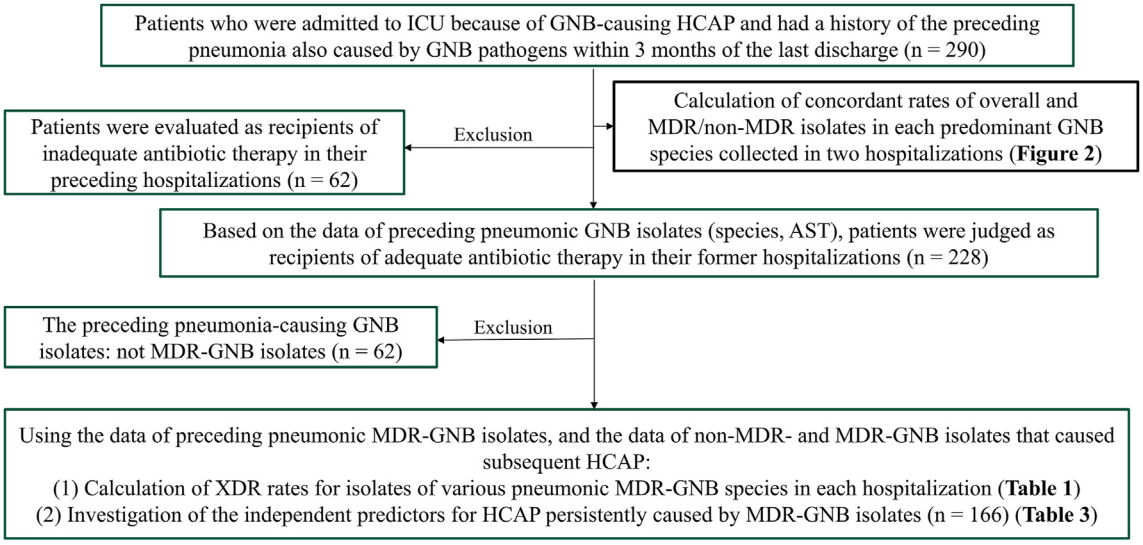

Figure 1. Steps for selecting patients with multidrug-resistant (MDR)- and non-MDR Gram-negative bacteria (GNB)-related healthcare-associated pneumonia who had received appropriate antibiotics for the treatment of pneumonia caused by MDR GNB in their preceding hospitalizations
ICU, intensive care unit. GNB, Gram-negative bacteria. HCAP, healthcare-associated pneumonia. AST, antibiotic susceptibility testing. MDR, multidrug-resistant. XDR, extensively drug-resistant.

Table 1 Distributions of isolates of the multidrug-resistant (MDR) Gram-negative bacteria (GNB) species, and rates of the extensively drug-resistant (XDR) GNB isolates that involved pneumonia among the enrolled patients who had received appropriate antibiotic(s) for the treatment of preceding pneumonia, and rates of concordant MDR GNB species between the two consecutive hospitalizations in respective species.

MDR GNB bacterial species, in the preceding pneumonia (no.)	Rates of XDR phenotypes in the MDR GNB isolates involving the preceding pneumonia, % (no.)	Concordant rates of MDR GNB species, between preceding pneumonia and subsequent HCAP, % (no.)	Rates of XDR phenotypes in the MDR GNB isolates involving the subsequent HCAP, % (no.)
Klebsiella pneumoniae (56)	21.4 (12/56)	85.7 (48/56)	43.8 (21/48)
Pseudomonas aeruginosa (40)	60 (24/40)	50 (20/40)	60 (12/20)
Acinetobacter baumannii complex (28)	71.4 (20/28)	92.9 (26/28)	92.3 (24/26)
Escherichia coli (18)	44.4 (8/18)	66.7 (12/18)	0 (0/12)
Proteus mirabilis (10)	0 (0/10)	40 (4/10)	0 (0/4)
Serratia marcescens (10)	40 (4/10)	60 (6/10)	33.3 (2/6)
Klebsiella oxytoca (4)	0 (0/4)	100 (4/4)	0 (0/4)

no., number. HCAP, healthcare-associated pneumonia.

of XDR phenotypes in the aforementioned three predominant MDR GNB species causing HCAP were 43.8%, 60%, and 92.3%, respectively. Additionally, it is noteworthy that 17 out of 48 concordant HCAP-causing MDR *K. pneumoniae* isolates (35.4%) exhibited phenotypes of non-susceptibility to ertapenem but susceptibility to anti-pseudomonal carbapenems. Furthermore, the concordance rates of carbapenem (imipenem, meropenem, or doripenem) resistance (CR) among MDR GNB isolates causing the preceding pneumonia and subsequent HCAP were as follows: 50% (4/8), 50% (4/8), and 94.4% (17/18) for *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* complex, respectively (not shown in Table 1). All 24 isolates of the HCAP-causing CR *A. baumannii* complex exhibited XDR phenotypes.

For the subgroups of MDR isolates of non-fermenting GNB (*P. aeruginosa*, and *A. baumannii* complex) and seven Enterobacterales species, the concordance rates of GNB species between the two consecutive hospitalizations did not differ significantly among 166 ICU-hospitalized patients [70.6% (48/68) vs. 75.5% (74/98); $P = 0.481$, Odds ratio (OR) 0.935, and 95% confidence interval (CI) 0.773–1.131, not shown in Table 1].

Distribution of MDR/XDR phenotypes and resistance genes in isolates of concordant GNB species associated with subsequent HCAP, and main antibiotic regimens prescribed for the treatment of patients with preceding pneumonia caused by concordant GNB with MDR/XDR phenotypes.

Table 2 presents detailed data on the MDR or XDR phenotypes, as well as the genes encoding resistant β-lactamases, among the concordant GNB isolates involved in HCAP. All patients received appropriate antibiotics for their pneumonia treatment within three days, as suggested by the consultant ID specialists, although divergent antibiotics were actually prescribed for the treatment of preceding pneumonia. Many MDR *K. pneumoniae* isolates harbored $bla_{\text{CTX-M-15}}$ (n = 26) and $bla_{\text{SHV-12}}$ (n = 19), while $bla_{\text{SHV-12}}$ was also detected in many XDR/non-CR *K. pneumoniae* isolates (n = 15). Moreover, $bla_{\text{KPC-2}}$ and bla_{VIM} were the main carbapenemase-encoding genes in XDR/CR *K. pneumoniae* (n = 4) and XDR/CR *P. aeruginosa* (n = 4) isolates, respectively. In contrast, diverse bla_{OXA} genes were detected in 24 isolates of the XDR/CR *A. baumannii* complex using multiplex PCR.

Table 2 Distribution of phenotypes (multidrug-resistant [MDR], extensively drug-resistant [XDR]) and resistance genes in isolates of the concordant Gram-negative bacterial (GNB) species associated with subsequent healthcare-associated pneumonia, and main antibiotic regimens prescribed for the treatment of patients with preceding pneumonia caused by MDR/XDR GNB.

Concordant MDR/XDR HCAP etiological microorganisms, and associated phenotypes (no.)	Main antibiotic regimens (no.) prescribed for the treatment of preceding pneumonia	Resistance genes (no.), for phenotypic MDR GNB isolates	Resistance genes (no.), for phenotypic XDR GNB isolates
Klebsiella pneumoniae (n = 48)	CFP-SUL (n = 8) HD-FEP (n = 3) HD-FEP + AMK (n = 1) Any carbapenem (n = 3) CZA (n = 3) IH-COL monotherapy (n = 18) IV-COL + IH-COL (n = 10) IV-COL + TGC + HD-MEM (n = 2)	MDR (n = 48) ESBL-encoding genes: CTX-M-15 (n = 26) CTX-M-14 (n = 2) SHV-12 (n = 19) Plasmidic AmpC-encoding genes: DHA-1 (n = 4) CMY-2 (n = 1)	XDR/non-CR (n = 17, and XDR/CR (n = 4) ESBL-encoding genes: SHV-12 (n = 15) Plasmidic AmpC-encoding genes: DHA-1 (n = 13) Carbapenemase-encoding genes: KPC-2 (n = 4)
Pseudomonas aeruginosa (n = 20)	HD-MEM + HD-SUL (n = 4) CFP-SUL (n = 3) CFP-SUL + AMK (n = 1) IV-COL + FOS (n = 2) CZA (n = 1) IV-COL + IH-COL (n = 6) IH-COL monotherapy (n = 3)	MDR (n = 8) ESBL-encoding genes: GES (n = 1) PER (n = 1)	XDR/non-CR (n = 8), and XDR/CR (n = 4) ESBL-encoding genes: GES (n = 1) PER (n = 2) Carbapenemase-encoding genes: VIM types (n = 4)
Acinetobacter baumannii complex (n = 28)	IV-COL + TGC (n = 14) IV-COL + HD-MEM (n = 6) IV-COL + HD-SUL (n = 3) IH-COL monotherapy (n = 5)	MDR (n = 2) ESBL-encoding genes: VEB (n = 2)	XDR/non-CR (n = 0) XDR/CR (n = 24*) Carbapenemase-encoding genes: OXA-24-like (n = 10) OXA-51-like (n = 8) OXA-72 (n = 8)

Abbreviations: no., number. MDR, multidrug-resistant. XDR, extensively drug-resistant. HCAP, healthcare-associated pneumonia. GNB, Gram-negative bacteria. CFP-SUL, cefoperazone-sulbactam. CZA, ceftazidime-avibactam. HD, high-dose. FEP, cefepime. AMK, amikacin. IH, inhalational. COL, colistin. IV, intravenous. TGC, tigecycline. MEM, meropenem. ESBL, extended-spectrum β-lactamase. SUL, sulbactam. FOS, fosfomycin. *Two CR/XDR-*A. baumannii* isolates harbored two different bla^{OXA} genes.

Distribution of the four main GNB species involved in pneumonia between two hospitalizations, and concordance rates between preceding pneumonia and subsequent HCAP for isolates of each predominant GNB species with either MDR or non-MDR phenotypes

Fig. 2 depicts the overall pneumonia-causing GNB isolates (irrespective of resistance phenotypes and species concordance between two hospitalizations). Notably high rates exceeding 60% were observed for isolates of *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* complex species (71%, 69.7%, and 66.7%, respectively). These rates were significantly higher compared with that of *Escherichia coli* (22.2%; all *P* values, <0.05). Moreover, in contrast to MDR *E. coli* isolates (22.2%), statistically higher rates of concordance were evident for isolates of MDR *K. pneumoniae* (85.7%) and MDR *A. baumannii* complex (92.9%), which caused the preceding pneumonia and subsequent HCAP (both *P* values, <0.001). The difference in concordance rates between MDR *P. aeruginosa* and MDR *E. coli* isolates was nearly significant ($P = 0.082$).

After receiving appropriate antibiotic treatments, the group of patients with prior pneumonia caused by MDR *P. aeruginosa* had higher concordance rates for both overall isolates and those with MDR phenotypes compared to the group with prior pneumonia caused by concordant non-MDR *P. aeruginosa* isolates [69.7% (46/66) and 50% (20/40) vs. 30.8% (8/26)]. However, despite the observed variation, this difference between the MDR and non-MDR subsets did not reach statistical significance ($P = 0.137$).

Univariate analysis between patients who had HCAP persistently caused by either MDR GNB or non-MDR GNB isolates

Table 3 presents the proportions of various demographic features (gender, age, and a CCI score of $\geq$4 points), physiological severity, significant co-morbidities, recipients of prolonged high-dose corticosteroids, the predominant three GNB causing HCAP (regardless of species concordance between the two hospitalizations), and other healthcare-associated factors among patients with HCAP persistently caused by either MDR GNB (n = 126) or caused by non-MDR GNB (n = 40) isolates. None of the enrolled patients received chemotherapy or T- or B-cell immunosuppressive agents due to debilitating conditions. Additionally, patients who received mechanical ventilation support also utilized a tracheostomy tube.

Between the two HCAP subgroups, the following variables were more likely ($P \leq 0.15$) to be associated with HCAP persistently caused by MDR GNB: DM, chronic structural lung disorders, an interval of $\leq$30 days between the two hospitalizations, tracheostomy tube use alone, and the use of tracheostomy along with mechanical ventilator support, evaluated using the chi-square test.

Independent predictors for HCAP persistently caused by MDR GNB species

Table 4 presents the independent factors predicting the development of HCAP persistently caused by MDR GNB in patients treated with appropriate antibiotics for the

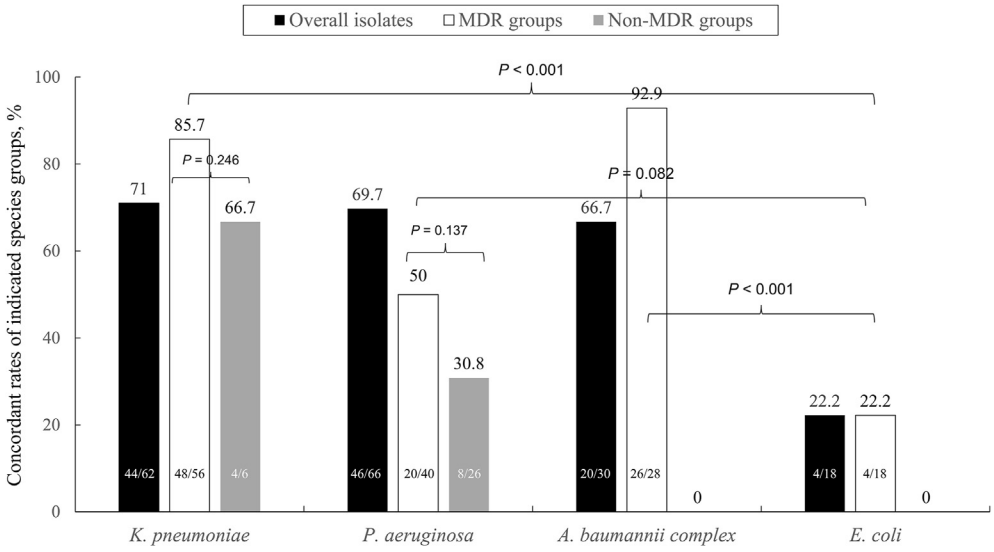

Figure 2. Differences in proportions of overall Gram-negative bacterial (GNB) isolates responsible for pneumonia in the two consecutive hospitalizations, regardless of resistance phenotypes and species concordance, and differences in the rates of concordance in overall isolates and those with multidrug-resistant (MDR) phenotypes between the subsets of MDR GNB isolates causing preceding pneumonia and healthcare-associated pneumonia (HCAP), as well as the rates of concordance in overall isolates and those with non-MDR phenotypes between the subgroups of non-MDR GNB isolates that caused preceding pneumonia and subsequent HCAP, in each predominant GNB species. The number (no.) at the bottom of each bar denotes the number of isolates (positive no./total no.).

Table 3 Comparison of demographic features, important co-morbidities, physiological severity, clinical factors, use of specified drugs, and the top three species of Gram-negative bacteria (GNB; irrespective of species concordance between the two hospitalizations) for patients who had received appropriate antibiotic(s) for the management of preceding pneumonia caused by multidrug-resistant (MDR) GNB in their former hospitalizations (n = 166), and were re-admitted to intensive care unit within 3 months because of recurrent Gram-negative pneumonia [healthcare-associated pneumonia (HCAP) caused by either MDR GNB (n = 126) or non-MDR GNB (n = 40)].

Characteristics	Patients receiving appropriate antibiotics for the treatment of preceding pneumonia caused by MDR GNB had the subsequent HCAP also caused by isolates of concordant GNB species (n = 166)		Univariate analysis		
	HCAP caused by MDR GNB (n = 126), case no. (%)	HCAP caused by non-MDR GNB (n = 40), case no. (%)	Odds ratio	95% Confidence interval	*P* value
Age ≥65-years	22 (17.5)	8 (20)	0.814	0.422, 1.806	0.873
Gender, male	108 (85.7)	30 (75)	1.143	0.943, 1.386	0.145
Charlson co-morbidity index ≥4 points	10 (7.9)	0 (0)	0.921	0.875, 1.009	0.120
APACHE II ≥ 30 points	52 (41.3)	16 (40)	1.032	0.669, 1.591	1.000
Interval of ≤30 days between the last discharge date and the subsequent admission date for HCAP	56 (44.4)	8 (20)	2.222	1.160, 4.256	0.005
DM	56 (44.4)	8 (20)	2.222	1.160, 4.256	0.005
Long-term dialysis	8 (6.3)	2 (5)	1.270	0.281, 5.737	1.000
COPD	16 (12.7)	6 (15)	0.847	0.355, 2.017	0.789
Chronic structural lung disorders	32 (25.4)	1 (2.5)	13.277	1.752, 100.591	0.001
Malignancy	10 (7.9)	4 (10)	0.794	0.263, 2.393	0.745
Prolonged high-dose steroid therapy	42 (33.3)	12 (30)	1.111	0.651, 1.895	0.847
Neurological disorders	10 (7.9)	4 (10)	0.794	0.263, 2.393	0.745
Dementia	66 (52.4)	22 (55)	0.952	0.687, 1.319	0.856
Nursing home residence	82 (65.1)	22 (55)	1.183	0.869, 1.610	0.265
Tracheostomy tube use alone	94 (74.6)	6 (15)	4.974	2.362, 10.473	<0.001
Mechanical ventilation support	44 (34.9)	2 (5)	6.984	1.772, 27.531	<0.001
Klebsiella pneumoniae[a]	48 (38.1)	6 (15)	3.487	1.363, 8.922	0.007
Pseudomonas aeruginosa[a]	20 (15.9)	18 (45)	0.231	0.105, 0.506	<0.001
Acinetobacter baumannii complex[a]	28 (22.2)	2 (5)	5.429	1.232, 23.910	0.017

[a] Not limited in isolates with concordant species between the two consecutive hospitalizations.
MDR, multidrug-resistant. GNB, Gram-negative bacteria. HCAP, healthcare-associated pneumonia. no, number. APACHE, Acute Physiological and Chronic Health Evaluation. DM, diabetes mellitus. COPD, chronic obstructive pulmonary disease.

preceding MDR GNB-causing pneumonia. Co-morbidities of DM (OR 13.0, 95% CI 2.23–74.25) and chronic structural lung disorders (OR 183.0, 95% CI 7.26–4608.97), an interval of ≤30 days between two hospitalizations (OR 10.8, 95% CI 2.15–54.77), the use of a tracheostomy tube alone (OR 18.47, 95% CI 3.08–110.81), and subsequent HCAP caused by MDR *A. baumannii* complex (OR 11.9, 95% CI 1.87–189.12) were found to be the independent predictors for HCAP persistently caused by MDR GNB. Conversely, MDR *P. aeruginosa* was a negative independent predictor for HCAP due to concordant MDR GNB (OR 0.05, 95% CI 0.006–0.417).

Discussion

This Taiwanese survey presents the following key findings. First, a high level of species concordance in MDR isolates of *K. pneumoniae*, *A. baumannii* complex, *P. aeruginosa*, and *Klebsiella oxytoca* was observed between the episodes of preceding pneumonia and subsequent HCAP (Table 1). Second, the use of a tracheostomy tube alone, an interval of ≤30 days between two hospitalizations (despite appropriate antibiotic use for the treatment of the preceding MDR GNB-causing pneumonia), MDR *A. baumannii* as the causative microbe of preceding pneumonia, and co-morbidities of DM and chronic structural lung disorders were independent predictors for the development of HCAP pertinaciously caused by MDR GNB (Table 3). Third, this is the first survey to report that MDR *P. aeruginosa*, identified as a microbial etiology of the preceding pneumonia, serves as a negative predictor for MDR GNB-causing HCAP.

The present survey shows that the interval of no more than 30 days between two hospitalizations, which indirectly reflects the poor functional status among these patients, strongly predicts the development of HCAP persistently

Table 4 Logistic regression analysis regarding predictors for healthcare-associated pneumonia persistently caused by multidrug-resistant (MDR) Gram-negative bacteria (GNB) among 166 patients who had received appropriate antibiotics for the treatment of preceding pneumonia also caused by MDR GNB in the former hospitalization courses.

Variables	OR (95% CI)	*P* value
DM	13.043 (2.291, 74.251)	0.004
Chronic structural lung disorders	182.965 (7.263, 4608.969)	0.002
Interval of ≤30 days between the last discharge date and subsequent admission date for GNB pneumonia	10.781 (2.150, 54.768)	0.004
Tracheostomy tube use alone	18.468 (3.078, 110.811)	0.001
Mechanical ventilator support	11.001 (0.596, 203.211)	0.107
Klebsiella pneumoniae	0.898 (0.156, 5.183)	0.904
Pseudomonas aeruginosa	0.051 (0.006, 0.417)	0.005
Acinetobacter baumannii complex	11.912 (1.867, 189.115)	0.043

OR, odds ratio. CI, confidence interval. DM, diabetes mellitus. GNB, Gram-negative bacteria.

caused by MDR GNB in Taiwanese patients (OR 10.8, Table 3). Webb et al. proposed the Drug Resistance in Pneumonia (DRIP) score calculation, consisting of four major and six minor risk factors.[25] This scoring system demonstrated notably superior predictive ability for identifying the emergence of MDR pathogens in HCAP compared to the standard HCAP criteria.[3] The DRIP score (≥4 points)[25] was found to significantly outperform the HCAP criteria[3] in predicting drug-resistant phenotypes in pathogens implicated in pneumonia, with a positive predictive value of 0.68 compared to 0.53. Notably, the findings of this survey align closely with the variables incorporated in the DRIP score, including recent antibiotic use within 60 days and a history of infection due to MDR pathogens within one year.[25]

A survey investigating hospitalized patients with HCAP in northern India revealed the presence of various comorbidities in these patients.[26] One meta-analysis study also demonstrated that patients with type 2 DM were at a two-fold risk of acquiring respiratory tract infections caused by drug-resistant organisms (OR 2.35, and 95% CI 1.49−3.69).[17] This finding corresponds to the observation from the present survey (OR 2.22, Table 2). Additionally, in line with the 2016 HAP treatment guidelines,[3] chronic structural lung disorder predisposed patients to acquiring HCAP persistently caused by MDR GNB (Table 3).

In most critically ill patients hospitalized in the ICU, various indwelling intravenous catheters are used. Studies have indicated that biofilms rampantly develop on many implanted medical devices after usage for more than 7 days.[27] Biofilm formation is recognized as a crucial pathogenic mechanism contributing to microbiologic persistence, hindering the efficacy of appropriate antibiotic therapy.[28] Gil-Perotin et al. observed that the bacteria flora immersed in the biofilm of the endotracheal tube (made of biomaterials with an abiotic surface) closely matched (95%) the culture results from endotracheal aspirate.[28] This scenario could likely be extended to patients using the tracheostomy tubes, as also suggested by Raveendra et al.[27]

MDR strains isolated from the tracheostomy tube were shown to be significantly associated with recent nosocomial infections.[29] Consistent with the present survey (Table 3), a Chinese study also indicated that the use of a tracheostomy tube independently predicted the development of pneumonitis caused by MDR GNB in vulnerable patients (OR 4.46).[24] In contrast, ventilator usage could not independently predict the development of HCAP persistently caused by MDR GNB in this survey (Table 4). This finding corresponds to the survey conducted by Raveendra et al.[27] This could be attributed to the consistent and regular replacement of the flexible trach adaptor and ventilator filter used by patients globally and in Taiwan who undergo tracheostomy combined with ventilator support.

A Taiwanese study uniquely revealed that nearly half (47.5%) of diabetic patients were found to be asymptomatically colonized with *K. pneumoniae* in the rectum.[30] Additionally, another study investigated 18 *K. pneumoniae* isolates colonizing the tracheostomy tubes and revealed that all isolates harbored $bla_{\text{CTX-M-1}}$, bla_{SHV}, bla_{TEM}, and *mrk*A adhesin genes.[31] In that investigation, 44.4% (8/18) of the studied *K. pneumoniae* isolates had variable potential of in vitro biofilm production on abiotic surfaces.[31] The present survey revealed that isolates of *K. pneumoniae* were cultured from 34.0% (32/94) of 94 patients who used tracheostomy tubes and had subsequent HCAP due to MDR GNB. This percentage significantly exceeded that of *P. aeruginosa* [21.3% (20/94)] and *A. baumannii* complex [17.0% (16/94)] (both *P* values, ≤0.05; not shown in Results). The distribution of GNB species associated with HCAP differed significantly from those reported by Jiang et al.[24] and Raveendra et al.[27] Although the causative etiology of *K. pneumoniae* was not an independent predictor for HCAP tenaciously caused by MDR GNB (Table 4), the findings of the other investigations[30,31] and the present survey suggest that MDR *K. pneumoniae* isolates pose a significant concern for Taiwanese HCAP patients who have used a tracheostomy tube. These isolates likely exhibit a high potential for adhesion and pertinaciously exhibit MDR phenotypes.

In this Taiwanese survey, it was found that MDR *A. baumannii* complex isolates, which had caused preceding pneumonia, exhibited a persistence rate of 92.9% (26/28) in subsequent HCAP (Table 1 and Fig. 2). Furthermore, MDR *A. baumannii* as the causative GNB etiology of preceding pneumonia was an independent predictor for the development of subsequent HCAP persistently caused by MDR GNB (OR 11.9, $P = 0.043$; Table 3). *A. baumannii* was validated to possess many characteristics (pili, cell surface hydrophobicity, secretion of biofilm-coating proteins, etc.)

facilitating adherence to epithelial cells of the human airway.[32] Furthermore, XDR-*A. baumannii* largely exhibits high resistance to disinfectants and is prone to colonization in ICU and healthcare facilities.[6,33] The characteristics of MDR *A. baumannii*[27,28] and the findings of the present survey suggest that this notorious pathogen is a tenacious HCAP etiology, emphasizing the need for heightened vigilance among Taiwanese HCAP patients.

In remarkable contrast to another study,[34] this survey showed that non-MDR *P. aeruginosa* isolates were the causative agents of HCAP in 50% of patients who had received appropriate antibiotics as therapy for the preceding MDR *P. aeruginosa*-related pneumonia (Table 1). Furthermore, MDR *P. aeruginosa*, as the causative microbe of preceding pneumonia, was a surprisingly negative predictor for MDR-HCAP (Table 3). We did not delve deeper into the root cause of this observation because investigating the actual airway environments in these debilitated patients is challenging. Consequently, we refer to other studies investigating the microbial evolution related to preceding airway MDR *P. aeruginosa* in patients receiving appropriate antibiotic therapy to propose plausible explanations for the negative predictor of MDR GNB.

Highly targeted antibiotic treatment and the implementation of stringent isolation measures for patients with pneumonia due to MDR/XDR *P. aeruginosa*, according to antibiotic stewardship policies,[12,35] likely result in considerable alterations of airway microbial environments in these patients. Additionally, in a murine model of peritonitis, an intraperitoneal inoculum of MDR *P. aeruginosa* isolates results in a significantly higher median concentration of plasma lipopolysaccharide than that of susceptible *P. aeruginosa* isolates, in parallel with a better survival duration observed in the former group of rats.[36] This finding suggests that the considerable potential of enhanced immunity elicited by MDR *P. aeruginosa*, with subsequent eradication of these MDR pathogens, is likely. Moreover, a survey regarding airway MDR *P. aeruginosa* isolates in patients with cystic fibrosis (CF) revealed that competitive exclusion between MDR *P. aeruginosa* and susceptible GNB in the same airway niche was validated when they were exposed to appropriate antibiotics.[37] The ecology dominated by MDR *P. aeruginosa* might revert to non-MDR ones after the discontinuation of the offending antibiotics.

The limitations of this survey are as follows: First, the findings of this Taiwanese multicenter survey might not be broadly applicable to other countries due to variations in GNB species distributions and resistance conditions worldwide. Second, the genospecies of *A. baumannii* complex isolates were not identified using the sequence of the 16 S—23 S ribosomal RNA gene intergenic spacer region.[38]

In conclusion, this survey unveiled a substantial resistance burden of GNB in Taiwanese patients with HCAP. Chronic structural lung disorders, DM, a short interval ($\leq$30 days) between the two hospitalizations, and the sole use of a tracheostomy tube were identified as independent predictors for patients with HCAP persistently caused by MDR GNB isolates, despite appropriate antibiotic treatments against MDR GNB responsible for the preceding pneumonia. The predominance of MDR *A. baumannii* complex as the causative microbe in the preceding pneumonia was also an independent predictor for HCAP caused by MDR GNB. Additionally, compared to isolates of MDR *A. baumannii* complex, MDR *K. pneumoniae* as the causative microbe of the preceding pneumonia is more likely to persist in patients using a tracheostomy tube who subsequently develop HCAP. A considerable heterogeneity in the airway environment of the debilitated patients with HCAP likely contributed to a significantly high rate of non-MDR phenotypes of *P. aeruginosa*. To prescribe appropriate antibiotic(s) for HCAP treatment in a timely manner, it is essential to identify predictors associated with HCAP persistently caused by MDR GNB.

Ethical approval

Min-Sheng General Hospital (Taoyuan, Taiwan) [MSIRB2024006, applied by Shio-Shin Jean], Far Eastern Memorial Hospital (New Taipei City, Taiwan) [111211-E, applied by Hou-Tai Chang], and Mackay Memorial Hospital (Taipei, Taiwan) [18MMHIS198e, applied by Li-Kuo Kuo], all approved this HCAP study.

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Credit authorship contribution statement

Li-Kuo Kuo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing — original draft. **Hou-Tai Chang:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing — original draft. **Shun-Chung Hsueh:** Conceptualization, Methodology, Project administration, Software, Validation, Visualization, Writing — original draft. **I-Min Liu:** Methodology, Software, Supervision, Writing — review & editing. **Po-Chuen Hsieh:** Formal analysis, Methodology, Project administration, Software, Supervision, Writing — review & editing. **Shio-Shin Jean:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing — original draft, Writing — review & editing.

Declaration of Generative AI and AI-assisted technologies in the writing process

Generative AI-assisted technologies were not used in the writing process of this manuscript.

Declaration of competing interest

None are declared.

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