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Brief Communication

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Antibiotic-Resistant Gram-negative Bacteria Carriage in Healthcare Workers Working in an Intensive Care Unit

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ABSTRACT

Little is known about antibiotic-resistant Gram-negative bacteria (GNB) intestinal carriage among healthcare workers (HCWs) in Vietnam. All HCWs at a tertiary intensive care units were asked to undertake weekly rectal swabs. Among 40 participants, 65% (26/40) carried extended spectrum β-lactamases (ESBL)/AmpC β-lactamase-producing *Escherichia coli.* Two HCWs colonized with ESBL/AmpC β-lactamase-producing *Klebsiella pneumoniae.* One HCW colonized with *Acinetobacter baumannii.* No one carried *Pseudomonas* spp.. A quarter (10/40) of HCWs were identified as persistent and frequent carriers. There is an urgent need to screen antibiotic-resistant GNB among HCWs and improve HCWs' hand hygiene compliance to reduce the transmission of antibiotic-resistant GNB in the hospital.

Keywords: Antibiotic-resistant Gram-negative bacteria; Intensive care units; Healthcare workers

Antibiotic-resistant Gram-negative bacteria (GNB) have emerged as important pathogens associated with high morbidity and mortality worldwide [1-3]. Antibiotic-resistant GNB may colonize the digestive tract, which is a possible source of later infections among critically ill patients in intensive care units (ICU) [4, 5]. Moreover, antibiotic-resistant GNB can be spread from patient to patient by healthcare workers (HCWs) [6, 7]. However, the burden of antibiotic-resistant GNB carriage among HCWs remains inconclusive in Vietnam.

To strengthen the antibiotic-resistant GNB prevention and control policy in Vietnam and comparable countries, a prospective study was conducted to examine the patterns of antibiotic-resistant GNB intestinal carriage among HCWs at the adult ICU, Hospital for Tropical Diseases (HTD) from October 28 to December 20, 2019. The study was approved by the Ethics Committee of the HTD, Vietnam (approval number 24/HDDD) and the University of New South Wales, Australia (approval number HC190730). All staff were invited to participate in the study. Written informed consent was obtained from participants. Swabs were taken on every Monday in eight consecutive weeks. The "Sterile Transport Swab" (Jiangsu, Kangjian Medical Apparatus Co., Ltd., Taizhou, China) was used, and the swabbing procedure was based on the HTD's infection control guideline. All participants were instructed by a qualified research assistant on sample collection. Participants were

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Author Contributions

Conceptualization: BTD, MCD, VVCN. Data curation: BTD, MCD, JC, VMHN, HHN, TBHB. Formal analysis: BTD, MCD. Investigation: BTD, MCD, JC, VMHN, HHN, TBHB. Methodology: BTD, MCD. Project administration: BTD, MCD. Resources: JC, VMHN, HHN, TBHB. Software: JC, VMHN, HHN, TBHB. Supervision: MCD. Validation: BTD, MCD, JC, VMHN, HHN, TBHB. Writing - original draft: BTD, MCD. Writing - review & editing: BTD, MCD, JC, VMHN, HHN, TBHB.

also asked to return their self-obtained rectal swabs within one hour of collection in a sealed envelope labelled with an identification code. It is noted that the quality of rectal swab samples was ensured provided that all participants are healthcare professionals working at this Adult ICU. Rectal swab samples were cultured on MacConkey agar (bioMérieux, Paris, France) and Xylose Lysine Deoxycholate agar (bioMérieux, France) to isolate GNB which are the major ICU pathogens in the HTD including *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp. and *Acinetobacter* spp. [4,5]. The bacterial identification was confirmed by the matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDITOF, Bruker, Bremen, Germany). Antibiotic susceptibility testing was conducted by the Kirby/Bauer disc diffusion method and interpreted using the 2015 Clinical and Laboratory Standards Institute (CLSI) guidelines against 7 antibiotics: amoxcillin-clavulanic acid, ceftriaxone, cefepime, ofloxacin, gentamicin, piperacillin-tazobactam and ticarcillin-clavulanate [8]. Specifically, ceftriaxone was replaced by ceftazidime for antibiotic susceptibility testing of *Pseudomonas* spp. and *Acinetobacter* spp. *E. coli* and *Klebsiella* spp. were further screened for extended spectrum β-lactamase (ESBL) and AmpC β-lactamase production. ESBL producers were detected using CHROMagar (CHROMagar, Paris, France). The double disc diffusion method was then used to detect ESBL activity using both cefotaxime and ceftazidime, alone and in combination with clavulanate. ESBL activity is considered if there is a ≥5mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate compared to the zone diameter of the agent when tested alone. Moreover, AmpC β-lactamase production was tested using CHROMagar C3G^{R} (CHROMagar, France). Then suitable colonies had an AmpC induction test to detect induced AmpC β-lactamases activity. A ceftazidime disc was placed near cefoxitin/imipenem. A flattening zone of 3rd cephalosporin toward the inducer (cefoxitin/imipenem) indicates the inducible AmpC β-lactamases. The microbiological methods used to identify antibiotic-resistant GNB from swabbing samples have been validated elsewhere [4, 5]. A questionnaire was used to collect baseline information about age, sex, profession, ICU working time, antibiotic exposure, and underlying diseases. There was no GNB outbreak or transmission in the study clinic during the study period.

In this study, antibiotic-resistant GNB included ESBL/AmpC β-lactamase-producing *E. coli*, ESBL/AmpC β-lactamase-producing *Klebsiella* spp., ceftazidime-resistant *Pseudomonas* spp., and multidrug-resistant *Acinetobacter* spp. (resistance to ≥3 classes of antibiotics). Based on participants' number of positive swab cultures with antibiotic-resistant GNB, participants were categorized into 4 groups including persistent carriers (having all 8 positive swab samples), frequent carriers (having 5 to 7 positive swabs), incidental carriers (having 1 to 4 positive cultures), and non-carriers (having all negative cultures) [9]. Persistent and frequent carriers were further grouped as prolonged carriers.

Data were analyzed using R statistical software. Descriptive analyses included frequency and percentage (95% confidence interval [CI]) for categorical data, and median (interquartile range [IQR]) for continuous data. Chi-squared test was used to examine the significant relationship between categorical variables. Mann-Whitney *U* test was utilized to compare continuous variables. Alpha was set at 5% level.

A total of 40 (67%, 40/60) HCWs agreed to participate in the study, including 7 physicians (64%, 7/11), 25 nurses (61%, 25/41), and all 8 nursing aids (**Table 1**). Among 40 participants, 88% (35/40) aged ≤40 years, 75% (30/40) were female, and 40% (16/40) had worked in the ICU over 5 years. After the first sampling, 25% (10/40) of participants carried antibiotic-resistant GNB. Based on the seven subsequent swab culture results, they were identified as

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Table 1. Demographic characteristics of 40 healthcare workers working in the adult ICU

Characteristics	Statistics[a]
Age (years)	
Median	33
IQR	27 - 36
Age groups	
≤30	15 (38)
31 – 40	20 (50)
41 – 50	5 (12)
Male	10 (25)
Female	30 (75)
Professions	
Medical doctors	7 (17)
Nurses	25 (63)
Nursing aids	8 (20)
Working time in ICU (years)	
Median	5
IQR	1 - 11
ICU length of working time (years)	
<1	9 (23)
1 – 5	15 (38)
6 – 10	4 (10)
11 – 15	10 (25)
16 – 20	1 (2)
>20	1 (2)
Underlying diseases	
Gastritis	8 (20)
Sinusitis	4 (10)
Diabetes mellitus	2 (5)
Gastroesophageal reflux disease	1 (3)
Rheumatoid arthritis	1 (3)
Thyroid cancer	1 (3)
No underlying disease	23 (58)

[a]Absolute count (percentage) for categorical variables, median and IQR for continuous variables.
ICU, intensive care unit; IQR, interquartile range.

persistent carriers (3/10), frequent carriers (3/10), and incidental carriers (4/10). Of the 30 HCWs with initially negative cultures, 47% (14/30) were tested negative continuously on all the remaining follow-ups, and thus finally categorized as non-carriers. Based on the seven subsequent swab culture results, the remaining 53% (16/30) of HCWs with initially negative cultures were identified as incidental carriers (12/16) and frequent carriers (4/16). Overall, the prevalence of antibiotic-resistant GNB persistent carriers (8%, 3/40), frequent (18%, 7/40), incidental (40%, 16/40), and non-carriers (35%, 14/40) were recorded. Regarding antibiotic susceptibility, around 50% of *E. coli* and *K. pneumoniae* was resistant to amoxicillin-clavulanic acid, and about 30% of them was resistant to ticarcillin-clavulanate. Both *E. coli* and *K. pneumoniae* were sensitive to piperacillin-tazobactam because their resistance rates were less than 2%. However, higher rates of resistance of *E. coli* were documented for ceftriaxone (25%) and gentamicin (11%) compared to *K. pneumoniae* with the rate of resistance of about 5% documented for each antibiotic. In contrast, *E. coli* was more resistant to cefepime (resistance rate of 18%) and ofloxacin (resistance rate of 22%) than *K. pneumoniae* (resistance rate to each antibiotic <2%). Most HCWs (65%, 26/40) carried intestinally ESBL/AmpC β-lactamase-producing *E. coli*, and 2 of them also colonized intermittently with ESBL/AmpC β-lactamase-producing *K. pneumoniae*. It is noted that ESBL/AmpC β-lactamase-producing *E. coli* was detected in persistent and frequent carriers, while ESBL/AmpC β-lactamase-producing *K. pneumoniae* was exclusively found in incidental carriers. The proportion of ESBL-producing *E. coli* carriage was 33% (13/40), AmpC β-lactamase-producing *E. coli* (18%, 7/40), and combined

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ESBL and AmpC β-lactamase-producing *E. coli* (10%, 4/40). Regarding the risk factors, none of our participants had antibiotic exposure during the study period. We found no significant association between intestinal carriage of antibiotic-resistant GNB and age, sex, working time in ICU, and underlying diseases (P >0.05). Professions were found to be likely associated with intestinal carriage (P = 0.09) provided that all (7/7) physicians carried antibiotic-resistant GNB, while 60% (15/25) of nurses and 50% (4/8) of nursing aids were antibiotic-resistant GNB carriers (data not shown). A single isolate of *A. baumannii* was cultured and none of HCWs colonized with *Pseudomonas* spp., making the patterns of intestinal carriage among ICU staff mostly describe the colonization of ESBL/AmpC β-lactamase producing *E. coli* and *K. pneumoniae*. *A. baumannii* was isolated in a nurse who did not carry any ESBL/AmpC β-lactamase-producing *E. coli* and *K. pneumoniae*. Regarding antibiotic susceptibility, this single isolate of *A. baumannii* was a susceptible strain and thus, the nurse carrying this isolate was not classified as an incidental carrier in our study. The prevalence of ESBL-producing *E. coli* was 48% (19/40, 95% CI: 33 - 63%), while that of ESBL-producing *K. pneumoniae* was 2.5% (1/40, 95% CI: 1 - 13%) because a study participant carrying ESBL-producing *K. pneumoniae* also carried ESBL-producing *E. coli*. The overall prevalence of HCWs carrying intestinally ESBL-producing organisms including *E. coli* and *K. pneumoniae* was 48% (19/40, 95% CI: 33 – 63%). In the general community in Vietnam, the prevalence of intestinal carriage of ESBL-producing *E. coli* was 51% (101/198, 95% CI: 44 - 58%) [10]. Another study found the oropharyngeal carriage rate of *K. pneumoniae* in the general community of 14% (145/1,029, 95% CI: 12 - 16%), of which 4% (6/145, 95% CI: 2 - 9%) was ESBL-producing *K. pneumoniae* [11]. A study examining the vaginal colonization of ESBL-producing bacteria in Vietnamese pregnant women found that *E. coli, Klebsiella* species were identified in 30% (918/3,104, 95% CI: 28 - 31%) of the vaginal swabs, with ESBL-producing *E. coli* was predominant (79%, 340/432, 95% CI: 75 - 82%), followed by *K. pneumoniae* (20%, 88/432, 95% CI: 17 - 24%) [12]. These reports and our findings demonstrate the high burden of ESBL-producing *E. coli* and *K. pneumoniae* in both the community and clinical settings in Vietnam. Our finding is in line with a report from Madagascar, a low-income country probably with comparable infection control policy where 49% (19/39, 95% CI: 34 - 64%) of HCWs tested positive for ESBL producers, primarily *E. coli* and *K. pneumoniae* [13]. However, our rate was higher than those reported from high-income countries such as Switzerland (15%, 6/41, 95% CI: 7 - 28%) [14] and the United States (4%, 15/379, 95% CI: 2 - 6%) [15]. Furthermore, several publications that have exclusively focused on ESBL-producing *E. coli* reported lower rates (*i.e.*, 3.4 - 21%) of intestinal carriage in HCWs compared to ours [16-18]. The prevalence of our HCWs with ESBL-producing *E. coli* colonization was 48% (19/40, 95% CI: 33 – 63%), while those of Egyptian and German HCWs were 21% (42/200, 95% CI: 16 – 27%) [17] and 4% (4/107, 95% CI: 1 – 9%) [18], respectively. Our rate was also higher than that of another study conducted in multiple rehabilitation centers in Israel, Italy, France, and Spain in which 3% (34/1001, 95% CI: 2 – 5%) of HCWs intestinally carrying ESBL-producing *E. coli*, and the highest prevalence was 11% among Spanish HCWs [16].

Our study is one of the few studies examining the prolonged intestinal carriage (up to 8 weeks) among HCWs worldwide provided that most published studies only focused on the presence or absence of antibiotic-resistant GNB [9, 19]. It is argued that transient negative fecal samples on the follow-up reflect quantitatively less shedding antibiotic-resistant GNB [19]. Thus, it is logical to infer prolonged carriage with increased risk of spreading antibiotic-resistant GNB. In our study, 25% (10/40, 95% CI: 14 – 40%) of HCWs were prolonged carriers. Interestingly, ESBL/AmpC β-lactamase-producing *E. coli* was common among prolonged carriers while ESBL/AmpC β-lactamase-producing *K. pneumoniae* was detected

only in incidental ones. In our study, swabs were taken on every Monday (*i.e.*, fixed time) in eight consecutive weeks in our study. Therefore, although only conventional microbiological method was utilized, we found that carriage of ESBL/AmpC β-lactamase-producing *E. coli* in our HCWs was not random. This allows us to further examine ESBL/AmpC β-lactamase-producing *E. coli* carriage as incidental, frequent and prolonged carriages. This has been proven in a recent publication, in which carriage of ESBL-producing *E. coli* differed between bacterial genotypes [9]. Therefore, knowledge about the duration of colonization with antibiotic-resistant GNB is crucial to implement adequate control measures in hospital with a focus on prolonged carriers and further molecular investigations are needed.

Overall, the incidence of HCWs acquired antibiotic-resistant GNB during the study was 40% (16/40, 95% CI: 26 - 55%). The potential sources for acquisition of antibiotic-resistant GNB in HCWs include occupation-related source (*i.e.*, related to caring for patients), healthcare-related source (*i.e.*, related to healthcare acquisition while being patients) and community-related acquisition [16]. In our study, no participant had been hospitalized in the previous 12 months, making the healthcare-related source less likely. Community-related acquisition such as contact with other ESBL carriers in the community setting may have been potential sources [16]. Indeed, it is documented that the prevalence of intestinal carriage of ESBL-producing *E. coli* in the Vietnamese community is up to 51% (101/198, 95% CI: 44 - 58%) [10]. There is a less occupational risk of carriage with antibiotic-resistant GNB in HCWs in developed countries [15, 18].

A recent study conducted in Vietnam found that if the infection prevention and control (IPC) practice, particularly hand hygiene is suboptimal, HCWs can acquire GNB from water sources in the healthcare setting [20]. Although we did not examine the levels of IPC compliance among our participants, the suboptimal compliance is well documented in Vietnam, regardless of healthcare settings [20-23]. We believe that breaches in IPC for hand hygiene may have been attributable to our high carriage rate. Considering the potential community source and suboptimal ICP, there is an urgent need for continuous surveillance of HCWs to detect antibiotic-resistant GNB and HCWs' compliance with hand hygiene moment 1, before touching a patient, to reduce the likelihood of transmission of antibiotic-resistant GNB to vulnerable patients [24].

Our study had some limitations. Firstly, genotyping method was not utilized to detect ESBLs and plasmid AmpC genes due to limited financial resources. However, all HCWs were prospectively screen for antibiotic-resistant GNB carriage for 8 consecutive weeks without any missing samples in our study. Hence, the risk of missing resistant organisms was less likely. Secondly, testing of carbapenem resistance was not carried out in this study due to limited financial resources. However, bacterial isolates have been stored for future study about carbapenem resistance. Thirdly, our study was a single-center study. Also, given the study required rectal samples, only 67% of ICU staff agreed to participate in our study. Thus, the study population may not be a good representative of the underlining population. However, to the best of our knowledge, our study is the first one in Vietnam, and among the very few studies in the world to examine the intestinal carriage of antibiotic-resistant GNB among ICU staff. The findings of our study serve as a foundation for future investigations of the magnitude of antibiotic-resistant GNB intestinal carriage in ICU staff and associated control measures.

In conclusion, an alarmingly high prevalence of intestinal carriage of antibiotic-resistant GNB has been detected in our participant, with ESBL-producing *E. coli* being the most predominant

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organism. There is an urgent need for continuous surveillance of HCWs to detect antibiotic-resistant GNB and HCWs compliance with moment 1, before touching a patient, to reduce the likelihood of transmission of antibiotic-resistant GNB to vulnerable patients.

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