Table 1: Multiplex polymerase chain reaction target

Viral pathogen	Target	Serotypes/subtypes/species detected
Adenovirus	Hexon gene	D,10,13,15,17,19,20,22e30,32,33,36-49,51,53,54, all 51 subtypes
Astrovirus	Outer capsid protein gene	1
Norovirus genogroup 1	ORF 1-2 junction	G1-2, G1-4
Norovirus genogroup 2	ORF 1-2 junction	G2-4
Rotavirus	Segment 7, NSP3 gene	A
Sapovirus	RNA dependant RNA polymerase/capsid genes	Genogroup I, II and IV

ORF: Open reading frame

sample. Negative control was used at the lysis buffer stage of the extraction process. The assay also included positive controls GASTRO PC (liquid) containing plasmids for Noro G1/G2, astro, rota and adenovirus.

The data were summarised and analysed using IBM's Statistical Package for the Social Sciences (SPSS) version 21 (IBM, Armonk, NY, USA).

Results

Of the 80 enrolled patients, viruses were detected in 55%. Single viral infection was seen in 33.7% of patients and mixed viral infections in 21.2%. Adenovirus was the most commonly associated virus (33.7%) followed by rotavirus (28.7%).

The highest co-infection was seen with adenovirus and norovirus (20.4%), followed by adenovirus and rotavirus (13.6%), sapovirus and rotavirus (4.5%) and norovirus, adenovirus and rotavirus (4.5%). Norovirus was present as co-infection in 20.4% of patients. Astrovirus and sapovirus each showed single infections in 1 (2.2%) patient. None of the vaccinated children was found to be positive for rota virus.

Rotavirus infection was the most common in the age group of 1–2 years (42.9%). Adenovirus infection was predominant in the age group of 3–4 years (66.7%). 35% of patients had fever of 2–3 days' duration, vomiting in 88.7% and abdominal pain in 56.8% of patients. Mild dehydration was seen in 65.9%, moderate dehydration in 29.5% and severe dehydration in 4.5% of patients. None of the virus was found to be significantly associated with any of the clinical symptoms.

Viral diarrhoea was mostly seen in April–July.

Discussion

In this study, the occurrence rate of viral diarrhoea was 55%, which corresponds with the findings of Imade and Eghafona, (42.6%) and Colomba *et al*. (59.1%).[2,6] 60% of the study population received Rotavac vaccine which contains live 116E strain, a naturally occurring reassortant strain G9P[11], containing one bovine rotavirus gene P[11] and ten human rotavirus genes. It is administered as 3-dose regimen, 4 weeks apart, beginning at 6 weeks of age. All doses of rotavirus vaccine should be completed by the age of 8 months.[7] 116E can cause mild gastroenteritis rarely. Bhandari *et al*. reported G9P[11] rotavirus gastroenteritis following administrations of ROTAVAC (approximately 1 event in 600 doses); no severe cases were seen.[7] In the present study, all the vaccinated child completed three doses of Rotavac vaccine by 4 months of age. Hence, probably, the rotavirus strains detected in the present study were of wild type. However, the assay that we performed for the detection of rotavirus was not designed to differentiate vaccine G9P[11] from wild G9P[11]; hence, we were not able to rule out contribution of the vaccine strains in causing diarrhoea in our study population.

In this study, overall, viral diarrhoea was more common below 2 years (68.1%), which corresponds with the findings of Carraturo *et al*. and Donà *et al*. with detection rate of 32% and 62%, respectively.[8,9] It is the period of activities for many children. Children usually learn to crawl, walk and sometimes even put their fingers into their mouths, which might be the route of transmission.[2]

In the present study, following diarrhoea, the most common clinical feature was fever (97.7%), followed by vomiting (90.9%) and abdominal pain (43.1%). Anbazhagi *et al*. noted diarrhoea (92%), followed by vomiting (83%) and fever (67%).[10] This is probably because the stool specimens were collected as early as possible within 2 days of onset of diarrhoea.

In the present study, viral diarrhoea mostly occurred in the summer months, i.e., April–July. It is possible that, over and above climatic changes multiple factors interact in certain geographical regions and oppose climatic influences. The waterborne transmission route may dominate during heavy rainfall, followed by flood during summer season in places like Assam.

In the present study, adenovirus was detected most frequently (33.7%), followed by rotavirus (28.7%). Borkakoty *et al*. found the adenovirus occurrence rate of 10.9% in Dibrugarh, Assam, which was lower than the present study, but higher than the other reports.[11,12] This implies that adenovirus burden is increasing in this region.

Conclusion

To conclude, it is only a matter of time that rotavirus-associated mortality and morbidity has steadily declined. It is still important to keep a strict vigilance on the other viruses causing gastrointestinal illness in Assam.

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Conflicts of interest

There are no conflicts of interest.

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Brief Communication

Diverse Aminoglycoside Phosphotransferase Types Conferring Aminoglycoside Resistance in *Enterobacteriaceae*: A Single-centre Study from Northeast India

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Abstract

The present study investigates the molecular basis of *aph*-mediated aminoglycoside resistance and their transmission dynamics in a tertiary care hospital of Northeast India. Two hundred forty one isolates (230 *Escherichia coli* and 11 *Klebsiella pneumoniae)* were collected and screened for aminoglycoside resistance genes. Various *aph* types were amplified using polymerase chain reaction (PCR) assay. Plasmid incompatibilty, horizontal transferability and ERIC-PCR based typing were carried out for all the positive isolates. Among them, 67 isolates showed the presence of *aph* gene. *Aph (3'')-IIIa* and *aph (3')-Via* were predominant and horizontally transferable. All the plasmids were of incompatibility I1 group. Twenty-eight different haplotypes of *E. coli* were found harbouring *aph* gene types. This study was able to identify diverse *aph* types in a single centre and their corresponding phenotypic trait.

Keywords: Aminoglycoside resistance, antibiotic resistance, aminoglycoside phosphotransferase, *Escherichia coli*

Introduction

Aminoglycoside antibiotics were among the frontline drugs used for therapeutic options in hospital settings and constitute as one of the potent agents for life-threatening infections caused by Gram-negative bacteria.[1] However, the efficacy of this antibiotic has been compromised by the emergence of acquired resistance and enzymatic modifications. The enzymes responsible are methyltransferase, acetyltransferase, nucleotidyltransferase and phosphotransferase. Aminoglycoside O-phosphotransferases (APHs) catalyse the transfer of a phosphate group to aminoglycoside molecule.[2] In recent studies, *Aph*-mediated aminoglycoside resistance is reported in *Escherichia coli* and *Klebsiella pneumoniae*.[3,4] In India, no previous study has been carried out to characterize *aph* gene with their corresponding resistance profile. Hence, the current study aimed to characterize *aph* variants and their transmission dynamics in a tertiary care hospital of Northeast India.

Materials and Methodology

Bacterial strains

A total of 241 consecutive nonduplicate clinical isolates of *E. coli* and *K. pneumoniae* were collected from Silchar Medical College and Hospital, Silchar, between June 2018 and January 2019 from the patients who were admitted or attended the OPD of the tertiary referral hospital. Clinical specimens used were urine, pus, aspirates and catheter tip. Isolates were identified based on cultural characteristics on CHROMagar (HiMedia, India) and standard biochemical reactions.[5]

Antimicrobial susceptibility testing

Minimum inhibitory concentration (MIC) of isolates against aminoglycoside antibiotics, namely kanamycin, tobramycin, gentamicin, netilmicin, amikacin and streptomycin (HiMedia, India) was determined by agar dilution method. Disc-diffusion method was also used for the detection of

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susceptibility pattern towards imipenem (10 µg), cefepime (30 µg), aztreonam (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg) and ciprofloxacin (5 µg). The results were interpreted in accordance with the Clinical and Laboratory Standards Institute guidelines 2017.[6]

Molecular detection of aminoglycoside O-phosphptransferases gene

Any organism that was resistant to at least one of the aminoglycosides was selected further for the molecular analysis. Two multiplex polymerase chain reaction (PCR) assays were performed targetting various *aph* genes, namely *aph* (2")-Ib, *aph* (2")-Ic, *aph* (2')-Id, *aph* (3')-IIb, *aph* (3')-I, *aph* (3")-IIIa, *aph* (3')-*Via* and *aph* (4)-Ia.[7] The PCR mixture was composed of 12.5 µl Go Taq green Master mix (Promega, Madison, USA); 10 pmol of each primer ~100 ng DNA template was prepared by boiling centrifugation method (82°C for 20 min). The PCR assay was performed in T100™ Thermal cycler (Bio-Rad, USA) with the condition as follows: initial denaturation at 94°C for 3 min, denaturation at 95°C for 20 s, annealing at 52°C for 45 s, extension at 72°C for 1 min and final extension at 72°C for 5 min.

Horizontal transferability assay

A horizontal transferability assay was done for all the 67 *aph* carrying isolates. Plasmid was extracted by QIAprep Spin Miniprep Kit (Qiagen, Germany) and isolated plasmids were subjected to transformation by heat shock method using *E. coli* DH5α as recipient.[8] Transformants were selected onto the Luria Bertani (LB) agar (HiMedia, Mumbai, Maharashtra, India) containing 2 µg/ml of kanamycin. Conjugation assay was performed using *E. coli*, harbouring *aph* as donor and azide-resistant *E. coli* J53 as recipient. Cells were mixed at a ratio of 1:5 donor-to-recipient and transconjugant was selected on LB medium (HiMedia, Mumbai, Maharashtra, India) containing 2 µg/ml of kanamycin and 100 µg/ml of sodium azide. Transformants and transconjugant were also confirmed by *aph* PCR assay.

Plasmid incompatibility typing

Plasmids harbouring *aph* genes were typed by PCR-based replicon typing to identify the different incompatibility (Inc) types.[9]

DNA fingerprinting by enterobacterial repetitive intergenic consensus-polymerase chain reaction

Enterobacterial repetitive intergenic consensus (ERIC)-PCR was performed to determine the clonal relatedness of all the isolates using primers ERIC-F (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC-R (5'-AAGTAAGTGACTGGGGTGAGCG-3'), and the banding patterns were determined by agarose gel electrophoresis.

Results

Of the 241 isolates, 230 were *E. coli* isolates and 11 of them were *K. pneumoniae*. Among them, 111 *E. coli* and 5 *K. pneumoniae* were found to be resistant to at least one of the aminoglycosides tested and were further selected for the molecular analysis. Among the study isolates, imipenem came up with the highest efficacy as *E. coli* and *K. pneumoniae* showed 89% and 73% susceptibility, respectively. However, against other antibiotics, susceptibility was very low [Supplementary Table 1]. While testing MIC, majority of the isolates were within susceptible range against tobramycin (143/241) followed by kanamycin (141/241), netilmicin (141/241), amikacin (136/241), gentamicin (131/241) and streptomycin (125/241). A total of 67 isolates (64 *E. coli* and 3 *K. pneumoniae*) were found to harbour single and multiple *aph* genes. Among them, 30 isolates were harbouring single *aph* gene types and 37 were found to harbour more than one *aph* types [Table 1]. All the *aph* gene types were conjugatively transferable, and Inc typing of the plasmids that harboured these genes showed that the isolates harbouring multiple *aph* genes were originated from a single IncI1 group [Figure 1] when transconjugants and transformants were confirmed by PCR assay for replicon typing and *aph* genes. DNA fingerprinting by ERIC-PCR suggested that 28 different haplotypes of *E. coli* were detected within this centre and were found to be responsible for the spread of this *aph* genes [Figure 2]. However, ERIC-PCR result showed that only a single haplotype of *K. pneumoniae* was responsible for the carriage of *aph* gene.

Discussion

Till the late 1990s, *aph (3")* family was more predominant among clinical isolates of Gram-negative pathogen.[2] In the current study, we found that most of our isolates were resistant to kanamycin and gentamicin. Another highlight of our study is the presence of diverse *aph* types within a single centre. We observed that *aph (3")* and *aph (2")* were more common than other types. The presence and maintenance of different *aph* genes in isolate from a single centre underscores their diverse origin. In other studies, *aph (3")-IIIa* was found to be responsible for kanamycin resistance.[10,11] In agreement with their studies, we too could select transformants and transconjugant receiving *aph* genes successfully on a screening media containing kanamycin, whereas the same was not successful with other aminoglycosides. Similar to our study, *aph (2")* was found conferring resistance to

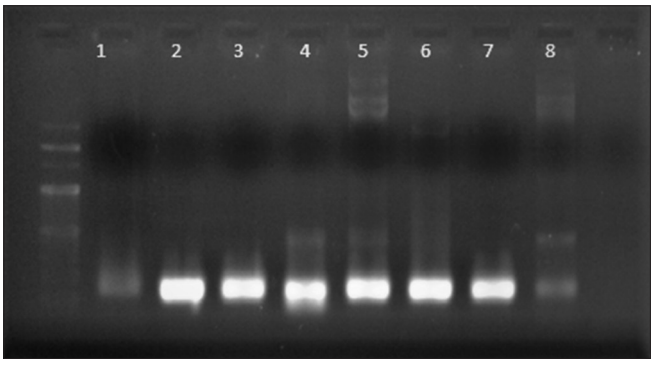

Figure 1: Inc I1 plasmid type (Lane 1–8)

Table 1: Genotypic phenotypic correlation of isolates based on the minimum inhibitory concentration results

Aph-positive single/multiple genes	Number of isolates	Resistance phenotype (MIC values are in μg/ml)
Aph (3'')-IIIa	7	Gen (MIC_{50}=16, MIC_{90}=32)
		Tob (MIC_{50}=16, MIC_{90}=64)
Aph (2'')-Ib	4	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=32, MIC_{90}=32)
Aph (3')-VIa	5	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
		Tob (MIC_{50}=16, MIC_{90}=16)
Aph (2')-Id	2	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
Aph (3')-IIb	2	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=32, MIC_{90}=32)
		Tob (MIC_{50}=32, MIC_{90}=32)
Aph (3')-I	4	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
Aph (4)-Ia	3	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
Aph (2'')-Ic	3	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
Aph (3')-IIb+aph (4)-Ia	2	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
		Tob (MIC_{50}=16, MIC_{90}=16)
Aph (2'')-Ib+aph (3'')-IIIa	1	Kan (MIC=64)
		Gen (MIC=32)
		Tob (MIC=16)
Aph (3')-IIb+aph (3'')-IIIa	4	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
		Tob (MIC_{50}=16, MIC_{90}=16)
Aph (2'')-Ic+aph (3'')-IIIa	2	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
		Tob (MIC_{50}=32, MIC_{90}=32)
Aph (2') Id+aph (3'') IIIa	2	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
		Tob (MIC_{50}=16, MIC_{90}=16)
Aph (2'') Ib+aph (3') I	1	Kan (MIC=64)
		Net (MIC=32)
		Tob (MIC=16)
Aph (3') I+aph (4) Ia	2	Kan (MIC_{50}=64, MIC_{90}=64)
		Net (MIC_{50}=32, MIC_{90}=32)
		Tob (MIC_{50}=16, MIC_{90}=16)
Aph (2') Id+aph (3')-I	1	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
		Tob (MIC_{50}=16, MIC_{90}=16)
Aph (3') IIb+aph (3') Via	1	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
		Tob (MIC_{50}=16, MIC_{90}=16)
Aph (2') Id+aph (3') Via	1	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
		Tob (MIC_{50}=16, MIC_{90}=16)
Aph (3') Via+aph (3'') IIIa	1	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
		Tob (MIC_{50}=16, MIC_{90}=16)
Aph (3') I+aph (3') Via	3	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
		Tob (MIC_{50}=16, MIC_{90}=16)
Aph (3')-IIb+aph (3'')-IIIa+aph (4)-Ia	1	Kan (MIC_{50}=64, MIC_{90}=64)
		Gen (MIC_{50}=16, MIC_{90}=16)
		Tob (MIC_{50}=16, MIC_{90}=16)

Contd...