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# High Carriage Rates of Multidrug-Resistant Gram-Negative Bacteria in Neonatal Intensive Care Units From Ghana

Appiah-Korang Labi,<sup>1,2</sup> Stephanie Bjerrum,<sup>4</sup> Christabel C. Enweronu-Laryea,<sup>5</sup> Prosper K. Ayibor,<sup>6</sup> Karen L. Nielsen,<sup>3</sup> Rasmus L. Marvig,<sup>7</sup> Mercy J. Newman,<sup>8</sup> Leif P. Andersen,<sup>3</sup> and Jorgen A. L. Kurtzhals<sup>2,3</sup>

<sup>1</sup>Department of Microbiology, Korle-Bu Teaching Hospital, Accra, Ghana, <sup>2</sup>Centre for Medical Parasitology at Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark, <sup>3</sup>Department of Clinical Microbiology, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark, <sup>4</sup>Department of Public Health, Global Health Section, University of Copenhagen, Copenhagen, Denmark, <sup>5</sup>Department of Child Health, University of Ghana Medical School, Accra, Ghana, <sup>6</sup>Department of Child Health, 37 Military Hospital, Accra, Ghana, <sup>7</sup>Centre for Genomic Medicine, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark, <sup>8</sup>Department of Medical Microbiology, University of Ghana Medical School, Accra, Ghana

**Background.** Carriage of multidrug resistant (MDR) Gram-negative bacteria (GN) in hospitalized neonates may increase the risk of difficult-to-treat invasive infections at neonatal intensive care units (NICUs). Data on MDRGN carriage among hospitalized newborns in Africa are limited.

**Methods.** We conducted a cross-sectional study at the NICUs of 2 tertiary hospitals in Ghana. Swabs from the axilla, groin, perianal region, and the environment were cultured, GN were identified, and antibiotic susceptibility was tested. We obtained blood culture isolates from neonates with sepsis. Whole-genome sequencing was used to characterize carbapenemase-producing *Klebsiella pneumoniae*. Typing was done by multilocus sequence typing (MLST) and single nucleotide polymorphism (SNP) analysis.

**Results.** A total of 276 GN were isolated from 228 screened neonates. Pathogenic GN were cultured in 76.8% (175 of 228) of neonates. *Klebsiella* spp (41.7%; 115 of 276) and *Escherichia coli* (26.4%; 73 of 276) were the commonest organisms. Carriage rates of MDRGN and third-generation cephalosporin resistant organisms were 49.6% (113 of 228) and 46.1% (105 of 228), respectively. Among *Klebsiella* spp, 75.6% (87 of 115) phenotypically expressed extended-spectrum  $\beta$ -lactamase activity, whereas 15.6% expressed carbapenemase and harbored *bla*<sub>OXA-181</sub> and *bla*<sub>CTX-M-15</sub>. Overall, 7.0% (16 of 228) of neonates developed GN bloodstream infection. In 2 of 11 neonates, sequencing showed the same identity between carriage and the bloodstream isolate. Length of stay before specimen collection and antibiotic use were independently associated with carriage rates, which increased from 13% at admission to 42% by day 2 and reached a plateau at 91% by day 15.

**Conclusions.** High carriage rates of MDRGN, including carbapenemase-producing enterobacterales may be an emerging problem in NICUs in Africa.

**Keywords.** carbapenemase; carriage; Ghana; multidrug resistant; neonates.

There is an increase in the global burden of infections with multidrug-resistant Gram-negative bacteria (MDRGN) in neonates, particularly in low-resource settings [1, 2]. Carriage of MDRGN, especially *Klebsiella pneumoniae* and *Escherichia coli*, is associated with late-onset neonatal bloodstream infections (BSIs) [3]. MDRGN can be transmitted from colonized individuals to other patients and persist in the environment due to poor infection prevention and control practices [4, 5]. MDRGN such as extended-spectrum  $\beta$ -lactamase (ESBL) and

carbapenemase-producing enterobacterales have been associated with difficult-to-treat infections, increased length of hospitalization, and increased risk of mortality [5, 6].

Screening critically ill patients for carriage of MDRGN helps in controlling the spread of antibiotic-resistant organisms [7] by allowing timely implementation of preventive measures [4, 8]. However, its usefulness in areas with endemic or rare occurrence of MDR organisms as well as its role in predicting sepsis remains controversial [9, 10].

In recent years, maternal-newborn interventions in low- and middle-income countries (LMICs) such as Ghana have led to more women giving birth in health facilities [11]. However, the infrastructural and human resources needed to provide effective hospital-based services are often deficient leading to overcrowding of neonatal intensive care units (NICUs) and increased risk for healthcare-associated infections [12, 13]. Few studies have examined the carriage of MDRGN in NICUs in sub-Saharan Africa. Local data are critical for developing evidence-based treatment guidelines and protocols for infection

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Correspondence: Appiah-Korang Labi, MBChB, MGCP, Department of Medical Microbiology, University of Ghana Medical School, P.O. Box 4236, Accra, Ghana ([quylabi2@gmail.com](mailto:quylabi2@gmail.com)).

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prevention and control. In this cross-sectional study, we conducted risk factor analysis for carriage of MDRGN and used whole-genome sequencing (WGS) to understand resistance mechanisms and sources of transmission in NICUs at 2 tertiary hospitals in Ghana.

## MATERIALS AND METHODS

### Study Design and Population

During the baseline period of an ongoing study evaluating infection control interventions against neonatal sepsis and MDRGN carriage (clinicaltrials.gov NCT03755635) [14], we conducted a cross-sectional study at the NICUs of 2 tertiary hospitals in Accra, Ghana. Data collection took place in September 2017 and January 2018. This timeline was chosen to ensure that neonates sampled the first time would have been discharged by the second sampling, thus reducing the chance of repeat sampling. Swabs were collected from all neonates on admission at the NICUs on a single day in September 2017 and in January 2018. To increase the proportion of newly admitted neonates in our dataset and thereby assess MDRGN carriage before admission, we collected swabs during the admission processes for a period of 14 days after the initial specimen collection.

### Study Sites

The NICU at Korle-Bu Teaching hospital (KBTH) is a 55-bed facility, with 3 cubicles for high-dependency care and a 5-bed kangaroo mother care ward. It admits approximately 2400 neonates a year [15]. The 37 Military Hospital (37MH) NICU has 20 beds, it is divided into 3 cubicles, it and admits approximately 800 neonates annually. The first-line empiric antibiotic used for neonatal sepsis at the KBTH was cloxacillin and amikacin, whereas empiric antibiotics used for sepsis treatment at the 37MH varied. Routine screening for MDRGN carriage was not standard practice at the 2 hospitals. Neonates were generally admitted on the day of birth usually without prior antibiotic administration. Thus, the microbiota of freshly admitted newborns is likely to reflect maternal microbiota.

### Clinical Data

Clinical data extracted from patient records on the day of specimen collection included maternal pregnancy history, birth weight, reason for admission, and antibiotic use before specimen collection. Data on maternal receipt of antibiotics and group B *Streptococcus* carriage were not recorded, and we did not screen mothers for carriage of MDRGN.

### Specimen Collection and Microbiology Analysis

Swabs were collected from neonates by a trained nurse using BBL culture swab liquid Amies Medium (Becton Dickinson, Franklin Lakes, NJ). Each sample was taken with a swab moistened in transport medium and gently rotated in the fossa of the

pinna, axilla, groin, and perianal region (in that order). These sites were chosen because of documented increased chance of isolating MDRGN [16]. All swabs were initially cultured on MacConkey agar plates at 35–37°C. Different GN colony morphotypes were identified and stored at –80°C and transported on dry ice to the Department of Clinical Microbiology, Rigshospitalet (Copenhagen, Denmark). Frozen isolates were thawed and cultured on lactose agar plates (SSI Diagnostica, Hillerød, Denmark) to recover GN, which were identified using MALDI Biotyper (Bruker Daltonics, Bremen, Germany).

During the study period, we conducted 3 environmental screenings at the KBTH (September 2017, October 2017, and January 2018, respectively) to understand the role of the environment in the spread of MDRGN infections. Sites (incubator doors, cots, trolley handles, door handles, weighing scales, tables, and desks) were screened using Replicate Organism Detection and Count (RODAC) plates filled with tryptic soy agar for flat surfaces; for uneven surfaces, a swab was taken.

Antibiotic susceptibility testing was performed on Müller-Hinton medium (SSI Diagnostica) by the Kirby Bauer disc diffusion method. All zone diameters were interpreted using European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [17]. Zone sizes within the intermediate resistance and absolute resistant range were classified as resistant. All enterobacterales were assessed for ESBL, and isolates with a meropenem zone <28 mm were assessed for carbapenemase expression. These tests were performed and interpreted according to EUCAST guidelines [18], using ROSCO (Taastrup, Denmark) phenotypic ESBL + AMPC and KPC + MBL + OXA-48 carbapenemase Kit. Multidrug resistance was defined as nonsusceptibility to  $\geq 1$  antibiotic in  $\geq 3$  antibiotic groups, with the following antibiotics used in the classification; gentamicin/amikacin, piperacillin tazobactam, meropenem, cefuroxime, cefotaxime, ciprofloxacin, and amoxicillin-clavulanic acid [19].

### Outcomes

Our primary outcome was the prevalence of GN carriage, including antibiotic susceptibility. We further determined the proportion of neonates who developed GN BSI by reviewing data on blood cultures performed during hospitalization. Data on BSI and corresponding bacterial isolates were made available through an ongoing prospective study assessing neonatal sepsis at the 2 study sites (clinicaltrials.gov NCT03755635) [14].

### Molecular Analysis

All *K pneumoniae* isolates with phenotypic carbapenemase production were whole-genome sequenced. Deoxyribonucleic acid (DNA) was purified with DNeasy Blood and Tissue kit (QIAGEN, Hilden, Germany). The isolates were sequenced on a MiSeq Instrument (Illumina Inc., San Diego, CA) using paired-end libraries (2 × 250 base pairs). Genome assemblies

were created with VelvetOptimiser v2.2.5 and annotated with Prokka v1.12.

*In silico* analyses of resistance genes, MLST and capsular typing were performed using the online platform of the Centre for Genomic Epidemiology of the Danish Technical University and Kaptive applying assemblies [20, 22]. To determine the source of BSI with *K pneumoniae*, we compared pairs of phenotypically similar isolates (carriage, bloodstream, and environment) in a single nucleotide polymorphism (SNP) analysis using BacDist [23].

#### Data Availability

Raw reads for the 7 *K pneumoniae* genomes used in SNP analysis in this study are available in the European Nucleotide Archive (ENA) under the accession number PRJEB37523.

#### Statistical Analysis and Definitions

Data were analyzed with STATA (version 12; StataCorp). Categorical data were compared using  $\chi^2$  test, Z score for proportions, and Wilcoxon rank-sum score for medians. Birth weight <2500 grams was classified as low birth weight. To identify factors associated with carriage of MDRGN as primary outcome and carriage of third-generation cephalosporin resistance as secondary outcome, we generated univariate and multivariate logistic regression models using the purposeful selection of covariates method [24]. We compared neonates with MDRGN with neonates who did not carry MDRGN, which included neonates who did not culture a GN organism. All variables associated with the outcome at a  $P < .2$  in univariate analysis were included in multiple regression analysis. This was followed by an iterative process of purposeful removal of covariates in the multiple logistic regression based on their nonsignificance at  $P > .1$ . Confounders were then removed if they did not change parameter estimate by at least 15%. We present results as crude and adjusted odds ratio (OR) with 95% confidence intervals (CIs). Two-tailed  $P < .05$  was considered significant.

#### Ethical Considerations

The study forms part of a larger project on neonatal sepsis given ethical approval by the Institutional Review Boards of KBTH (IRB/0025/2017) and 37MH (37MH-IRB IPN 144/2017). Informed consent was obtained from parents of neonates on admission. For day 1 or fresh admissions, administrative permission was sought to take swabs as part of the admission process. Carriage findings from the study were not disclosed to clinical staff or parents.

## RESULTS

#### Patient Characteristics

During the 2 periods of sample collection, 228 neonates were assessed for GN carriage. Median duration of stay at time of specimen collection was 3 days (interquartile range [IQR],

1–11). Mortality among the screened neonates was 9.6% (22 of 228). Ninety-two (40.4%) samples were collected on the first day of admission. Table 1 describes the main characteristics of included neonates overall and by hospital.

#### Antibiotic Use

Of the screened neonates, 52% (118 of 228) had received antibiotic treatment before specimen collection. In total, 291 antibiotic prescriptions were made for 118 neonates. The median duration of antibiotic therapy at the time of data collection was 8 days (IQR, 7–12). Most (73%, 86 of 118) had received a combination of 2 (IQR, 2–3) antibiotics. The top 5 antibiotics used were cloxacillin (38.8%, 113 of 291), amikacin (35.4%, 103 of 291), meropenem (8.2%, 24 of 291), ciprofloxacin (6.2%, 18 of 291), and gentamicin (3.1%, 9 of 291) (Figure 1).

#### Carriage of Multidrug-Resistant Gram-Negative Bacteria

A total of 276 GN organisms were isolated from 76.8% (175 of 228) neonates. Table 2 shows common organisms isolated and their respective antibiotic susceptibility patterns. The top 3 organisms isolated were *Klebsiella* spp (41.7%, 115 of 276), *E coli* (26.4%, 73 of 276), and *Enterobacter* spp (12.3%, 34 of 276).

Overall, the MDRGN carriage rate was 49.6% (113 of 228), whereas carriage of third-generation cephalosporin resistance was found in 46.1% (105 of 228) (Table 2). There was no statistically significant difference in the rate of MDRGN carriage between neonates admitted at KBTH (51.6%, 98 of 190) and 37MH (39.5%, 15 of 38) ( $P = .20$ ). Multidrug-resistant was most common among *Klebsiella* spp (87.0%, 100 of 115), followed by *Enterobacter* spp (70.6%, 24 of 34) (Table 2). For the identified *Klebsiella* spp, resistance rates were 78.3% (90 of 115) to cefotaxime, followed by 65.2% (75 of 115) to gentamicin and 43.5% (50 of 115) to amikacin. Resistance rates of *E coli* were 34.2% (25 of 73) to cefotaxime, 13.7% (10 of 73) to gentamicin, and 1.4% (1 of 73) to amikacin (Table 2).

#### Gram-negative bacteria in the environment

A total of 191 samples were taken from the KBTH NICU environment for culture. Forty sites cultured a total of 47 GN organisms. Fourteen (29.8%) of the organisms were *K pneumoniae* from the following sites: incubator doors (4); cots (9); and nurses trolley handle (1). The majority, 85.7% (12 of 14), were MDR, 78.6% (11 of 14) were ESBL positive, and 21.4% (3 of 14) were carbapenemase producing.

#### Resistance Mechanisms

Phenotypic expression of ESBL was found in 76.0% of *Klebsiella* spp and 32.8% of *E coli*. Among *Klebsiella* spp, 16.0% (18 of 115) had meropenem zone <28 mm and were genotypic carbapenemase producers, carrying the carbapenemase *bla*<sub>OXA-181</sub> as well as ESBL *bla*<sub>CTX-M-15</sub>. Thus, carriage rate of carbapenemase-producing *Klebsiella* spp was 7.9% (18 of

**Table 1. Sociodemographic and Clinical Characteristics of Neonates at Enrollment<sup>a</sup>**

Characteristics	All Neonates (N = 228)	Neonates Enrolled From KBTH (N = 190)	Neonates Enrolled From 37MH (N = 38)	P Value
Age of mother, years	30.0 [25.0–35.0]	30 [25–35]	29 [23.5–34.5]	.480
Parity	2.0 [2.0–3.0]	2 [1–3]	2 [1–3]	.750
Number exposed to antibiotics at the time of sample collection	118 [51.8]	98 [51.6]	20 [52.6]	.910
Number of antibiotics prescribed	2.0 [2.0–3.0]	2 [2]	2 [2–3.8]	.174
Duration of stay at the time of specimen collection	3.0 [1.0–11.0]	3 [1–13]	2 [1–6]	.146
Age at admission, median [IQR] (days)	1.0 [1.0–1.0]	1 [1]	1 [1]	.865
Sex		N = 187	N = 36	.315
Male	113 [50.7]	92 [49.2]	21 [58.3]	
Birth weight (grams)				
≥2500	101 [44.3]	76 [40.0]	25 [65.8]	.003
1500–2400	73 [32.0]	63 [33.2]	10 [26.3]	.820
1000–1400	45 [19.7]	42 [22.1]	3 [7.9]	
<1000	9 [4.0]	9 [4.7]	0 [0]	-
Mode of delivery		N = 190	N = 36	
Caesarean section	123 [54.4]	100 [52.6]	23 [63.9]	.210
Type of Birth		N = 187	N = 35	
Preterm	133 [59.9]	117 [62.6]	16 [45.7]	.016
GN colonization	176 [77.2]	151 [79.5]	25 [65.8]	.067
MDRGN colonization	113 [49.6]	98 [51.6]	15 [39.5]	.170
GN BSI	16 [7%]	14 [7.4]	2 [5.3]	-

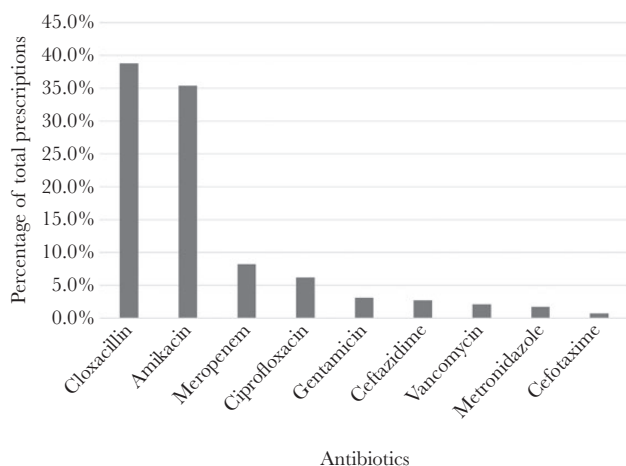
Abbreviations: 37MH, 37 Military Hospital; BSI, bloodstream infection; GN, Gram-negative bacteria; IQR, interquartile range; KBTH, Korle-Bu Teaching Hospital; MDR, multidrug resistant.

<sup>a</sup>Data are presented as medians with IQRs and N [%] where appropriate; statistical level of significance set at  $P < .05$ .

228). Carbapenemase-producing organisms were only found at KBTH.

#### Risk Factors for Carriage of Multidrug-Resistant Gram-Negative Bacteria

Antibiotic administration before specimen collection was associated with carriage of MDRGN in univariate (crude OR, 15.0; 95% CI, 7.9–28.6) and multivariate analysis (adjusted OR



**Figure 1.** Proportion of antibiotic type prescribed before specimen collection (n = 291).

[aOR], 11.1; 95% CI, 5.5–22.1) (Table 3). MDRGN carriage was also associated with duration of admission before specimen collection in univariate (crude OR, 1.1; 95% CI, 1.05–1.14) and multivariate (aOR, 1.04; 95% CI, 1.01–1.07) analysis. MDRGN carriage rate increased with duration of hospitalization from 13% for neonates screened at day 1 of admission to 42% by day 2, 47% by day 3, and reached a plateau at 91% by day 15 (Supplementary Figure 2). Carriage of third-generation cephalosporin-resistant organisms was associated with antibiotic use in univariate (crude OR, 16.10; 95% CI, 8.3–31.10) and multivariate analysis (aOR, 12.87; 95% CI, 6.41–25.8). The duration of stay before sampling was also associated with carriage of third-generation cephalosporin-resistant organisms in univariate (crude OR, 1.07; 95% CI, 1.04–1.10) and multivariate analysis (aOR, 1.02; 95% CI, 1.01–1.04) (Table 4). In subgroup analysis of neonates who had received antibiotics, the duration of antibiotic use was significantly associated with carriage of an MDR GN (aOR, 1.16; 95% CI, 1.03–1.30), ie, the odds of carrying an MDR GN increased 1.16 times for each day of antibiotic exposure (Supplementary Table S1). Likewise, duration of antibiotic use was independently associated with carriage of third-generation cephalosporin-resistant organisms among neonates on antibiotics (aOR, 1.19; 95% CI, 1.02–1.41) (Supplementary Table S2). Furthermore, in this subset of patients, low birth weight and vaginal delivery



**Table 2. Antibiotic Resistance and Resistant Phenotypes Among Neonates With Carriage of Gram-negative Bacteria<sup>a</sup>**

Antibiotic	<i>Klebsiella</i> spp N = 115	<i>Escherichia coli</i> N = 73 (%)	<i>Enterobacter</i> spp N = 34	<i>Pseudomonas aeruginosa</i> N = 20	<i>Acinetobacter</i> spp N = 21	<i>Citrobacter</i> spp N = 4
Ampicillin	115 [100]	54 [74]	34 [100]	-	-	4 [100]
Amoxiclav	95 [82.6]	34 [46.6]	15 [44.1]	-	-	1 [75]
Gentamicin	75 [65.2]	10 [13.7]	16 [47.1]	3 [15.0]	11 [52.6]	0
Amikacin	50 [43.5]	1 [1.4]	14 [41.2]	0	7 [33.3]	0
Aztreonam	90 [78.6]	25 [34.2]	17 [50]	18 [90]	-	1 [25]
Ceftazidime	91 [79.1]	23 [31.5]	17 [50]	3 [15.0]	-	1 [25]
Cefotaxime	90 [78.3]	25 [34.2]	16 [47.1]	-	-	1 [25]
Cefuroxime	91 [79.1]	24 [32.9]	16 [47.1]	-	-	1 [25]
Ciprofloxacin	81 [70.4]	20 [27.4]	15 [44.1]	2 [10.0]	7 [33.3]	1 [25]
Mecillinam	7 [6.1]	1 [1.4]	3 [8.8]	-	-	1 [25]
Meropenem <sup>b</sup>	1 [.9]	0	0	2 [10.0]	7 [33.3]	0
Tazobactam piperacillin	41 [35.7]	9 [12.3]	8 [23.5]	3 [15.0]	-	1 [25]
MDR	100 [87.0]	40 [54.8]	24 [70.6]	3 [15]	7 [33.3]	2 [50]
ESBL phenotype	87 [75.6]	24 [32.8]	-	-	-	0
Carbapenemase producing	18 [15.6]	0	0	-	-	-

Abbreviations: *Acinetobacter* spp, *Acinetobacter baumannii*-17, *Acinetobacter nosocomialis*-3, *Acinetobacter iwoffi*-1; carbapenemase producing, genotypic carbapenemase-producing organisms; *Citrobacter* spp, *Citrobacter koseri*-3, *Citrobacter freundii*-1; *Enterobacter* spp, *Enterobacter cloacae*-22, *Enterobacter kobei*-6, *Enterobacter aerogenes*-4, *Enterobacter hormaecheae*-2, *Enterobacter asburiae*-1; ESBL phenotype, extended-spectrum  $\beta$ -lactamase-producing organisms; EUCAST, European Committee on Antimicrobial Susceptibility Testing; *Klebsiella* spp, *Klebsiella pneumoniae*-109, *Klebsiella variicola*-4, *Klebsiella oxytoca*-2; MDR, multidrug-resistant nonsusceptibility to  $\geq 1$  antibiotic in  $\geq 3$  antibiotic groups.

<sup>a</sup>Data are presented as N [%].

<sup>b</sup>Carbapenem resistance determined by EUCAST disc diffusion.

were independent risk factors for carriage of MDR and third-generation cephalosporin-resistant GN.

#### Bloodstream Infections During Admission

During admission, 7.0% (16 of 228) of the neonates had GN BSI; 13 (81.3%) were MDR, 11 (84.6%) of which were caused by ESBL-producing *K pneumoniae*. Two (18.2%) of the neonates with BSI caused by ESBL-producing *K pneumoniae* were carriers of phenotypically similar organisms. The pairs were ST37/capsular type KL158 and ST37/capsular type KL15. In both cases, BSI had occurred before swabs were taken. Single nucleotide polymorphism

analysis of WGS data showed zero SNP difference between each carriage-bloodstream pair of isolates. The ST37 paired isolates (carriage/bloodstream) were closely related genetically to 3 environmental *K pneumoniae* isolated 3 months before admission of the neonate with an SNP difference of 2–8.

#### DISCUSSION

The major finding of this study was a high rate of MDRGN carriage among neonates at 2 NICUs in Ghana. The carriage rate increased with duration of hospitalization and was

**Table 3. Factors Associated With Carriage of Multidrug-Resistant Gram-Negative Bacteria in Neonates Admitted to Neonatal Intensive Care Units<sup>a</sup>**

Characteristic	MDR GN Present [n = 113]	Crude Odds Ratio [95% CI]	P Value	Adjusted Odds Ratio [95% CI]	P Value	
Admitting weight (grams)	$\geq 2500$	47 [41.6]	1.00			
	1500–2499	35 [31.0]	1.06 [0.58–1.93]	.854		
	1001–1499	24 [21.2]	1.31 [0.65–2.66]	.448		
	$\leq 1000$	7 [6.]	4.02 [0.80–20.31]	.092		
Duration of stay before sample collection (days)	9.0 [4.0–22.0]	<b>1.10 [1.05–1.14]</b>	<b>&lt;.001</b>	<b>1.04 [1.01–1.07]</b>	<b>.023</b>	
Age of mother (years)	29.0 [24.0–35.0]	0.97 [0.93–1.01]	.152			
Parity	1.0 [1.0–3.0]	0.83 [0.67–1.03]	.092			
Sex	Male	52 [46.0]	0.68 [0.40–1.16]	.159		
Type of delivery	Caesarean section	58 [51.3]	0.78 [0.46–1.32]	.350		
Type of gestation	Preterm	72 [64.9]	1.51 [0.88–2.60]	0.133		
Prolonged PROM <sup>b</sup>	Yes	9 [10.4]	3.46 [0.72–16.62]	.121		
Antibiotic Use	Yes	92 [81.4]	<b>15.00 [7.87–28.57]</b>	<b>&lt;.001</b>	<b>11.08 [5.54–22.15]</b>	<b>&lt;.001</b>

Abbreviations: CI, confidence interval; GN, Gram negative; MDR, multidrug-resistant; PROM, premature rupture of membranes.

<sup>a</sup>Data are presented as medians with interquartile ranges and n/N (%) where appropriate. Significant variables are presented in bold.

<sup>b</sup>Statistical level of significance set at  $P < .05$ .

**Table 4. Factors Associated With Carriage of Third-Generation Cephalosporin-Resistant Bacteria in Neonates Admitted at Neonatal Intensive Care Units<sup>a</sup>**

Characteristic		3rd-Gen Present n = 105	Crude Odds Ratio [95% CI]	PValue	Adjusted Odds Ratio [95% CI]	PValue
Admitting weight (g)	≥2500	44 [41.9]	1.00			
	1500–2499	32 [30.5]	1.01 [0.55–1.86]	.972		
	1001–1499	22 [21.0]	1.24 [0.61–2.51]	.551		
	≤1000	7 [6.7]	4.53 [0.90–22.91]	.067		
Duration of stay before specimen collection (days)		9.0 [4.0–22.0]	<b>1.07 [1.04–1.10]</b>	<b>&lt;.001</b>	<b>1.02 [1.01–1.04]</b>	<b>.044</b>
Age of mother (years)		29.0 [24.0–35.0]	0.97 [0.93–1.02]	.205		
Parity		1.0 [1.0–3.0]	0.84 [0.68–1.04]	.111		
Sex Male		47 [44.8]	0.64 [0.38–1.08]	.097		
Type of delivery	Caesarean section	55 [52.4]	0.86 [0.51–1.44]	.566	0.95 [0.49–1.83]	.876
Type of gestation	Preterm	68 [65.4]	1.54 [0.89–2.65]	.119		
Prolonged PROM <sup>b</sup>	Yes	8 [9.8]	2.31 [0.57–9.06]	.231		
Antibiotic use	Yes	88 [83.8]	<b>16.05 [8.27–31.13]</b>	<b>&lt;.001</b>	<b>12.87 [6.41–25.83]</b>	<b>&lt;.001</b>

Abbreviations: CI, confidence interval; GN, Gram negative; MDR, multidrug resistant; PROM, premature rupture of membranes; 3rd-Gen, third-generation cephalosporin.

<sup>a</sup>Data are presented as medians with interquartile ranges and N (%) where appropriate. Significant variables are presented in boldface.

<sup>b</sup>Statistical level of significance set at  $P < .05$ .

strongly associated with antibiotic use. Carriage of MDR *Klebsiella* spp, *E coli*, and *Enterobacter* spp was frequent; these isolates were resistant to universally recommended antibiotics (ampicillin, gentamicin) for empirical treatment of neonatal sepsis [25, 26]. In 2 neonates, we found an indication of possible endogenous BSI with MDR carriage organisms. This is in line with existing literature which shows that carriage of a drug-resistant organism is likely to serve as source of later infection [3].

To the best of our knowledge, this is the first report of *bla*<sub>OXA-181</sub> carbapenemase-producing organisms in Ghana. OXA-181 belongs to the OXA-48 type class D group of  $\beta$ -lactamases. These enzymes can hydrolyze penicillins at a high level and carbapenems at a low level but do not affect extended-spectrum cephalosporins and are not susceptible to most  $\beta$ -lactamase inhibitors [27].

Important reservoirs of OXA-48-type carbapenemases are countries in North Africa, the Middle East, Turkey, and India [28]; however, important reservoirs of OXA-181-type carbapenemases are in the Indian subcontinent [28]. In Africa, carbapenem resistance mediated by *bla*<sub>OXA-181</sub> has been documented mainly in nonneonatal populations [29, 30]. In Morocco and Algeria, carriage of OXA-48 carbapenemase-producing enterobacteriales in neonates was 1.8% [31] and 1.6%, respectively [32] compared with 7.9% found in our study. The relatively higher rate of OXA-181 in our study could indicate a possible outbreak at the KBTH NICU.

Organisms expressing OXA-48-like carbapenemases are difficult to identify using routine phenotypic resistance testing methods [28]. This requires specialized but simple techniques, which may not be routinely available in laboratories in LMICs. We used EUCAST guidelines that have been found to be comparable to other methods for phenotypic identification of

carbapenemase production [18, 33] and could be implemented in low-resource settings.

Similar to MDR, third-generation cephalosporin resistance was common among ESBL *Klebsiella* spp and *E coli*. These rates were higher compared to other reports from Africa [31]. In our recent multicenter study from Ghana, 64% antibiotic use was recorded among neonates, but cephalosporins (9.8%) were not commonly prescribed [34]. Our study corroborates the low use of these agents in neonates in Ghana. This suggests that other factors such as noncephalosporin antibiotic use, the NICU environment, and maternal colonization may play an important role in the selection and transmission of cephalosporin-resistant organisms.

In our study, we showed that invasive infection may have involved endogenous microbiota of affected neonates as indicated in literature [3]. Our data further suggest the possible role of environmental persisting organisms such as *K pneumoniae* in carriage and infection.

Risk factors associated with carriage of MDR GN and third-generation cephalosporin-resistant enterobacteriales in previous studies include prolonged exposure to antibiotics, increasing number of days of formula feeding, low birth weight, exposure to cephalosporins, prolonged hospitalization, and use of invasive devices [32, 35]. In our setting, exposure to antibiotics and prolonged hospitalization were the main risk factors identified for carriage of MDR and third-generation cephalosporin-resistant GN. More than 10% of neonates were colonized with MDRGN on arrival to the NICU. On the other hand, the fact that MDR colonization took place within few days of admission in the majority of patients suggests that antimicrobial stewardship and enhanced infection prevention will be critical strategies for the control of MDRGN infections.

Neonatal intensive care units in low-resource settings face the challenge of low staff to patient ratio, limited space resulting in sharing of cots and incubators, and reuse of single-use devices [36]. These practices may facilitate the spread of MDRGN among neonates with the possibility of outbreaks [37]. The high rates of carbapenemase-producing enterobacteriales and ESBLs in the NICUs coupled with previously documented poor hand hygiene practices among healthcare workers in Ghana [38, 39] suggest that these organisms may also spread beyond NICUs if practices are not optimized.

Screening for MDRGN is previously found to be cost intensive, resulting in excess use of antibiotics, and of limited benefit to patients in areas where MDRGN are rare or endemic [3, 10]. Thus, the usefulness of screening in low-resource settings with high prevalence of MDRGN has been questioned [31]. However, in the absence of routine diagnostic microbiology services to inform choice of antibiotics, screening may be useful to design rational empiric treatment policies. The current recommendations suggest that susceptibility to carbapenems should be reported as found in enterobacteriales with OXA-181 [18, 28], but its usefulness in treating such cases remains in doubt [28]. Other agents like colistin or ceftazidime-avibactam combinations may be useful for managing these infections, although the cost may prohibit their use in low-resource settings.

This study has limitations. Specimens were collected at 2 different time periods, and context-specific interventions or events between the periods may have directly affected the carriage rate. In addition, empirical therapy for treating infections varied at both sites. The cross-sectional nature of the study does not allow for proper evaluation of associations between carriage of MDRGN and duration of stay. We also did not have enough neonates who cultured GN organisms that were not MDR for comparison with MDRGN-positive neonates. The carriage rates may have been underestimated because recovery of GN is higher from rectal swabs than from perianal swabs [16], which were used in this study. The study was conducted in tertiary hospitals; thus, our findings may not be generalizable to nontertiary facilities. Nevertheless, this study highlights the role that MDRGN may play in NICUs in LMICs.

## CONCLUSIONS

We found high external carriage of MDRGN among hospitalized neonates with high levels of resistance to recommended antibiotics for severe neonatal infections. Carbapenemase production mediated by *bla*<sub>OXA-181</sub> was found among *K pneumoniae*. There is a need to improve antibiotic use and infection control and prevention practices in NICUs to limit the potential impact of MDRGN in hospitalized neonates in low-resource settings.

## Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of

the authors, so questions or comments should be addressed to the corresponding author.

**Supplementary Figure 1A.** Proportion of antibiotic type prescribed before specimen collection at KBTH (n = 246).

**Supplementary Figure 1B.** Proportion of antibiotic type prescribed before specimen collection at 37MH (n = 55).

**Supplementary Figure 2.** Proportion of multidrug-resistant Gram-negative bacteria compared with days of admission before specimen collection.

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## References

- Agarwal R, Sankar J. Characterization and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. *Lancet Glob Health* 2016; 4:e752–60.
- Eibach D, Campos CB, Krumkamp R, et al. Extended spectrum beta-lactamase producing Enterobacteriaceae causing bloodstream infections in rural Ghana, 2007–2012. *Int J Med Microbiol* 2016; 306:249–54.
- Folgori L, Tersigni C, Hsia Y, et al. The relationship between Gram-negative colonization and bloodstream infections in neonates: a systematic review and meta-analysis. *Clin Microbiol Infect* 2018; 24:251–7.
- Taconelli E, Cataldo MA, Dancer SJ, et al.; European Society of Clinical Microbiology. ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients. *Clin Microbiol Infect* 2014; 20 (Suppl 1):1–55.
- Moshiri T, Mahida N, Soo S. Multi-resistant Gram negative Enterobacteriaceae in paediatrics: an infection prevention and control challenge. *Paediatr Child Health* 2018; 28:196–200.
- Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. *Clin Microbiol Infect* 2016; 22:416–22.
- Wilson AP, Livermore DM, Otter JA, et al. Prevention and control of multi-drug-resistant Gram-negative bacteria: recommendations from a Joint Working Party. *J Hosp Infect* 2016; 92 (Suppl 1):S1–44.
- Stapleton PJ, Murphy M, McCallion N, et al. Outbreaks of extended spectrum beta-lactamase-producing Enterobacteriaceae in neonatal intensive care units: a systematic review. *Arch Dis Child Fetal Neonatal Ed* 2016; 101:F72–8.
- Walker O, Babb C, Karampatsas K, et al. Screening for colonisation with gentamicin-resistant Gram-negative organisms on the neonatal unit: does positive screening predict sepsis? *Arch Dis Child Fetal Neonatal Ed* 2019; 104:F113–4.
- Seidel J, Haller S, Eckmanns T, Harder T. Routine screening for colonization by Gram-negative bacteria in neonates at intensive care units for the prediction of sepsis: systematic review and meta-analysis. *J Hosp Infect* 2018; 99:367–80.
- Montagu D, Sudhinaraset M, Diamond-Smith N, et al. Where women go to deliver: understanding the changing landscape of childbirth in Africa and Asia. *Health Policy Plan* 2017; 32:1146–52.
- Fischer D, Schlößer RL, Kempf VAJ, et al. Overcrowding in a neonatal intermediate care unit: impact on the incidence of multidrug-resistant Gram-negative organisms. *BMC Infect Dis* 2019; 19:357.



13. Rameshwarnath S, Naidoo S. Risk factors associated with nosocomial infections in the neonatal intensive care unit at Mahatma Gandhi Memorial hospital between 2014 and 2015. *South Afr J Infect Dis* **2018**; 33:93–100.
14. Neonatal Sepsis at Neonatal Intensive Care Units in Ghana. Available at: <https://clinicaltrials.gov/ct2/show/NCT03755635>. Accessed 22 November 2019.
15. KBTH. Korle Bu Teaching Hospital, 2012 Annual Report. Accra, Ghana: Korle-Bu Teaching Hospital; **2013**.
16. Weintrob AC, Roediger MP, Barber M, et al. Natural history of colonization with Gram-negative multidrug-resistant organisms among hospitalized patients. *Infect Control Hosp Epidemiol* **2010**; 31:330–7.
17. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 8.0. **2018**; Available at: <http://www.eucast.org>. Accessed 4 March 2018.
18. EUCAST Guidelines for Detection of Resistance Mechanisms and Specific Resistances of Clinical and/or Epidemiological Importance Version 2.0. **2017**; Available at: [http://www.eucast.org/resistance\\_mechanisms/](http://www.eucast.org/resistance_mechanisms/). Accessed 4 March 2018.
19. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* **2012**; 18:268–81.
20. Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* **2012**; 67:2640–4.
21. Larsen MV, Cosentino S, Rasmussen S, et al. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol* **2012**; 50:1355–61.
22. Wick RR, Heinz E, Holt KE, Wyres KL. Kaptive web: user-friendly capsule and lipopolysaccharide serotype prediction for klebsiella genomes. *J Clin Microbiol* **2018**; 56:e00197–18.
23. GitHub - MigleSur/BacDist: Snakemake Pipeline for Bacterial SNP Distance and Phylogeny Analysis. Available at: <https://github.com/MigleSur/BacDist>. Accessed 6 August 2019.
24. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. *Source Code Biol Med* **2008**; 3:17.
25. Fuchs A, Bielicki J, Mathur S, et al. Reviewing the WHO guidelines for antibiotic use for sepsis in neonates and children. *Paediatr Int Child Health* **2018**; 38: S3–15.
26. Ministry of Health Ghana National Drugs Programme. Standard Treatment Guidelines. Seventh Edition. Accra, Ghana: Ministry of Health; **2017**.
27. Stewart A, Harris P, Henderson A, Paterson D. Treatment of infections by OXA-48-producing Enterobacteriaceae. *Antimicrob Agents Chemother* **2018**; 62:e01195–18.
28. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. *J Antimicrob Chemother* **2012**; 67:1597–606.
29. Uwaezuoke NS, Kieffer N, Iregebu KC, Nordmann P. First report of OXA-181 and NDM-1 from a clinical *Klebsiella pneumoniae* isolate from Nigeria. *Int J Infect Dis* **2017**; 61:1–2.
30. Jacobson RK, Manesen MR, Moodley C, et al. Molecular characterisation and epidemiological investigation of an outbreak of blaOXA-181 carbapenemase-producing isolates of *Klebsiella pneumoniae* in South Africa. *SAMJ South Afr Med J* **2015**; 105:1030–5.
31. Arhoun B, Oumokhtar B, Hmami F, et al. Rectal carriage of extended-spectrum  $\beta$ -lactamase- and carbapenemase-producing Enterobacteriaceae among hospitalised neonates in a neonatal intensive care unit in Fez, Morocco. *J Glob Antimicrob Resist* **2017**; 8:90–6.
32. Mairi A, Touati A, Ait Bessai S, et al. Carbapenemase-producing Enterobacteriaceae among pregnant women and newborns in Algeria: prevalence, molecular characterization, maternal-neonatal transmission, and risk factors for carriage. *Am J Infect Control* **2019**; 47:105–8.
33. Haldorsen B, Giske CG, Hansen DS, et al.; NordicAST CPE Study Group. Performance of the EUCAST disc diffusion method and two MIC methods in detection of Enterobacteriaceae with reduced susceptibility to meropenem: the NordicAST CPE study. *J Antimicrob Chemother* **2018**; 73:2738–47.
34. Labi AK, Obeng-Nkrumah N, Nartey ET, et al. Antibiotic use in a tertiary healthcare facility in Ghana: a point prevalence survey. *Antimicrob Resist Infect Control* **2018**; 7:15.
35. Giuffrè M, Geraci DM, Bonura C, et al. The increasing challenge of multidrug-resistant Gram-negative bacilli: results of a 5-year active surveillance program in a neonatal intensive care unit. *Medicine (Baltimore)* **2016**; 95:e3016.
36. Allegranzi B, Bagheri Nejad S, Combescure C, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. *Lancet* **2011**; 377:228–41.
37. Dramowski A, Aucamp M, Bekker A, Mehtar S. Infectious disease exposures and outbreaks at a South African neonatal unit with review of neonatal outbreak epidemiology in Africa. *Int J Infect Dis* **2017**; 57:79–85.
38. Yawson AE, Hesse AAJ. Hand hygiene practices and resources in a teaching hospital in Ghana. *J Infect Dev Ctries* **2013**; 7:338–47.
39. Asare A, Enweronu-Laryea CC, Newman MJ. Hand hygiene practices in a neonatal intensive care unit in Ghana. *J Infect Dev Ctries* **2009**; 3:352–6.