Preplanned Studies

Dissemination of Antibiotic Resistance Genes Among Patients with Diarrhea — Freetown, Sierra Leone, 2018

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Summary

What is already known about this topic?

Antibiotic resistance (AR) is a serious public health threat worldwide. However, the AR and antibiotic resistance genes (ARGs) data from West Africa, especially from Sierra Leone, are limited.

What is added by this report?

The study revealed ARGs' common dissemination, and multiplex antibiotic resistance genes in one sample. Genes bla_{NDM} and $bla_{\text{OXA-48-like}}$ were first discovered in Sierra Leone.

What are the implications for public health practice?

Basic information is provided for AR research and surveillance and highlights that effective AR surveillance among diarrhea patients is necessary for Sierra Leone and West Africa.

Antibiotic resistance (AR), a major public health problem in developed, developing, and undeveloped countries, has increased with rapid globalization. Due to logistical issues and a rapidly migrating population in Africa, AR has become a complicated issue for the general public. Although medical hygiene and public health system in Africa have improved, and the use of antibiotics has also increased in many African countries (*1*–*3*), AR has become a prevalent issue. Sierra Leone is categorized as one of the most undeveloped countries in the world with bacterial diarrhea as a common and major disease throughout the country. Antibiotics are an effective treatment option for this disease. Since the medical and public health system in Sierra Leone is still in its infancy, the available data related to AR are limited. Here, 17 antibiotic resistance genes (ARGs)/ antibiotic resistance gene (ARG) groups in the stool samples of 56 diarrhea patients were detected in Freetown, Sierra Leone, 2018. Nine ARGs/ARG groups were detected as positive, and most of the samples carried at least 2 ARGs/ARG groups. Two stool samples carrying 7 ARGs/ARG groups highlighted the complexity of ARGs in Freetown, Sierra Leone. Genes bla_{NDM} and $bla_{\text{OXA-48-like}}$ are the first reported ARGs from Sierra Leone. The diversity and dissemination data of ARGs in Freetown of Sierra Leone are expected to complement the antibiotic resistance data of West Africa and highlight the need for continued monitoring of antibiotic resistance.

A total of 56 acute diarrheal stool samples were obtained from six sentinel hospitals of Freetown, Sierra Leone between May 2018 and December 2018 (Table 1). Most samples were collected in July and October (n=10, each), while only two samples were collected in May and December.

A total of 17 ARGs/ARG groups, including 249 genes types/subtypes, were detected using the probe method of real-time PCR. The real-time PCR primers and minor groove binder (MGB)-conjugated fluorescent probes used were reported elsewhere (*4*), except the *tet*(A) gene. The *tet*(A) gene was detected using the forward primer (5'-CAT TCT GCA TTC ACT CGC CCA GGC AAT GAT-3'), reverse primer (5'-GAA GCA AGC AGG ACC ATG ATC GGG AAC GC-3'), and the 6-carboxyfluorescein (FAM)-labeled *tet*A-specific probe (5'-GAT TGC CGA CGG CAC AGG CTA CAT CCT GCT TG-3').

Seventeen ARGs/ARG groups were detected among the 56 diarrhea stool samples, and the ARG positive detection rates ranged from 0 to 92.9% (Table 2). *int*I1, IS*CR*1, $bla_{\text{CTX-M}}$ E groups, and *tet*A gene positive rates were over 50%. Eight ARGs/ARG groups were not detected, including $bla_{\text{CTX-M}}$ A, *qnr*S, *aac*(6')-Ib-cr, *cfr*, *fex*A, *mcr*-1, *arm*A, and *aac*(6')-Ie-*aph*(2')-Ia. At least one ARG/ ARG group was detected from all of the stool samples, and most samples showed ARG/ ARG group coexistence. Forty-two (75%) of the stool samples carried more than two ARGs/ARG groups, and two samples carried seven ARGs/ARG groups. Seventeen different ARG coexistent types were detected. Carbapenem resistance encoding genes bla_{NDM} and $bla_{\text{OXA-48-like}}$ were also detected. bla_{NDM} and bla_{SHV} A genes coexisted in some stool samples.

TABLE 1. Information of diarrheal stool surveillance samples collected from six sentinel hospitals in Sierra Lenoe, 2018.

Month of collection	Sample number	Sentinel hospital
May	2	MHOS (2)
June	9	SZ (1), LH (8)
July	10	SZ (1), LH (3), EH (6)
August	9	SZ (6), LH (3)
September	5	SZ (3), EH (2)
October	10	LH (3), WH (5), RH (2)
November	9	SZ (8), LH (1)
December	2	SZ (1), LH (1)
Subtotal	56	MHOS (2), SZ (20), LH (19), EH (8), WH (5), RH (2)

Abbreviation: SZ=Sierra Leone-China Friendship Hospital; LH=Lumley Hospital; EH=Emergency Hospital; WH=Waterloo Hospital; RH=Rokupa Government Hospital; MHOS=Ministry of Health of Sierra Leone.

TABLE 2. Positivity information of ARGs/ARG groups detected by real-time PCR in 56 acute diarrheal stool samples from Freetown in Sierra Leone, 2018.

ARG	Number of positive samples (%)	ARG	Number of positive samples (%)
bla_{NDM}	6 (10.7)	*cfr*	0 (0)
bla_{CTX-M} A	0 (0)	*fex*A	0 (0)
bla_{CTX-M} E	32 (57.1)	*mcr*-1	0 (0)
bla_{SHV} A	9 (16.1)	*arm*A	0 (0)
$bla_{OXA-48-like}$	1 (1.8)	*aac*(6')-Ie-*aph*(2')-Ia	0 (0)
bla_{PER}	1 (1.8)	*tet*A	44 (78.6)
*qnr*A	1 (1.8)	*int*I1	52 (92.9)
*qnr*S	0 (0)	IS*CR*1	30 (53.6)
aac(6')-Ib-cr	0 (0)		

Abbreviation: ARG=antibiotic resistance gene.

IS*CR*1 and *int*I1 genes always coexisted with other ARGs/ARG groups. bla_{NDM} gene group coexisted with more than four other ARGs/ARG groups and was detected in five stool samples. Gene group $bla_{OXA-48-like}$ was the first group to be observed in Sierra Leone. Further details of ARGs/ARG groups' coexistence are summarized in Table 3.

DISCUSSION

From this short-term surveillance, serious AR has already been noticed, with multidrug resistance and first reported carbapenemase-encoding genes bla_{NDM} and $bla_{OXA-48-like}$ from Sierra Leone.

Isolating bacteria from stool samples is a complex process that includes the probability of bacterial culture having some variable and uncontrollable parameters. In addition, due to the long purchasing period of ordering and receiving special reagents in Sierra Leone, many bacteria cannot be isolated and studied for AR. Considering the existing non-affluent laboratory infrastructure and equipment of Sierra Leone's lab, ARGs/ARG groups were directly detected from the stool samples by real-time PCR.

Previous studies about the rapid detection of ARGs by real-time PCR among samples are limited. These studies mainly reported ARG detection among isolates. For instance, a report from Tunisia reported that class 1 integrons, bla_{TEM}, $bla_{CTX-M-1}$, bla_{OXA-1}, and bla_{CMY-16} were positive in *Klebsiella pneumoniae* isolates, and the bla_{OXA-48} gene was associated with many other ARGs (5). A study from Egypt demonstrated a high prevalence of resistance to β-lactam antibiotics through ESBLs and AmpC β-lactamases production among the *Acinetobacter baumannii* isolates (6). Rapid detection of ARGs by real-time PCR may reveal the existence of ARGs/ARG groups in diarrhea patients more rapidly and objectively. Our study provided a convenient and accurate method for AR routine surveillance in Sierra Leone, Africa.

TABLE 3. Coexistence of ARGs/ARG groups in 56 acute diarrheal stool samples from Freetown in Sierra Leone, 2018.

Number of ARGs	Coexisting ARGs/ARG groups	Number of samples	Total number of samples
1	*int*I1	6	6
2	bla_{CTX-M} E-*int*I1	3	8
	*int*I1-IS*CR*1	2	
	*tet*A-*int*I1	3	
3	bla_{CTX-M} E-bla_{SHV} A-*tet*A	2	18
	bla_{CTX-M} E-*int*I1-IS*CR*1	1	
	bla_{CTX-M} E-*tet*A-*int*I1	4	
	bla_{NDM}-*int*I1-IS*CR*1	1	
	*tet*A-*int*I1-IS*CR*1	10	
4	bla_{CTX} E-*tet*A-*int*I1-IS*CR*1	6	14
	bla_{CTX-M} E-bla_{SHV} A-*tet*A-*int*I1	2	
	bla_{CTX-M} E-*tet*A-*int*I1-IS*CR*1	5	
	bla_{SHV} A-$bla_{OXA-48\ like}$-*tet*A-*int*I1	1	
5	bla_{CTX-M} E-bla_{SHV} A-*tet*A-*int*I1-IS*CR*1	3	6
	bla_{NDM}-bla_{CTX} E-*tet*A-*int*I1-IS*CR*1	1	
	bla_{NDM}-bla_{CTX-M} E-*tet*A-*int*I1-IS*CR*1	2	
7	bla_{NDM}-bla_{CTX} E-bla_{PER}-*qnr*A-*tet*A-*int*I1-IS*CR*1	1	2
	bla_{NDM}-bla_{CTX-M} E-bla_{SHV}A-*qnr*S-*tet*A-*int*I1-IS*CR*1	1	
	Subtotal		56

Abbreviation: ARG=antibiotic resistance gene.

Some highly prevalent ARGs/ARG groups were previously reported from Africa, such as *tet*(A), *int*1, and bla_{CTX-M} E. A study that included patients with clinical features of healthcare-associated infections in an urban tertiary hospital in Sierra Leone showed a resistance rate of 1.3% for carbapenem-resistant Enterobacteriaceae but did not reveal related ARGs (*7*). Carbapenemase-encoding genes bla_{NDM} and $bla_{OXA-48-like}$ were the first reported in Sierra Leone, West Africa. Some investigators have isolated carbapenem-resistant Enterobacteriaceae isolates carrying bla_{NDM} and bla_{OXA-23} genes from various east-African countries including Kenya, Uganda, Tanzania, Ethiopia, and Rwanda (*2*). The ARGs in our collected stool samples included tetracycline-, fluoroquinolones-, carbapenem-resistance genes, ESBLs and integrase 1 encoding genes. The findings of this study are consistent with those of previously published studies and demonstrate that carbapenemase genes are disseminated in West Africa. Many common ARGs/ARG groups may also spread in Sierra Leone, which could reduce the effectiveness of anti-infective treatment, accelerating the spread of AR. The presence of the carbapenem-resistance gene along with other ARGs in stool samples is critical and may result in the dissemination of ARGs.

Coexistence of multiple ARGs/ARG groups was discovered in the tested samples. 89.3% (50/56) and 75.0% (42/56) of the samples carried more than two and more than three groups of ARGs, respectively. Two stool samples carried seven types of ARGs, simultaneously. Six stool samples carried a single *int*I1 gene that could contribute to ARGs transmission and integration (*5*). The fact that no other ARGs/ARG groups were detected simultaneously might be because of the limited ARG groups screened in this study. Detection of more ARGs should be the focus of future studies. This study found the diversity and complexity of ARGs in stool samples obtained from Freetown, Sierra Leone. Previous studies demonstrated that multidrug resistance had spread in some African countries. A study in Ghana found that >50% and 7.3% of the *Escherichia coli* isolates obtained from pregnant women were positive for bla_{TEM} and *aph*(*3*)-Ia, respectively (*8*). A study from Egypt revealed that 75.4% of the tested gram-negative isolates harbored at least one extended-spectrum β-lactamase-encoding gene — bla_{CTX-M}, and bla_{SHV} (*9*). In Tunisia, an extensively drug-resistant clinical isolate of *Proteus mirabilis* carried plasmid-mediated resistance to

carbapenems (bla_{NDM-1}), cephalosporins (bla_{CMY-4}), aminoglycosides (*aph*3-VIa and *aph*3-Ia), and fluoroquinolones (*qnr*A6) (*10*). The dissemination of ARGs in Africa is alarming and should be given more attention in routine surveillance.

Our investigation revealed that the common types of ARGs detected in diarrheal stool samples collected in this study included *tet*(A), *int*1, IS*CR*1, and bla_{CTX-M} E. These ARGs might be related to the use or misuse of the above antibiotics. Although no carbapenems antibiotics were available for sale in any hospital or pharmacy during our investigation, carbapenemase-encoding genes bla_{NDM} and $bla_{OXA-48-like}$ were detected in the diarrheal stool samples. The findings of this study provide the direction of research for future studies. By expanding on the origins of sample collection, types of ARGs screened, and antibiotics investigated in Sierra Leone, further detailed insights can be obtained about the dynamics of AR in Sierra Leone.

This small-scale study revealed AR present situation among a panel of diarrheal stool samples collected from Sierra Leone, but was also subject to some limitations. First, only 56 of stool samples from several public sentinel hospitals in Freetown were involved in this study, because of restrictions for medical technology, sample transportation, and socioeconomic and behavioral factors. Second, limited ARGs were detected here because of long purchase period of special reagents in Africa. AR informantion of *int*1, IS*CR*1, *tet*(A), bla_{CTX-M} E common dissemination, bla_{NDM} gene existence, and multiplex ARGs in one sample, provides basic information for AR research and surveillance and highlights that continued effective AR surveillance is necessary for Sierra Leone and West Africa.

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