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Epidemiology and Clonal Spread Evidence of Carbapenem-Resistant Organisms in the Center of Care and Protection of Orphaned Children, Vietnam

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Abstract

Objective: To determine the prevalence of colonization and transmission of carbapenem-resistant Gram-negative organisms in order to develop of an effective infection prevention program. **Design**: Cross-sectional study with carbapenem-resistant organisms (CRO) colonization detection from the fecal specimens of 20 Health Care Workers (HCWs) and 67 residents and 175 random environment specimens from September 2022 to September 2023. Setting: A Care and Protection Centre of Orphaned Children in South of HCM City. **Participants:** It included 20 HCWs, 67 residents, and 175 randomly collected environmental specimens. **Method**: Rectal and environmental swabs were collected from 20 HCWs, 67 residents (most of them were children), and 175 environmental specimens. MELAB Chromogenic CARBA agar plates,

Card NID, and NMIC-500 CPO of the BD Phoenix TM Automated Microbiology System and whole genome sequencing (WGS) were the tests to screen, confirm CROs, respectively and determine CRO colonization and transmission between HCWs, residents, and the environment. **Result**: We detected 36 CRO isolates, including 6, 11 and 19 CROs found in 6 HCWs, 10 residents and 19 environments. The prevalence of detectable CRO was 30% (6/20) in HCWs, 14.92% (10/67) in residents, and 10.86% (19/175) in environmental swabs in our study. WGS demonstrated CRO colonization and transmission with the clonal spread of *E. coli* and *A. nosocomialis*, among HCWs and residents (children). **Conclusion**: Significant CRO colonization and transmission was evident in HCWs, residents, and the center environment. Cleaning and disinfection of the environment and performing regular hand hygiene are priorities to reduce the risk of CRO colonization and transmission.

Keywords

Carbapenem-Resistant Organisms, Contamination, Hand Hygiene, Whole Genome Sequencing, Infection Prevention

1. Introduction

Extended-spectrum β-lactamase (ESBL) producing Gram-negative pathogens, such as *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), often carry genes for ESBL production, reported firstly in 1983 and spread in the community since the late 1990s [1] [2]. A study in 2022 estimated a global pooled prevalence of ESBL *E. coli* intestinal carriage in the community at 16.5% [3].

Carbapenemase enzymes capable of hydrolyzing a wide range of β-lactam antibiotics confer significant antimicrobial resistance, such as class A KPC enzyme in *K. pneumoniae,* widespread in the United States and endemic in some parts of Europe, class B metallo-β-lactamases commonly present in *Pseudomonas aeruginosa and Enterobacteriaceae family* worldwide, class D OXA-type enzymes found in *A. baumanii* and *Enterobacteriaceae* family worldwide, particularly in Europe and North Africa. The antibiotic-resistant genes for carbapenemases appear on mobile genetic elements, which allow for the dissemination of genes between Gram-negative species [4].

The detection of hospital-acquired carbapenemase-producing *Enterobacteriaceae* preceded community-acquired isolates with *Klebsiella pneumoniae* harboring both carbapenemases (KPCs) and β-lactamases detected in a North Carolina hospital in the USA in 1996 [5].

Prevalence of infections caused by carbapenem resistant *Enterobacteriaceae* associated with community acquisition or onset was estimated between 7.7% - 29.5% globally and from 5.6% - 10.8% in the USA (2017), with higher prevalence in Asia (21.3%) [6].

Non-fermenting Gram-negative organisms, *A. baumanii* and *P. aeruginosae,* have multiple mechanisms for antimicrobial resistance: carbapenem resistance

through porin mutations, efflux, carbapenemase production, derepressed AmpC, or a combination of several mechanisms, and mobile genetic elements from other Gram-negative species [7]. The prevalence of carbapenem resistance in carbapenem-resistant organisms (CRO) is a clinical concern in immunocompromised patients with hospital-acquired pneumonia. Nosocomial spread and outbreaks associated with CROs, as well as transfer of the genetic resistant mechanisms of CROs to other Gram-negative bacteria [8] [9]. The CRO outbreaks in Intensive Care Units where patients have undergone procedures, such as mechanical ventilation, and treated with prior antibiotics. Outbreaks often present on contaminated items in the hospital environment [9].

The increasing rate of carbapenem-resistance Gram-negative bacteria (including CRE, such as carbapenem-resistant E. coli, K. pneumoniae, and. Ent. cloacae, and non-CRE, for example, *A. baumanii* and *P. aeruginosae*), is a healthcare threat globally with several mechanisms. In particular, the emergence of plasmid-mediated acquired carbapenemases is a top concern because these enzymes can hydrolyze penicillins, cephalosporins, monobactams, and carbapenems, which are the first-line and last-resort antibiotics, and antibiotic-resistant gene transfer is potential between bacterial species. These CROs increased their prevalence and carbapenemase production, and multiple reports showed nosocomial outbreaks with high rates of morbidity and mortality [10]-[12].

Vietnam has high rates of antibiotic-resistant Gram-negative bacteria; the COMPACT II study published in 2012 reported a prevalence of 35% of tested isolates resistant to carbapenems. The highest rate of resistance of *A. baumanii* was 89.5%, followed by *P. aeruginosa* (46.7%), and relatively lower rates in *Enterobacteriaceae* (5.6%) [13]. A study using next-generation sequencing to map resistance genes demonstrated high levels of multi-drug resistant *A. baumanii* isolates reported in Vietnam, and most of those isolates were susceptible to colistin [14].

Antimicrobial resistance is a major public health threat in many parts of the world, including Vietnam, an example of high rates of antibiotic-resistant Gram-negative bacteria in healthcare settings. Transmission of resistance mechanisms of community and nosocomial isolates can appear between organisms, and non-fermenters especially are implicated in nosocomial outbreaks. There is little literature regarding the implication of these organisms in pediatric long-term residential facilities [14] [15]. We performed this study to determine the prevalence and transmission of resistant Gram-negative organisms in the Center of Care and Protection of Orphaned Children in Southern Vietnam and to develop an effective infection prevention program.

2. Method

2.1 Definitions

CRE: Carbapenem-resistant Enterobacteriaceae are resistant to carbapenems, regardless of mechanism (includes ESBL, AmpC, porins, carbapenemase production);

CPE: Carbapenemase-producing Enterobacteriaceae have a genetic element that codes for carbapenemase production e.g. NDM, KPC;

CRO: Carbapenem-resistant organisms include all gram negative organisms and all resistance mechanisms;

CPO: Carbapenemase-producing organisms includes all gram negative organisms that produce a genetically coded carbapenemase [8].

2.2. Design

A cross-section study was implemented in South Vietnam from September 2022 to May 2023 at The Care and Protection of Orphaned Children Center, whose area is about 2000 m^2, including a building consisting of three floors in which the first and general floors are sections used as bedrooms and patient-care section. On the general floor, there is a section used for preparing food in the kitchen section. There is a small chicken and vegetable farming section to supply food for feeding patients, almost children, residing in this center because they are orphans.

The majority of children in this center have a disability related to cerebral palsy; many of the children have experienced complications of immobility and infection, to be admitted to local hospitals and exposed to broad-spectrum antibiotics. This center has been operating since 2000 and caring for children of the range of ages from 4 to 26 years old. In this study, we used the word resident for the children living at this center.

All 20 healthcare workers (HCWs) and 67 residents (including children) participated in the study. We observed and determined 303 environmental samples in the Center of Care and Protection of Orphaned Children. But we randomly chose some samples in the environment, based on the Yamane formula [16], was $n = 175$, $n = \frac{N}{1 + N \cdot e^2}$, Where n = sample size required ($n = 175$), N = the total number of environmental samples was 307 (N) observed, and determined in the center of care and protection of orphan children, e = 0.05, allowable error (%).

This study received an approval from the Board of Directors on Ethics in Biomedical Research at Thien Phuoc Nhan Ai Center of Care and Protection of Disabled Children, Vietnam, under the approval number 026/2565. The written informed consent was obtained from HCWs and the guardians of children.

Most of the children residing at the center have a physical and (or) mental disability. The residents' guardians participated in interviews to collect information. The HCWs at the Center are Catholic Sisters who provide nursing and personal care (wound care, bathing, feeding etc.) in 8 - 10 hour shifts and six days a week. All residents and staff of the center were included in this study (20 HCWs and 67 residents (most of them were children)).

For HCWs, all HCWs were female 100%, while the prevalence of female patients was 26.9 %. The range of HCW's age was from 20 to 66 years old (Max = 66, Min = 20). Almost all HCWs were over or equal to 27 years old, and one was 20, while the children's age was from 4 to 26 years old.

Most HCWs graduated high school ($n = 9$, 45%). Of 20 HCWs, two graduated

(10%) from the Nursing College and were registered nurses, one (5%) was a pharmacist who graduated from Pharmacy College graduation, twelve children carers, one cleaner, two Food providers, one guard, one manager of the Center, one pharmacist, and two registered nurses.

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For residents ($N = 67$), 10% are children with normal health, while 90% have mental and physical disabilities (most of them are children with cerebral palsy and other disorders (epilepsy, hydrocephalus, hyperactivity, mental retardation, osteogenesis imperfecta).

The proportion of HCWs with a duration of years for professional work of HCWs under five years was 50%, and 50% of HCWs have worked for more than five years, while more than 67% of residents have resided for over five years in the Center of Care and protection Of Orphan Children. This result implied that HCWs and orphan patients have lived, worked, and had many opportunities to contact for a long time.

There was 100% HCWs have worked seven days per week and 90% worked over 8 hours per day. This remarkable finding showed that HCWs had much time to contact patients in performing healthcare practice to patients in this healthcare setting. Thus, CRO transmission happened as an evitable event while HCWs cared for and treated patients at this center (as shown in part 3, 5 and 6 of Table 1).

Table 1. Demography characteristics of HCWs and residents.

					<= 20	20 - 30	> 30 - 40	> 40	Min/Max	Notes		
1) Female	H (20)	(n, %)	20 (100)	2) Age (years)	1 (5.00)	4 (20)	5 (25)	10 (50)	20.00/66.00	≥27 yrs old is cut-point		
	Rsd (67)	(n, %)	18 (26.9)		62 (92.5)	5 (7.5)			4.00/26.00			
			Cc	Cln	FP	Gd	Mgr of Center	Pharm	RN	N occu		
3) Occu	H (20)	(n, %)	12 (60)	1 (5)	2 (10)	1 (5)	1 (5)	1 (5)	2 (10)	0 (0)	4) 7 ds/p W	20 (100)
	Rsd (67)	(n, %)	0	0	0	0	0	0	0	67 (100)		4 (6)
			<5 yrs	>5 - 10	> 10 - 20		0 (hrs)	0.5	4	7	8	10
5) Drt of PW/Rsd	H (20)	(n, %)	10 (50)	3 (15)	7 (35)	6) N of hrs for PW per D				2 (10)	10 (50)	8 (40)
	Rsd (67)	(n, %)	22 (32.84)	37 (55.22)	8 (11.94)		63 (94.0)	3 (4.5)	1 (1.5)			

CC = Children carer, **Cl*n*** = Cleaner, **FD** = Food provider, **Gd** = Guard, **Mgr of Ct** = Manager of Center, **Pharm** = Pharmacist, **RN** = Registered nurse, **No OCCU** = No occupation; **Drt of PW (HCW)/Rsd (Yrs)** = Duration of Professional work (HCW)/residents (years), **7 ds/p W** = 7 days per week, **PW** = Professipnal work, **N of hrs for PW per D** = Number of hours for professional work.

Exclusion criteria were defined as non-consent, known CRO/CPO colonization or infection in 6 months prior to the study or active infection at the time of the study. However, none of the residents or HCW fulfilled any exclusion criteria hence all were included.

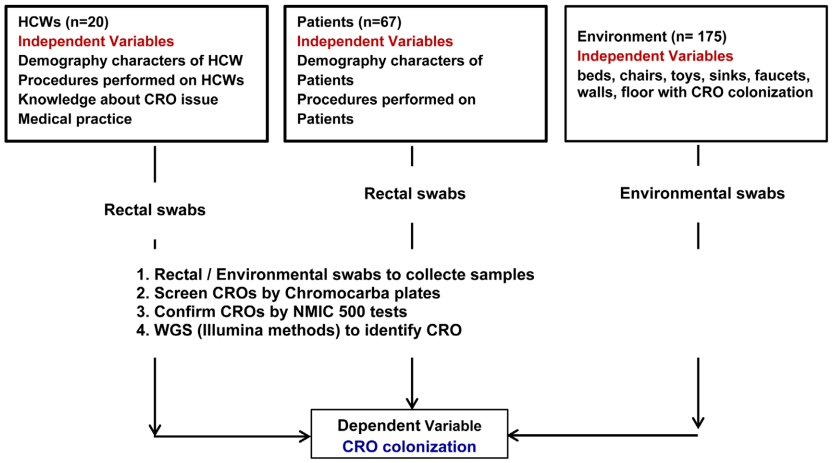
Residents and HCW were allocated a participant code, and isolates were allocated a laboratory code for tracking.

2.3. Sample Collection

The samples from residents and HCW were collected on a sterile rectal cotton sampling swab inserted approximately 1 cm into the anal canal and rotated for 10 seconds. The swab was then put into individual vials of Amies transport medium and transported on dry ice to a reference hospital within 6 hours of collection.

The environmental samples included surfaces and furnishings in the center using a sterile cotton swab. An area of approximately 10 cm × 10 cm was sampled on environmental surfaces. The swabs were placed in Amies transport medium and processed in the same way as for the rectal swabs.

We showed the relationship between variables, the characteristics in our study in the Conceptual Framework as shown in Figure 1.



Demography characters of HCWs/residents: Age (years), previous antibiotic treatment, chronic disease, previous hospital stay, past surgical intervention, duration of treatment, Duration of professional work (based on number of years and hours per day); **Procedures performed on HCWs/residents:** Peripheral IV catheter insertion, urinary catheter insertion; **Knowledge/Attitude of HCWs** in prevention and control of CRO (questionnaires used to evaluate Knowledge/Attitude of HCWs before; **Medical practice in prevention and control of CRO**.

Figure 1. Conceptual framework and process of screen, confirm and identify CROs.

2.4. Microbiology Methods

The sample swabs were plated onto MELAB Chromogenic CARBA agar plates and incubated at 37o Celsius. Purity cultures of suspect colonies were performed on blood agar and followed species identification by Card NID, antimicrobial susceptibility testing, and phenotypic carbapenemase detection using the NMIC-500

CPO Detect panel of the BD Phoenix TM Automated Microbiology System.

The reference strains in the NMIC 500 test to confirm susceptibilities are E. coli ATCC® 25922, K. pneumoniae ATCC BAA-1705™, and P. aeruginosa ATCC™ 27853.

2.5. Whole Genome Sequencing (WGS)

We discovered thirty-six CRO isolates in HCWs, residents, and the environment. However, we chose twelve CROs detected from HCWs and residents who had close contact with HCWs: HCWs feed food or bath residents, etc. CROs belonged to the same species with a high ratio of identical antibiotic-resistant phenotypes between CRO isolates and the number of CROs chosen to perform WGS available to our study financial resources. So, we selected twelve CRO isolates from HCWs and residents (as shown in Table 2), to determine whether the potential CRO transmission/contamination occurred between HCWs and residents in these chosen CROs. Extraction and concentration of DNA was performed in Vietnam (as shown in Table 2) and the samples sent to Charles River Laboratories in Australia for sequencing.

Table 2. Antibiogram of 12 CROs suspected with close relations based on the identifical rate of antibiotic-resistant phylogetype/BD.

(a)						
Sample	1	2	3	4	5	6
Study code	**H006**	**C4003**	**C4003**	**H007**	**H019**	**H008**
WGS Code	**6276065**	**6276066**	**6276063**	**6276074**	**6276064**	**6276068**
Isolates	***E. coli* Class D**	***E. coli* Class D**	***K. pneumoniae***	***E. coli***	***E. coli* class B**	***Ent. cloacea***
ETP	**R**	**R**	**R**	**R**	**S**	**R**
IP	**S**	**I**	**R**	**I**	**I**	**R**
MP	**S**	**S**	**R**	**S**	**S**	**R**
AK	**S**	**S**	**S**	**S**	**S**	**S**
AM	**R**	**R**	**R**	**R**	**R**	**R**
AMS	**R**	**R**	**R**	**R**	**R**	**R**
AZM	**R**	**R**	**S**	**R**	**S**	**R**
CZ	**R**	**R**	**R**	**R**	**X**	**R**
FEP	**S**	**S**	**R**	**R**	**S**	**R**
FOX	**R**	**R**	**R**	**S**	**S**	**N**
CAZ	**R**	**R**	**S**	**R**	**S**	**R**
CZA	**S**	**S**	**S**	**S**	**S**	**R**
CRO	**R**	**R**	**R**	**R**	**S**	**R**

Continued

CXM	R	R	R	R	S	R
CIP	R	I	R	R	R	R
CST	X	X	X	X	X	X
FO	S	S	N	S	S	N
GEN	R	S	S	R	S	R
LVX	R	X	R	R	R	X
MIN	S	S	R	S	S	S
NFN	S	S	R	S	S	R
NOR	R	S	R	R	S	S
TZP	R	R	R	R	I	R
TGC	S	S	I	S	S	S

(b)

Sample	7	8	9	10	11	12
Study code	C3011	C3022	H009	C2020	C2012	C3002
WGS Code	6276071	6276070	6276073	6276072	6276069	6276067
Isolates	*A. baumannii*	*A. baumannii*	*A. baumannii*	*A. baumannii*	*A. baumannii*	*A. baumannii*
ETP	R	R	R	R	R	R
IP	S	S	S	S	S	S
MP	S	S	S	S	S	S
AK	S	S	S	S	S	S
AM	R	R	R	R	R	R
AMS	S	S	S	S	S	S
AZM	R	R	R	R	R	R
CZ	R	R	R	R	R	R
FEP	N	N	S	I	S	S
FOX	R	R	R	R	R	R
CAZ	S	S	R	S	S	S
CZA	N	N	N	N	N	N
CRO	X	X	X	X	X	X
CXM	R	R	R	R	R	R
CIP	S	S	S	S	S	S
CST	X	X	X	X	X	X

Continued

FO	R	R	R	R	R	R
GEN	S	S	S	S	S	S
LVX	N	N	N	N	N	S
MIN	S	S	S	S	S	S
NFN	N	N	N	N	N	R
NOR	N	N	N	N	N	N
TZP	S	S	S	S	S	S
TGC	N	N	N	N	N	N

"I" means intermediate and **"R"** means resistance, **"X"** means the MICs of antibiotics are in the range of sensitive or intermediate, **"N"** is an antibiotic not recommended for the treatment of infections.

2.6. At Charles River Laboratories in Australia

The genomic DNA was quantified with Qubit High-sensitivity assays using a Qubit 4.0 Fluorometer (Thermo Fisher Scientific, USA). The samples were purified using AMPure XP (Beckman Coulter, USA) with a 1X volume ratio to elute samples with low EDTA Tris-HCl buffer. Samples were normalized to 50-200ng and processed using the Illumina DNA Library Prep kit (Illumina, USA) according to the manufacturer's protocols using 5 PCR cycles for indexing. The performance of Quantification and size estimation of the libraries were on both the Qubit 4.0 Fluorometer (Thermo Fisher Scientific, USA) and the 4200 Tape Station System (Agilent, USA). The samples were normalized to 4nM and then pooled into a new microfuge tube. The pooled library was diluted to 1nM and sequenced on the MiniSeq Sequencer (2 × 150 bp paired-end reads) (Illumina, USA) using MiniSeq High Output 300 Cycle flowcell.

3. Results

In total there were 36 CROs identified from 262 samples (including rectal swabs from 20 HCWs and 67 residents, and 175 environmental samples), and 6 ESBL producing *E. coli* from residents and HCWs. From the HCWs, 4 were colonised with a CRE (20%) and 2 with a CRO (10%). Additionally there were 3 HCWs carrying an ESBL producing organism (15%) giving a total of 45% of HCW colonized with a resistant gram negative organism. In the residents 14.93% were colonized with a CRO, with the majority being *A. baumanii* (10.44%). One resident (child) had 2 distinct CRE (*E. coli* and *K. pneumoniae*). There were an additional 3 residents with carriage of ESBL producing *E. coli* giving a total of 19.4% residents carrying a resistant Gram-negative organism. From the environmental samples there were 19 CRO isolated, with *A. baumanii* being the most commonly identified organism (as shown in Table 3, Table 4). In this study, we did not collect raw food samples to investigate CRO on or in these samples.

Table 3. Prevalence of resistant Gram-negative organisms.

HCWs (*N* = 20)		Residents (N= 67)			Environment (N= 175)	
CRO & CRE	**n, %**	**CRO & CRE**	**n, %**		**CRO**	**n, %**
*Ent. cloacae*_Class B	1 (5.00)	*E. coli*_Class D	1 (1.49)	†*K. pneumonia* Class D	*A. baumannii*	8 (4.57)
E. coli	1 (5.00)	*A. baumannii*	7 (10.45)		*A. faecalis*	4 (2.29)
*E. coli*_Class B	1 (5.00)	*B. cepacia* complex	1 (1.49)		*P. aeruginosa*	1 (0.57)
*E. coli*_Class D	1 (5.00)	*P. aeruginosa*	1 (1.49)		*P. putida*	4 (2.29)
A. baumannii	2 (10.00)				*S. maltophilia*	2 (1.14)
Total	**6 (30.00)**	**Total**	**10 (14.93)**		**Total**	**19 (10.86)**
ESBL		**ESBL**				
E. coli	3 (15.00)	E. coli	3 (4.50)			
Total CRO + ESBL	**9 (45.00)**	**Total CRO + ESBL**	**13 (19.40)**			

†: 2 CRE isolates from one child.

Table 4. Distribution and detection of CROs in environmental samples.

Environmental Samples	Total of environmental samples (1)	N of samples randomly collected (2)	N of samples of CRO detected (3)	identified CRO (4)	% identified CRO (3)/(2)
Faucet handle for hand washing	23	13	1	*A. baumannii*	15.4 (2/13)
Faucet handle for cooking and cleaning vegetable in kitchen			1	*P. aeruginosa*	
Electric train game for children	14	8	1	*P. putida*	12.5 (1/8)
Washing machine	4	2	1	*A. baumannii*	50 (1/2)
Children chair 1 for lunch and dinner in kitchen	42	24	1	*P. putida*	8.3 (2/24)
Children chair 2 for lunch and dinner in kitchen			1	*S. maltophilia*	
Soil sample collected inside chicken small farm	2	1	1	*P. putida*	100 (1/1)
Contact area (palm) between hand of HCW and hand of children	12	7	1	***A. baumannii***	28.6 (2/7)
Contact area in palm of HCW			1	***A. baumannii***	

Continued

Sample collected from outpart of feeding tube and inner wall of piston used to pump food to feed a disabled child	2	1	1	*A. faecalis*	100 (1/1)
Toilet bowl in room 2	14	8	1	*A. faecalis*	25 (2/8)
Toilet bowl in room 3			1	*A. faecalis*	
Toothbrush shelf for children in room 1	7	4	1	*S. maltophilia*	25 (1/4)
children wood bed in room 4	61	35	1	*A. baumannii*	8.6 (3/35)
children plastic bed 1 in room 4			1	*A. baumannii*	
children plastic bed 2 in room 4			1	*A. baumannii*	
Tables for lunch and dinner	21	12	1	*A. baumannii*	25 (3/12)
			1	*A. faecalis*	
			1	*P. putida*	
Wall of all rooms	28	16	0	Not detected	0 (0/16)
Floor	9	5	0	Not detected	0 (0/5)
Door handle of rooms and toilets	14	8	0	Not detected	0 (0/8)
Pillow	54	31	0	Not detected	0 (0/31)
	307	**175**	**19**		

N = number.

*A. **baumannii*** was the most common CRO organism isolated in the environment and was found around a faucet, washing machine, the palm of HCW, and residents. A. faecalis isolates were identified in 2 of the toilet bowls, in an infant feeding tube, and on one of the dining tables. A resident (child) receiving feeds from the apparatus colonized with A. faecalis did not have this organism found on rectal screening. However, we did isolate a carbapenem-resistant E. coli from the rectal swab. P. putida isolates were found on a toy train, a dining chair, soil from the chicken coop, and a dining table. CROs were not present in any of the samples of the walls or floor ($n = 21$) (as shown in Table 4).

Whole Genome Sequencing

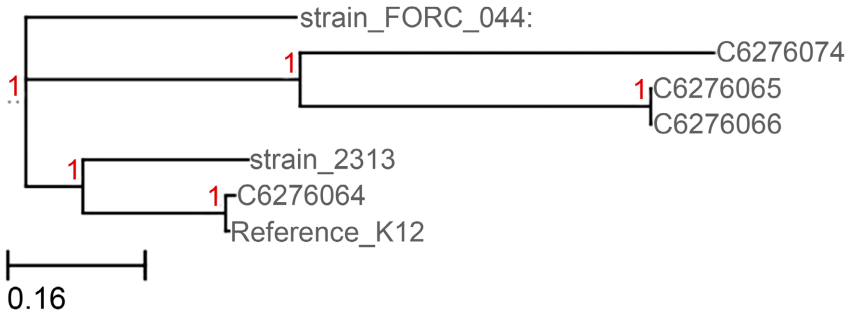
To determine CRO transmission, WGS was performed on 12 isolates from residents and HCWs who had close contact (feeding/bathing/care), comparing

isolates of the same species with similar antibiotic-resistant phenotypes (≥ 80%) profiles. In detail, we used 25 antibiotics to determine the susceptibility or resistance of CROs in our study (Table 2). So, the ratio of identical antibiotic-resistant phenotypes

- between C4003 and H006 was 80% (20/25),
- between C3022 and H009 was 84% (21/25),
- between C3011 and H009 was 92% (23/25),
- between C3022 and C3011 was 92% (23/25).

Moreover, we added two random outgroups of Escherichia coli (FORC_044 and strain_2313) and used K12 as the reference genome for SNP calling. Most strains (6276064, 6276065, 6276066, 6276074, FORC_044, strain_2313) have from 40,000 to 80,000 different SNPs pairwise between any two genomes. Therefore, they are unrelated isolates. Two samples (6276065 (H006) and 6276066 (C4003)) had only 16 different SNPs and are closely strongly related, and the number of genomes of *E. coli* is about 4,608,319 genomes. Hence, the SNP difference between 6276065 and 6276066 was 0.0003% (16/4,608,319). So, the genomic identity between 6276065 and 6276066 was 99.9997% (100% - 0.00034%). This result is evidence of a cross-contamination event between H006 and C4003.

Based on the Phylogram of *E. coli* isolates of Figure 2, the 6276065 (H006) and 6276066 (C4003) isolates were from an identical clone, as described on Figure 2. This is clear evidence for *E. coli* cross-contamination/transmission between a child and a healthcare worker. There was a cross-contamination event between H006 and C4003.



Phylogram in Newick format (**strain_FORC_044:** 0.316839165, (**C6276074:** 0.485110495, (**C6276065:** 0.000092635, **C6276066:** 0.000000005) 1.000: 0.407502424) 1.000: 0.318098382, (**strain_2313:** 0.193857825, (**C6276064:** 0.010811940, **Reference_K12:** 0.004323809) 1.000: 0.163951729) 1.000: 0.063587173).

Figure 2. Phylogram of *E. coli* isolates showing 6276065 and 6276066 are the identical strain.

To determine if *E. coli* transmission between the 6276065 (H006) and 6276066 (C4003) were linked epidemiologically we investigated the characteristics of Demography/ antibiotic use/ procedures related to and performed on the H006 (an HCW) and a patient (C4003) (as shown in part (a) (H006 (an HCW) and a resident (C4003)) in Table 5).

1) Characteristics of Demography/antibiotic use/procedures related to and

performed on H006 (HCW) and C4003 (resident, child):

The H006 did not have a history of antibiotic use of around one year, hospital admission out of the center, surgical operation, or peripheral or urethral catheter use before participating in our study. In contrast, C4003, 5-year-old female child, was admitted to a pediatric hospital to treat pneumonia, and the type of antibiotics used to treat pneumonia was Augmentin 1000 mg two times around 60 days before participating in our study (as shown in Table 5).

2) There was antibiotic resistance loss of gentamycin and norfloxacin in the child sample (as shown in Table 2)

- No known antibiotic genes were deleted or inserted between the two samples.
- Table 6 shows gene gain in the child sample, though not in the canonical antibiotic resistance genes: These genes are identified using the Patric annotation system, based on the CARD/NCBI databases, and virulence genes based on the VFDB database.

Table 5. Characteristics of Demography/antibiotic use/procedures related to and performed on HCWs and patients.

(a)						
Study Code	**H006**	**C4003**	**H007**	**H019**	**H008**	**C3011**
WGS code	**6276065**	**6276066**	**6276074**	**6276064**	**6276068**	**6276071**
Sex	Female	Female	Female	Female	Female	Male
Location of work /residence	F1	F1	F1	F1 & G	F1	F1
Age	35	**5**	66	50	27	**10**
Professional	Children carer	no job	Children carer	Manager	Children carer	no job
How long to work or reside in the Center (Years)	1	4	17	19	4	**0.25**
Number of days for working per week	7	0	7	7	7	0
Number of hours for working per day	8	0	8	10	8	0
Antibiotic use	**No**	**Yes**	**Yes**	**Yes**	**Yes**	No
Reason	**No**	**Pneumoniae**	Bacterial tonsillitis	Knee ligament tear	Bacterial tonsillitis	No
Number of times of antibiotic use for 1-year BEF study	**0**	2	4	1	2	0
The last time of antibiotic use. How long ago was it? (days)	**0**	60	30	365	14	No

Continued

Types of used antibiotics	Not use	AMC (1000 mg) × 2	Not remember	Not remember	AMC (1000 mg) × 2	Not use
Number of antibiotic-use days in last time	0	5	4	4	7	0
Past hospital admission out of Center for 6 months BEF study	No	No	No	Yes	No	No
Past hospital admission in of Center for 6 months BEF study	No	Yes	No	No	No	No
Number of days for Past hospital stay out of Center	0	5	0	4	0	0
Chronic diseases	0	Cebreral palsy	0	0	0	Hyperactivity
Past Surgical history	No	No	No	No	Yes	No
Past surgical history, How long ago was it? (years)	No	No		1	5	No
Past perperal catheter use	No	No	No	No	yes	No
Past urithral catheter use	No	No	No	No	Yes	No
Number of hospital stay (days) about 1 year	0	5	0	4	0	0
CRO_1	*E. coli* Class D	*E. coli* Class D	*E. coli*	*E. coli* class B	*Ent. cloacea*	*A. baumannii*
CRO_2		K. pneumoniae				

(b)

Study Code	C3022	H009	C2020	C2012	C3002
WGS code	6276070	6276073	6276072	6276069	6276067
Sex	Female	Female	Male	Male	Male
Location of work/residence	F1	F1	F1	F1	G
Age	8	56	14	16	12
Professional	no job	Children carer	no job	no job	no job
How long to work or reside in the Center (Years)	5	17	5	6	10
Number of days for working per week	0	7	0	0	0

Continued

Number of hours for working per day	0	8	0	0	0
Antibiotic use	No	No	No	**Yes**	No
Reason	No	No	No	Sacral decubitus ulcer	No
Number of times of antibiotic use for 1-year BEF study	0	0	0		No
The last time of antibiotic use. How long ago was it? (days)	No	No		30	No
Types of used antibiotics	Not use	Not use	Not use	AMC (625 mg)	Not use
Number of antibiotic-use days in last time	0	0	0	10	0
Past hospital admission out of Center for 6 months BEF study	No	No	No	No	No
Past hospital admission in of Center for 6 months BEF study	No	No	No	Yes	No
Number of days for Past hospital stay out of Center	0	0	0	0	0
Chronic diseases	Cebreral palsy	Elevated Liver Enzymes	Cebreral palsy	Cebreral palsy	intellectual and motor disability
Past Surgical history	No	No	No	No	No
Past surgical history, How long ago was it? (years)	No	No	No	No	No
Past perperal catheter use	No	No	No	No	No
Past urithral catheter use	No	No	No	No	No
Number of hospital stay (days) about 1 year	0	0	0	10	0
CRO _1	***A. baumannii***	***A. baumannii***	***A. baumannii***	***A. baumannii***	***A. baumannii***
CRO_2					

BEF = Before.

Table 6. Antibiotic and virulence gene count by Patric between the 2 closely related E. coli isolates.

	Source of annotation	H 006	C4 003
	Victors	3	3
Antibiotic Resistance	CARD	94	94
Antibiotic Resistance	NDARO	17	17
Antibiotic Resistance	PATRIC	75	75
Drug Target	DrugBank	**392**	**394**
Drug Target	TTD	59	59
Transporter	TCDB	864	864
Virulence Factor	PATRIC_VF	**203**	**207**
Virulence Factor	VFDB	98	98
Virulence Factor	Victors	**232**	**236**
		6,276,065	**6,276,066**

This data comes from annotation of the genomes using Patric (https://www.bv-brc.org/).

3) The child sample has gained four virulence genes that lost antibiotic resistance (as shown in Table 7).

Table 7. SNP mutations differing between 6276065 and 6276066 in or near coding regions.

Contig	U00096	U00096	U00096	U00096
Position	3768881	857877	1062446	1440170
Mutation type	Complex	Complex	Complex	Complex
Mutation event	TACCAT=>CGCCAA	GTCCTCG=>ATCTTCC	TAT=>CAG	GTTGAGTGCC=>ATTCAGCGCG
Mutation effect	Noncoding/unknown	Synonymous	Noncoding/ unknown	Synonymous
Gene		ybiU DUF1479 domain-containing protein		ydbK putative pyruvate-flavodoxin oxidoreductase

4) The mutation effect of ydbK changed several amino acids and might cause a change in antibiotic resistance.

From these points of view determined above, the gain of antibiotic resistance made a lot more sense:

We can list some significant notes, including

a) There were very few mutations, four mutations, between the C3004 isolate and HCW (H006) isolate. It suggested that a short evolution time existed between two isolates.

b) Strong selective pressure (for example, antibiotic therapy) for this functional

mutation happened shortly. It suggested the HCW (H006) isolate gained antibiotic resistance from an ancestor, which was antibiotic-sensitive.

c) Child sample has maintained the ancestral sensitivity. HCW (H006) and (C4003) might have been contaminated from a common source (Child-infected HCW) or conversely. It meant that if the child had the ancestral strain (which was also most similar to the public reference genome), It suggested the infection from the Child (C4003) to HCW (H006) OR a common infection source.

Continuously, we compared the genomics and applied the MALDI-based typing of the 12 CRO samples. Specifically, we focused on the CRO samples, as shown in Table 2. We suspected that some infections may be transmission/contamination events within an orphanage due to antibiotic profiling of these isolates demonstrated high resistance (Table 2).

To track the CRO transmission/contamination in six A. baumanii isolates, with WGS code as shown in Table 7 underwent with WGS. We applied the Snippy 4.6.0 software to count SNPs (single nucleotide polymorphism) different between the suspected isolates and the Orthoani software tool for ANI (average nucleotide identity) comparison to determine whether two or more isolates are the same or differentiated species. We also used the Acinetobacter nosocomialis M2 reference genome (GCF_005281455.1_ASM528145v1) to compare these isolates. The number of genomes of Acinetobacter nosocomialis is 3,940,614 genomes.

So, the rate of genome different (as shown in Table 8).

- between A. seifertii and 6276067_isolate was 1.07% (42,246/3,940,614),
- between A. baumannii and 6276069_isolate was 0.72% (28,554/3,940,614),
- between 6276071and 6276070_isolate was 0.0683% (2693/3,940,614),
- between 6276073 and 6276070_isolate was 0.0240% (941/3,940,614),
- between 6276073 and 6276071_isolate was 0.0450% (1775/3,940,614),

It means that the rate of genome identity

- between A. seifertii and 6276067_isolate was 98.93% (100% - 1.07%),
- between A. baumannii and 6276069_isolate was 99.28% (100% - 0.72%),
- between 6276071 and 6276070_isolate was 99.932% (100% - 0.0683%),
- between 6276073 and 6276070_isolate was 99.976% (100% - 0.0240%),
- between 6276073 and 6276071_isolate was 99.955% (100% - 0.0450%).

Lee's Study showed that when the ratio of ANI (average nucleotide identity) of two or more strains with the same species is over > 95%, two or more species belong to a unique clone [17]. Based on research results of Lee about the rate of ANI, our study results determined that the 6276067_ isolate was *A. seifertii*, the 6276069_isolate was *A. baumannii*, **the 6276071, 6276070, and 6276073_isolate belonged to an identical clone.**

To improve the taxonomic accuracy of the Acinetobacter sp. samples typing of three isolates, including the 6276071, 6276070, and 6276073_isolate, we calculated the ratio of average nucleotide identity (ANI) of these three isolates by using Ortho ANI.

The ratios of ANI between 6276070, 6276071, 6276073_isolate and *A. nosocomial* were 97.6676, 97.4891, and 97.8075% (Table 9). All these ratios were over 95%

[17], while the ratios of ANI between 6276070, 6276071, 6276073_isolate, and *A. baumanii* (or *A. seifertii*) were below 95%. So, the 6276070, 6276071, and 6276073_isolate were *A. nosocomialis* and belonged to an identical clone. This data clearly shows that **6276070 (C3022), 6276071 (C3011) and 6276073 (H009)** are very closely related and **link health care worker H009 to residents (children) C3022 and C3011 by a contamination event**.

Table 8. Number of SNPs different between Acinetobacter sp. Isolates (from 6276067 to 6276073).

	6276067	**6276069**	**6276070**	**6276071**	**6276072**	**6276073**	***A. baumannii***
6276067	0						137,315
6276069	137,683	0					**28,554**
6276070	107,480	110,830	0				
6276071	107,410	110,794	**2693**	0			
6276072	137,890	**27,818**	110,599	110,597	0		
6276073	137,315	110,717	**941**	**1775**	110,506	0	
A. seifertii	**42,246**	137,683	107,480	107,410	137,890	107,178	133,408

Table 9. ANI matrix of isolates and 2 public reference genomes.

***Ortho ANI values calculated from the OAT software* [17]**	**6276070_asm**	**6276071_asm**	**6276073_asm**	***A. nosocomialis***	*A. seifertii*	*A. baumanii*
6276070_asm		**97.6561**	**98.8262**	**97.6676**	91.9344	91.5521
6276071_asm			**99.347**	**97.4891**	91.8987	91.5963
6276073_asm				**97.8075**	91.9274	91.4095
A. nosocomialis					92.2549	91.363
A. seifertii						89.7719

The results of phylogram of *A. nosocomialis* isolates, including 6276070 (C3 022), 6276071 (C3 011) and 6276073 (H009) showed all 3 isolates belonge to an identical clone (as described in Figure 3).

Phylogram in Newick format (**C6276071:** 0.050761002, **GCF_005281455_reference** 5.008689352, (**C6276070:** 0.013087841, **C6276073:** 0.000000005) 0.436: 0.000000005).

Figure 3. Phylogram of *A. nosocomialis* isolates showing 6276070, 6276071, 6276073 are the identical strain.

Based on part (a): 6276070 and part (b): 6276071, and 6276073 in **Table 5**, three isolates, including 6276070 (C3 022), 6276071 (C3 011), and 6276073 (H009), did not have risk factors: antibiotic use, hospital stay in and out of Center of Care and Protection of Orphan Children, the past surgical operation, peripheral and urethral catheter use. These three isolates were closely associated with a contamination event described above. It suggests that the origin of transmission may come from the environment.

We detected some carbapenem-resistant genes in 12 CRO samples, including the OXA-48 family, TEM family, CTX-M family, NDM family, OXA-1 family, OXA-51 family, ADC family (*Acinetobacter* spp), and other antimicrobial-resistant genes, and show in detail the antimicrobial-resistant genes in another paper.

4. Discussion

Our study showed significant carriage of resistant Gram-negative organisms in residents (19.4%) and HCWs (45%), with the majority having carbapenem resistance. Resistant organisms were most frequently Enterobacteriaceae in HCWs, but in residents, there were significantly more non-fermenter organisms that would be associated with environmental contamination. This result was supported by detecting non-fermenter organisms in 10.86% of environmental samples, with the Acinetobacter calcoaceticus-baumannii complex being the most prevalent organism in children and environmental samples.

Some studies detected the carriage rate of extended-spectrum β-lactamase–producing Gram-negative bacteria (ESBLs). For example, in 50 HCWs in the ICU, the ESBLs carriage rate in rectal swabs was 21.43%, in which K. pneumoniae and E. coli were the ESBLs-producing isolates detected in this study, with the rate of 33.3%, and 18.75%, respectively [18], at the prolonged care facilities [19] [20], the ESBLs carriage rate in rectal swabs significantly changed from 3.5% to 21.4 %. One study performed at five rehabilitation centers in four European countries showed the carriage rate of ESBL in HCWs was 3.5% of the 1001 HCWs screened [20].

In another prospective, longitudinal, observational Swiss study at four veterinary institutions (2018), researchers detected only 2 in 108 (1.9%) HCWs were colonized with hyperepidemic clones of Carbapememase-producing *E. coli* (CP-Ec), including ST410-producing OXA-181 and ST167 producing NDM-5. Nevertheless, these CP isolates were molecularly identical to CP detected in some dogs and cats cared for and treated at the same institutions [21].

A study at the US National Institutes of Health Clinical Center from November 2013 to February 2015 showed that healthcare personnel (HCP) or microbiology laboratory staff had a history of regularly close patient contact, they would have a prevalence of ESBL colonization of 4% (15/379), higher than staff without frequent contact history with patients or bacterial specimens (2.9%, 11/376). However, the difference between the prevalence of ESBL in HCWs and the control group was not statistically significant, with P = 0.55. There were no HCPs

colonized with CROs. This result suggested that ESBL can reside on HCWs through close contact between HCWs and patients [22].

However, these studies did not find carbapenem-resistant organisms in HCWs.

In a 2021 study investigating the carriage of resistant Gram-negative organisms in HCWs in a Vietnamese Intensive Care Unit, 26 of the 40 (65%) were found to carry an ESBL or Amp C β-lactamase producing E. coli, two were colonized with an ESBL or Amp C β-lactamase producing *K. pneumoniae* and one had a carbapenem-resistant *A. baumanii* [23].

A point prevalence screening study in 3 Vietnamese ICUs reported CRE point prevalence of 79.4% in the Neonatal Intensive Care Unit (NICU), 84.2% in the Paediatric Intensive Care Unit (PICU) and 60.7% in the Surgical Intensive Care Unit (SICU) with *K. pneumoniae* and *E. coli* being the most commonly detected organisms; a significant number of these were demonstrated to have been acquired in the ICUs [24].

The difference in prevalence and organisms reflects the difference in risk factors with ICUs being more metropolitan, and having sicker patients with higher exposure to antibiotics. However, one important thing is all studies described above did not find CRO transmission between HCWs and children.

Our study results suggest that if the isolates are the same species and the ratios of identical antibiotic-resistant phenotypes of these isolates are ≥ 80%, the potential for these isolates to belong to an identical clone is very high. However, we only performed WGS on 12 CRO isolates. Hence, it is necessary to perform a study with a larger sample to validate our findings.

In this study, we detected Carbapenem-resistant *E. coli* (CR *E. coli*) transmission events between *E. coli* of C4003 and *E. coli* of H006 samples, and another event, three *A. nosocomialis* contamination between an HCW (H009) and two residents (children) (C3022 and C3011). It highly suggests *E. coli* transmission and *A. nosocomialis* contamination between HCWs and residents in this center. This finding is the first detection of carbapenem-resistant *E. coli* and *A. nosocomialis* transmission/contamination at a center of care and protection of orphan children between two HCWs and three residents (children) in Vietnam. The transmission source may have come from E. coli of a resident (a child) (C4003) or the source of *A. nosocomialis* contamination from the environment. CRO contamination/transmission in HCWs, residents, and the environment is an urgent issue for the Center for Care and Protection of Orphaned Children. So, cleaning the environment, disinfecting the devices/equipment used to care for and treat residents, and encouraging HCWs and residents to comply with hand hygiene to prevent CRO contamination/infection are essential priorities to decrease CRO contamination at this center. Next, targeted antibiotic use appropriate to treat infectious diseases is an important measure to prevent bacterial antibiotic resistance.

The advantages of WGS are to facilitate outbreak investigation, detect emerging strains, predict their clinical importance [25], and be a valuable tool to predict phenotypic resistance of the clinically prescribed antibiotics to treat bacterial

infections [26].

5. Conclusion

This study demonstrated the high prevalence of organisms in a remote Center of Care and Protection of Orphan Children, where vulnerable children reside, and the transmission/contamination of antibiotic-resistant organisms, such as carbapenem-resistant organisms, between HCWs providing personal care and the residents (children). Cleaning the environment, disinfecting the devices/equipment used to care for and treat residents, and encouraging HCWs and residents to comply with hand hygiene are essential to decrease CRO contamination at this center. This study also demonstrated the utility of WGS in determining clonality and contamination/transmission, which can be a tool in Infection prevention programs in this setting.

6. Study Limitations

The limitations of this study were the single site, a Center of Care and Protection of Orphan Children in a remote region, with a small population of 87 participants in our research, and we performed the WGS for only 12 of 36 CRO isolates in our study. Thus, sequencing for all 36 CROs isolated from the environment, HCWs, and residents may have shown the emerging transmission patterns and the additional targets for Infection Prevention.

Although our study results had the limitation of detecting the patterns of CRO transmission, it also showed CRO transmission was a significant issue necessary for detecting early and controlling effectively in healthcare settings.

Ethics Statement

The study protocol must be evaluated and approved by the Board of Directors of Ethics Biomedical research at Thien Phuoc Nhan Ai Center of Care and protection of Disabled Children, N˚ 3/CSTPNA-QĐ.

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Author Contributions

The concept, design, data acquisition, analyses and interpretation of study findings were conducted by Nguyen Van Kim. All coauthors contributed towards the content, review and ultimate write-up of the manuscript. Larry Croft did the bioinformatic analysis. Yin Peng Lee extracted DNA, made libraries and sequenced the samples. Tara Cassidy managed the WGS component of the project. Rebecca Johnson, and Le Ha Tam Duong for providing administrative support.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of the manuscript.

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Abbreviations and Acronyms

Abbreviations of Antibiotics

AK	Amikacin
AM	Ampicillin
AMC	Amoxicillin-clavulanic acid
AZM	Aztreonam
CZ	Cefazolin
FEP	Cefepime
FOX	Cefoxitin
CAZ	Ceftazidime
CZA	Ceftazidime/avibactam
CRO	Ceftriaxone
CXM	Cefuroxime
CIP	Ciprofloxacin
CST	Colistin
FO	fosfomycin
GEN	Gentamicin
LVX	Levofloxacin
MIN	Minocycline
NFN	Nitrofurantoin
NOR	Norfloxacin
TZP	Piperacillin/tazobactam
TGC	Tigecylin
SXT	Trimethoprim-sulphamethoxazole
I	Intermediate
R	Resistance
X	MICs of antibiotics are in the range of sensitive or intermediate
N	Is an antibiotic not recommended for the treatment of infections

Abbreviations Related to Study

ANI	Average nucleotide identity
CPE	Carbapenemase-producing Enterobacteriaceae
CPO	Carbapenemase-producing organisms
CRE	Carbapenem-resistant Enterobacteriaceae
CRO	Carbapenem-resistant organisms
ESBL	Extended-spectrum β-lactamase
HCW	Health Care Workers
HCP	Healthcare personnel
NICU	Neonatal Intensive Care Unit
PICU	Paediatric Intensive Care Unit
SICU	Surgical Intensive Care Unit
SNP	Single nucleotide polymorphism
WGS	Whole Genome Sequencing
B. cepacia	*Burkholderia cepacia complex*
A. baumannii	*Acinetobacter baumannii*
A. faecalis	*Alcaligenes faecalis*
E. cloacae	*Enterobacter cloacae*
E. coli	*Escherichia coli*
K. pneumoniae	*Klebsiella pneumoniae*
P. aeruginosa	*Pseudomonas aeruginosa*
P. putida	*Pseudomonas putida*
S. maltophilia	*Stenotrophomonas maltophilia*